A Safety Report on Ashwagandha Root Extract

An Evaluation of the risk assessment

made by DTU Food Institute 2020

Prepared by
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and
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Summary

The emerging health challenges have resulted in rise in global use of herbal supplements. Ashwagandha is one of the herbal supplements having multi-dimensional health effects. Traditional use of Ashwagandha is documented in Ayurveda text since centuries. Various properties of this botanical have been validated in systematic research including clinical trials, studies on animals and other laboratory investigations. The beneficial effects are specific to use of roots and specific extraction methods that follow traditionally validated principles and practices.

The rising popularity of Ashwagandha based supplements may have resulted in various market preparations with various extraction methods (e.g purified extracts) from various parts of the herb. The concerns about safety of Ashwagandha needs systematic analysis of studies. This document presents research synthesis with a focus on safety of Ashwagandha root. The analysis presents data from 79 human clinical studies, 29 Toxicity studies, and 130 preclinical studies. The report also discusses observations by DTU Food Institute and presents data driven responses to the comments.

The published research suggests that ARE (Ashwagandha root extract) is clinically beneficial for reducing stress and anxiety by balancing cortisol levels, it enhances memory and cognition, increases sex drive, endurance, strength, muscle size, and muscle recovery and improves sleep and quality of life. The data vouch its safety as no serious adverse event is reported in any of the clinical trials. Trends from animal studies shows efficacy and safety of ARE. Animal studies include acute and long-term toxicity assessment including reproductive and developmental toxicity. The KSM-66 Ashwagandha extract, which is derived from traditional practices of Ayurveda, is safe and effective for above mentioned indications.

The report by the DTU Food Institute (published in May 2020) needs a reconsideration due to two main reasons:

1. The report is not taking into consideration the different properties concerning function and safety between different plant parts of Ashwagandha
2. There is evolving evidence on safety and efficacy of Ashwagandha published since 2020 (more than 400 papers)

Background:

This report is based on an extensive literature search conducted to understand and demonstrate the safety of the Ashwagandha Root (*Withania somnifera*) Extract (ARE). This safety report highlights the safety of ARE with KSM-66 Ashwagandha (Ixoreal Biomed Inc, USA) as an example.

Recent report "Risk assessment of the root from *Withania somnifera*" prepared by DTU Food Institute (Fødevareinstituttet) and submitted to The Dietary Supplement Group (Kosttilskudsgruppen), The Danish Veterinary and Food Administration (Ref.: DTU DOCX. No. 19/1030299, dated May 15, 2020) discusses risk assessment of ARE, but the DTU report mentions that it was unable to find reports identifying the various active substances in the various plant parts. The DTU mentions that there is information about the root to be used as an abortifacient. DTU also mentions that there are animal studies that show a negative effect on sex hormones, immune system and thus these effects could pose a potential safety risk in humans. Lastly, DTU also mentions the possible impact of Ashwagandha on the thyroid gland.

On this background, there is need to analyse the studies referred by DTU and provide detailed answers to the conclusions that DTU has raised. The research on ARE shows that DTU's assumption concerning the safety risks when consuming Ashwagandha does not apply to a well-specified and well-studied extract of ashwagandha root and needs re-thinking on the conclusions made in the report.

The importance of distinguishing between different plant parts when doing a safety assessment is an important aspect while discussing safety of natural products. When evaluating the taste or nutrition of the apple fruit, one ordinarily would not draw conclusions from findings about the apple plant's stem or the leaves. Similarly, when evaluating the properties of the ashwagandha root, one ought not to rely on findings related to the other parts of the plant. Yet, in criticizing the use of ashwagandha root, the DTU cites papers that are not specifically about the root part of the plant. Furthermore, what the DTU presents from the literature is rather skewed, meaning that there are findings and qualifications in the scientific literature that counterbalance some of DTU's assessments, but the DTU does not talk about these counterbalancing facts and factors. Even among the papers that the DTU does cite, in some cases the DTU focuses on only some sections of the papers when other parts of the paper are not fully consistent with the conclusions that the DTU draws from those papers. All this is very surprising because the DTU is a highly respected organization. One would, therefore, expect DTU to be very systematic and objective about the ashwagandha root, building up the facts and then drawing conclusions from the facts. In this case, however, it seems as though the DTU has already decided that the ashwagandha root should be banned and then searched the literature for papers that are consistent with that decision. Perhaps the DTU is prejudiced against ashwagandha because it comes from India which is an unfamiliar country. It is unclear what other explanation is there to explain the fact that the DTU has chosen to ignore the large volume of positive evidence on ashwagandha root benefits and is focusing on the small minority set of papers on infrequent adverse events that are possibly random events. For almost any consumable material on which there is a large literature (like rice or milk), one would

expect there to be some papers that identify adverse effects that are possibly random events. Presumably, we would not react to that by banning rice or milk, because we would weigh the infrequent adverse effects of wheat or milk against the larger literature and empirical evidence. For the ashwagandha root, there is a large literature and empirical evidence of millions of people over multiple centuries consuming the root products safely and with strong beneficial effects.

Dr. Senia Johansson (Department of Medicinal Chemistry, Biomedical Centre, Uppsala University, Sweden), has the following statement on DTU not distinguishingly adequately the literature on ashwagandha root versus leaves, and ignoring the large literature on positive benefits of ashwagandha root versus infrequent adverse effects that may be random events:

"In conclusion, to ensure the safety and effectiveness of food supplements / dietary supplements that contain plant extracts, it is crucial to conduct a comprehensive safety evaluation on the specific part of the plant used in the supplement, such as the root extract. The content of several substances has been shown to vary significantly different between the root and aerial parts of *Withania somnifera* (L.) Dunal, as indicated in Tables 1 and 2 (***Annexure - I***). Evaluating the entire plant, leaves or fruit and implying the same conclusion for other parts like root, as was done in the DTU assessment, can lead to misleading results since different parts of a plant have unique functions, structures, and chemical compositions that can affect the supplement's safety and efficacy, making it difficult to compare them and draw meaningful conclusions. Concluding the safety of Ashwagandha root (*Withania somnifera* (L.) Dunal) based on studies of leaves or the entire plant can also be deceptive because other parts of the plant, such as the leaves, contain different bioactive compounds, including high amounts of withaferin A and withanone that are present in much lower concentrations in the root. Thus, the safety and efficacy of supplements with Ashwagandha root extract are likely to differ from one containing extracts from other plant parts. Consequently, it is crucial to perform a safety assessment of the exact plant part utilized in the supplement to guarantee its safety and effectiveness for consumers."

Another important aspect is type of extract used for the study. The science of Ayurveda suggests use of whole root extracts rather than isolated phytochemicals. Although the purified extract leads to potentiation of efficacy, the toxicity of isolated compounds becomes a challenge. This fact is highlighted by many researchers working in the field (Patwardhan B, 2016 & Patwardhan B and Chagutru R, 2016). This is the reason, the distinction between ARE and PAE (Purified Ashwagandha Extract) has to be noted while discussing the safety of Ashwagandha.

This report is a research synthesis on the safety of ARE. The data from authentic sources like PubMed, Scopus, and Ayush Research Portal databases were searched using the keywords Ashwagandha or *Withania somnifera* in combination with terms such as toxicity, safety, preclinical, etc. The selections were limited to English language reports. A PubMed database search returned a total of 1413 articles were located and the studies investigating the biochemical profile, phytochemistry, molecular docking and cloning, review articles, and duplicate publications were excluded. After excluding the studies mentioned above, we have

compiled the data from 79 human clinical studies, 29 Toxicity studies, and 130 preclinical studies **(*Annexure II – V- Compiled Data*).** The report presents research synthesis on ARE and PAE with the relevant data and its interpretation with the focus on safety of Ashwagandha.

Introduction to *Withania somnifera* (L.) Dunal

Ashwagandha, scientifically known as *Withania somnifera* (L.) Dunal which belongs to the Solanaceae family is one of the most revered adaptogenic botanicals and anti-stress agents in Ayurveda, the traditional system of medicine (Mishra, L.C and Singh B.B., 2000, WHO Monograph 2009). Brekhman and Dardymov proposed in 1969 the term adaptogen as an innocuous agent that increases non-specific resistance against harmful factors or physical, chemical, biological, and psychological "stressors", normalizing the homeostasis of individuals. Traditionally Ashwagandha root has been used for its anti-inflammatory, anti-stress, antioxidant, memory-boosting, rejuvenating, and aphrodisiac properties.

The most prominent and clinically evaluated part of the plant is the roots. Ashwagandha roots have been used for over 4000 years as an important ingredient. The uses of Ashwagandha root are documented in various Ayurvedic textbooks, as well as standard references such as the United States Pharmacopoeia, British Pharmacopoeia, Indian Pharmacopoeia Commission, the Health Canada monograph, and the World Health Organization's monograph for Ashwagandha, list only the root and recommend the usage of roots and not the aerial parts of the Ashwagandha plant.

Despite having a long history of use, no serious concerns have been reported and it is considered the safest botanical in Ayurveda (Indian system of Medicine). The Ministry of AYUSH, Government of India has recently issued an advisory to the manufacturers of Ashwagandha to refrain from using leaves to prepare Ashwagandha products (***ref. L-1 1O11/9/2021-DCC, dated October 06, 2021***). The Indian authority advises the use of roots of Ashwagandha for the preparation of single or compound formulations.

It is not just historical or traditional use, but also several studies conducted on Ashwagandha have proven the safety, efficacy, and tolerability of Ashwagandha in various study models. The most clinically documented extract is KSM-66 Ashwagandha root-only extract with around 25 completed randomized, double-blind, placebo-controlled studies conducted on healthy but stressed individuals.

Safety documented in clinical trials on Ashwagandha root

The safety and efficacy of Ashwagandha have been evaluated in 79 clinical studies (***list attached as annexure II***). Data from these clinical studies suggest that Ashwagandha root extract may help reduce stress, anxiety, and fatigue and improve sleep, stamina, endurance, general well-being, sexual performance, male infertility, and cognitive function. A total

population of more than 5020 participants has been included in these 79 studies with 2862 participants in the Ashwagandha group and 2158 participants in the control group.

The adverse events reported are 3.10% with Ashwagandha and 3.10% in the placebo/control group. No serious adverse attributed to Ashwagandha are reported. All the adverse events reported in these studies have been minor and resolved within a short period without any medical intervention or sequelae. The frequency and severity of Ashwagandha's side/adverse effects are reported to be similar to control and placebo. Most importantly, these reported adverse events were not serious and they do not raise any suspicion of hepatotoxicity, thyroid toxicity, or negative effect on sex hormones.

Safety Studies of Ashwagandha

Acute toxicity studies have shown purified Ashwagandha Root Extract to be safe up to 2000 mg/kg body weight in rats, which is much more than the therapeutic dose. The chronic toxicity studies of purified ARE (90 days in rats) at a maximum dose of 2000 mg/kg body weight did not induce any observable toxic effects. About 29 toxicity studies have been conducted with Ashwagandha and a list of them has been provided in ***Annexure-IV***.

In the 90-day study conducted using KSM-66 Ashwagandha, one hundred (50 male and 50 female) rats were used. The animals were assigned to six groups viz G1-Control, G1R-Control recovery, G2-500mg/kg, b.wt, G3-1000 mg/kg, b.wt G4 and G4R-2000 mg/kg,b.wt. No mortality and morbidity were observed in any of the animals in the control, treated, and recovery groups during the study. No adverse clinical signs were observed in the control, treated, and recovery groups of animals from day one of test item administration till the end of the observation period. No test item-related changes were observed in the control, control recovery, treated, and high-dose recovery group animals in haematology, electrolytes, and biochemistry parameters. No changes in thyroid hormones T3, T4, and TSH were observed as analysed on day 91 in the control and treated groups on day 91 and Control Recovery and High dose Recovery group at the end of the observation period. Under the conditions of this study and based on the toxicological endpoints evaluated, the test item KSM-66 Ashwagandha Root Extract was observed to be 2000mg/kg safe when administered orally for a period of 90 days in Wistar rats. Hence the No-Observed Adverse Effect Level (NOAEL) of KSM-66 Ashwagandha Root Extract is found to be 2000 mg/kg/day by oral route.

Withania somnifera root extract: Efficacy and Mechanism of Action

As an adaptogen, the ashwagandha root significantly helps to manage stress (Chandrasekhar K et al, 2012; Choudhary D et al, 2017; Salve J et al, 2019, Priyanka G. et al, 2020; Kaur J et al, 2022). Ashwagandha has a GABA-mimetic effect, which reduces the over-excitation of neurons, thereby producing calmness, and reducing stress and anxiety (Candelario M et al, 2015). This GABA-mimetic activity might also play a potential role in inducing gonadotropin-release hormone secretion and improving hormone balance. Ashwagandha improves the body's defense against disease by improving cell-mediated immunity. It also possesses potent

antioxidant properties that help protect against cellular damage caused by free radicals (Panda S et al, 1997).

The root extract of Ashwagandha has been shown to induce alanine transaminase activity which increases alanine in seminal fluid leading to a less oxidative stress index and improved semen quality (Gupta A et al, 2013). Ashwagandha also normalizes the lactate, phenylalanine, glutamine, citrate, and histidine in the seminal fluid which can improve the enzymatic processes in the tricarboxylic acid cycle (TCA) and fatty acid metabolism (Mahdi AA et al, 2011; Kyathanahalli CN et al, 2014). Ashwagandha is neither a sedative nor a stimulant, it just creates a perfect balance and brings the body to homeostasis. If we are low on energy, Ashwagandha helps in boosting energy levels and helps in reducing stress levels.

KSM-66 Ashwagandha – Unique Process of Preparing root extract

KSM-66 Ashwagandha is a standardized aqueous root-only extract that is produced using a unique proprietary extraction process, based on "Green Chemistry" principles, without using alcohol or any other chemical solvent. KSM-66's extraction process entails pre-treating the ashwagandha roots with milk. For this reason, it contains milk constituents. Such pre-treatment is consistent with the process described by traditional Ayurveda healers and texts. The milk pre-treatment is important in our extraction process because this is in fact what leads to the retention of both hydrophilic components and lipophilic components of the raw root, which in turn leads to a full-spectrum extract of such high potency. KSM-66 Ashwagandha root is different from other alcoholic and hydro-alcoholic extracts because it does not upset the delicate balance of various constituents found in crude ashwagandha root; rather, it retains and potentiates the synergism in the whole root.

The treatment with milk follows the traditional practice of Ashwagandha use that maximizes the therapeutic benefits and minimizes the possibility of adverse events even when administered in large amounts.

KSM-66 Ashwagandha's unique extraction process yields the optimum percentage of withanolides, retaining the other important bioactive of the plant, which is required for the efficacy of the herb. KSM-66 withanolide content is measured by HPLC and is found to be of >5% concentration. KSM-66 Ashwagandha is standardized to the following withanolides: Withanolide A, Withanone B, Withaferin A, Withanoside IV, Withastromonolide A, and Withanone. KSM-66 Ashwagandha contains negligible amounts of Withaferin-A and Withanone (below detection level). The certificate of analysis of the KSM-66 Ashwagandha root extract clearly indicates that the alkaloid content is below the detection level. In addition to numerous clinical studies on KSM-66 Ashwagandha, it has been studied for several toxicological effects in various in vitro and animal models.

Clinical safety of KSM-66 Ashwagandha® root extract

So far 19 human clinical studies have been published using KSM-66 Ashwagandha for various therapeutic applications. In addition, 8 studies have been completed out of which 3 studies are under peer review for publication, and another 24 studies on KSM-66 are ongoing. All studies are conducted on healthy but stressed individuals and are closely monitored for adverse events. KSM-66 Ashwagandha is known to promote balance in the body and has been clinically proven to:

- Reduce stress and anxiety by balancing cortisol levels
- Enhance memory and cognition
- Increase endurance, strength, muscle size, and muscle recovery
- Improve sleep and quality of life
- Improve sexual function in both men and women and normalize testosterone in men
- No changes in laboratory parameters that could possibly suggest hepatic toxicity, renal toxicity, or disruption of hormonal status including sex hormones and thyroid hormones
- Normalize thyroid function

In these studies, clinical safety was assessed based on the frequency of adverse events reported by the participants. Also, the Patient's Global Assessment of Tolerability to Therapy (PGATT) was considered by the physician to assess the tolerability of the extract. Mild adverse events were reported for both Ashwagandha and the placebo group participants in 8 of the published studies. Participants did not report any adverse events in the other 10 studies.

The total number of participants in all these 19 published studies was 1114 out of which 577 received KSM-66 Ashwagandha and 537 received placebo. Only about 3.98% (23/577) participants receiving KSM-66 Ashwagandha reported adverse events (mild severity), whereas 5.02% (27/537) participants receiving placebo reported mild adverse events. According to the investigators, no serious adverse events were reported in the studies and it did not raise any suspicion of toxicity with daily consumption of ashwagandha. Thus, it may be mentioned that KSM66 Ashwagandha was well-tolerated among the participants of the studies published.

Ixoreal, the maker of KSM-66 is committed towards the goal providing of safe and effective products. In doing so a study is in progress to assess the efficacy and safety of the KSM-66 Ashwagandha on 1200 participants across the globe. Another study that evaluates the long-term safety of Ashwagandha supplementation for one year is currently ongoing. Further studies evaluating the safety and efficacy of KSM-66 on thyroid function, sleep, male sexual function, female sexual function, hair, and skin health, and many more conditions are also ongoing.

Verma et al (2021) evaluated the safety of KSM-66® ashwagandha root extract in healthy adult volunteers. In this randomized, double-blind, placebo-controlled, and parallel-group study, 80 healthy participants (40 males, 40 females) were randomized in a 1:1 ratio to receive either Ashwagandha 300 mg or a placebo of the same dosage, twice daily, orally for 8 weeks. The authors of the study concluded that the outcomes did not indicate any untoward effects in any of the treated volunteers. No statistically significant change or abnormality was observed in the

considered parameters including liver parameters and thyroid hormonal profile in both groups. No adverse events were reported by any of the participants in this study. Results of this study indicate that intake of KSM-66 Ashwagandha® at up to 600 mg per day in divided doses is very well tolerated. Currently, one long term study (One-year duration) with 200 participants is on ongoing to evaluate the safety of KSM-66 Ashwagandha.

Several toxicity studies have been performed on the KSM-66 Ashwagandha such as Acute oral toxicity, 28 Days Repeated Oral Dose Toxicity, Genotoxicity (acute and 28 days repeated dose by comet assay), 90-Day Repeated Dose Oral Toxicity, In vivo Mammalian Erythrocyte Micronucleus Test, Bacterial Reverse Mutation Test, In Vitro Chromosomal Aberration Test and Thyroid Toxicity in Zebrafish. A detailed summary of these toxicity studies has been provided in ***Annexure V***. The dose used in the 90-day toxicity (2000mg/kg/bwt) on rats is 200 times higher than the normal dose for humans.

The Global Presence Of KSM-66 Ashwagandha (Billions Of Doses Consumed):

KSM-66® is marketed and has significant market distribution all over the world. It was developed over 14+ years of R & D. It has been on the market for just over 11 years, but it is already in more than 1600 products in 45 countries and in many from major supplements companies across the globe like Pfizer, Walmart, Mars, Nature's Bounty, GNC, GSK, CVS, Nestle, Unilever, Bayer to name a few. KSM-66® Ashwagandha is used in plenty of delivery formats, the most common being capsules, tablets, soft gels, and powders. It is also used as an ingredient in various functional foods and beverages like gummies, bars, chocolates, non-alcoholic beverages, tea, coffee, and processed fruit and vegetable juices.

According to Ixoreal, the maker of KSM-66, in the last 6 years alone, around 3326120000 doses (Over three billion) of KSM-66 Ashwagandha have been sold worldwide, and there is no report of adverse events associated with KSM-66 Ashwagandha usage. To sum up, the KSM-66 Ashwagandha root extract is extremely safe to use and is one of the most widely available dietary supplements on the market.

Responses to the claims/concerns raised by the DTU

DTU's Introduction Summary:

The DTU risk assessment report mentions two rat experiments conducted using ethanol and methanol root extracts. The study with the ethanol root extract shows effect on platelets and coagulation while the study with methanol extract does not show the corresponding effect.

Comment: The difference in the effects with different extracts demonstrates the importance of the extraction with various solvents. KSM-66 Ashwagandha is neither extracted using ethanol nor methanol, but it is extracted using a unique proprietary extraction method that took 14 years to develop and it is based on the Ayurvedic tradition, where only milk and water should be

used during the extraction process. In the study mentioned, all the hematological values were well within the normal reference range (Patel et al 2016).

The DTU risk assessment report mentions that the root is used as an abortifacient and even the dose at which this effect is exerted is unknown.

Comment: There is no known literature evidence that demonstrates that Ashwagandha root is used as an abortifacient. In fact, a study by Prabhu & Panchapakesan, 2015 evaluated the prenatal developmental toxicity potential of *Withania somnifera* root extract in rats. This study used a hydroalcoholic extract of WS root in pregnant rats up to 2000 mg/kg body weight/day. There was no evidence of maternal or fetal toxicity. Also, *Withania somnifera* root extract caused no changes ($p < 0.05$) in the body weight of parental females, number of corpora lutea, implantations, viable fetuses, external, skeletal, and visceral malformations.

No evidence of maternal or fetal toxicity was observed. *Withania somnifera* root extract caused no changes ($p < 0.05$) in the body weight of parental females, number of corpora lutea, implantations, viable foetuses, external, skeletal, and visceral malformations. Under the conditions of the study, the no-observed effect level (NOEL) of *Withania somnifera* root extract for maternal and developmental toxicity was concluded to be 2000 mg/kg/day.

Also, reproductive and developmental toxicity using KSM-66 Ashwagandha is completed and the study suggests that KSM-66 Ashwagandha is safe and no toxicity was observed. The animals were assigned to four groups viz G1-Control, G2-500mg/kg, b.wt, G3-1000 mg/kg, b.wt G4 - 2000 mg/kg,b.wt. Dosing of both sexes started 2 weeks prior mating. Males were dosed from pre-mating until the minimum dosing period of 28 days is completed and sacrificed. Females were dosed during pre-mating, mating, gestation, and at least thirteen days after delivery (PPD 13) or and up to a day before necropsy.

The DTU risk assessment report mentions that majority of the clinical trials have been performed in men who already suffer from poor sperm quality and have altered levels of sex hormones.

Comment: Chauhan S et al 2022 and Wankhede S et al 2015 studied the effect of KSM-66 Ashwagandha on Testosterone levels in healthy men. The results of the study demonstrated that KSM-66 Ashwagandha though increased testosterone levels in healthy men but this increase was within the normal reference range. Another study using KSM-66 Ashwagandha, which is under peer review demonstrated a significant increase in both free and total testosterone levels in healthy men undergoing resistance training.

Apart from the studies on healthy men, Dongre, S. et al 2015, Ajgaonkar A, et al 2022, and Gopal, S et al 2021 studied the effect of KSM-66 on women's health. KSM-66 Ashwagandha significantly increased sexual desire, libido, satisfaction, and also estrogen levels in healthy women.

The DTU risk assessment report mentions that there are animal studies that indicate an effect of sex hormones after dosing with Withania somnifera which could potentially pose a risk to the user's health.

Comment: The studies that show a negative effect were conducted using the aqueous extracts of the leaf and other plant parts than the root which has different content and combinations of active substances. One should be careful in extending the finding from this study to well-defined root extracts. Traditionally it is only the Ashwagandha root that has been used as an aphrodisiac.

Taxonomy, natural occurrence, and chemotypes:

The DTU risk assessment report mentions the occurrence of various morphological forms and chemotypes and due to lack of information on ingredients and chemotypes, the DTU does not want to elaborate on the ingredients and chemotypes.

Comment: KSM-66 Ashwagandha is the only major branded Ashwagandha, whose manufacturers own the entire supply chain in producing the extract. The makers have their own farms extending to 1000s of acres where Ashwagandha is grown. The seeds from the berries are separated, and the roots are harvested. The leaves and the other plant parts are used as compost. These seeds are sown again and this way the KSM-66 Ashwagandha comes from the same chemotype.

Substances in the roots and other parts of the plant:

Alkaloids:

The DTU risk assessment report mentions that there are several studies that have been published on the total content of alkaloids and it ranges from 0.16%-0.91%. The total alkaloid content in the dried leaves is 2.10% and this is higher than the alkaloid content found in the roots.

Comment: The data from various studies denotes that the alkaloidal content of the Indian ashwagandha roots have an overall alkaloid content that ranges from 0.13% to 0.31% (Mirjalili MH, et al 2009; Lal et al., 2012; Lal et al., 2014; Lal, 2015; Susheel G et al 2017, Ganguly B et al, 2018 and Kumar et al., 2022).

The certificate of analysis of **KSM-66 Ashwagandha root extract** clearly indicates that the amount of **alkaloids** is **below the detection level.**

Withanolides:

The DTU risk assessment report mentions that there are several studies that have been published on the total content of Withanolides in the root are around 0.1-1.11% and the amount of Withaferin A is around 0.06-0.89%. The berries of the various Indian specimens, the total

withanolide content found was 2.95% - 3.35%. The amount of Withaferin A in the stems was 1.51mg/g.

Comment: Trivedi et al. (2017) identified a total of 43 withanolides in the hydroalcoholic extract of Ashwagandha root using LC/MS, GC/MS and NMR. Namdeo AG et al 2011, and Johri et al 2005 have performed the metabolic characterization of *Withania somnifera* from different regions in India using NMR spectroscopy and have found that Withaferin A is approximately 0.92% in roots and around 22.31% in leaves. Also, Withaferin A is cytotoxic in nature and is used in the treatment to kill the cancer cells. However, the application of Withaferin A on healthy cells is not recommended, precisely because of this cytotoxic effect.

KSM-66 Ashwagandha is standardized extract and the withanolide content is measured by HPLC and is found to be of **>5%** concentration. Also, the certificate of analysis of **KSM-66 Ashwagandha root extract** clearly indicates that **Withaferin A** is **below the detection level**.

General Toxicity Studies: Root

Patel et al 2016: *The DTU pointed out that in a study published by Patel et al 2016, two studies were conducted to evaluate the acute single dose toxicity and sub-acute toxicity of Ashwagandha root extract. The extract used in this study was an ethanolic extract with 4.5% Withaferin A. The acute experiment was performed with 5 female rats, each orally administered with a single dose of the extract at 2000mg/kg body weight. No macroscopic pathological changes were observed at the Day 15 before the rats were euthanized.*

The same authors conducted a subacute toxicity study in young healthy rats of both sexes and these were divided into six groups each having five males and five female rats. The animals were dosed with the extract for 28 days in doses of 0, 500, 1000 or 2000mg/kg body weight/day after which they were euthanized.

The DTU notes that during the study, statistically significant increases in the packed cell volume and mean corpuscular volume levels were elevated in both 1000mg/kg and 2000mg/kg body weight. Also, in females the white blood cell levels were elevated at both 1000mg/kg and 2000mg/kg body weight.

Comment: The authors of the study state that the increases in the hematological parameters in both female and male rats were within the normal reference range and the increases in these values were not considered as toxicologically relevant. The author of the study comes to a different conclusion and mentions that compared to the control group in sub-acute toxicity study, administration of extract did not show any toxicologically significant treatment related changes in clinical observations, ophthalmic examination, body weight gain, feed consumption, clinical pathology evaluation, and organ weight. Terminal necropsy did not reveal any treatment related gross or histopathological findings. Based on this study, the no-observed-adverse-effect-level of WSE is 2000 mg/kg body weight, the highest level tested.

Also, the extract contains 4.5% Withaferin A. Withaferin A is cytotoxic in nature and is used in the treatment to kill the cancer cells. However, the application of Withaferin A on healthy cells is not recommended, precisely because of this cytotoxic effect. The certificate of analysis of **KSM-66 Ashwagandha root extract** clearly indicates that **Withaferin A** is **below the detection level**.

The authors also recommend a 90-day toxicity study, which has already been done on KSM-66 Ashwagandha root extract. The 90-Day Repeated Dose Toxicity study was performed with KSM-66 Ashwagandha (2000mg/kg) and the results shows that no test item-related changes observed in haematology parameters and biochemical parameters in any of the treated groups when compared with control groups. No clinical signs of toxicity were observed in the control, treated, and recovery groups of animals from the day of test item administration till the end of the observation period (***Annexure – V***).

Prabhu et al 2013: *The DTU pointed out that in a study published by Prabhu et al 2013, two studies were conducted to the acute single dose toxicity and sub-acute toxicity of methanolic Ashwagandha root extract. The methanolic extract of Ashwagandha root contains 0.04% Withaferin A.*

In the acute experiment, five female rats received a single dose of the extract orally (through a gastric tube, dissolved in distilled water) of 2000 mg extract/kg body weight. No deaths or behavioural changes were observed during the 14 days of the experiment, and no pathological changes were seen in the macroscopic examination.

In the subacute experiment rats were dosed daily for 28 days with the extract (dissolved in distilled water) at doses of 0 (control), 500, 1000 and 2000 mg/kg body weight. There were no statistically significant differences seen between the dosed groups and the control group in feed consumption and fluid intake, body weights and growth, organ weights, haematological and biochemical parameters or in the macroscopic and histopathological studies.

The DTU Food Institute finds it unusual that not a single one of the many tested parameters differs statistically significantly from the control group.

Comment: The authors recommend a 90-day toxicity study, which has already been done on KSM-66 Ashwagandha root extract, which comes to the same conclusion as Prabhu that there were no statistically significant changes between the KSM-66 group and control.

In addition to the above studies, several researchers including Sahni YP et al, 2014; Jain H et al 2010; and Sharma S et al 1985, have found that Ashwagandha root extract is safe and devoid of any toxicity.

General Toxicity Studies: Berries

Duke 1985: *The DTU pointed a study where the berries that are occasionally eaten by children caused severe symptoms from the gastrointestinal tract.*

Comment: KSM-66 Ashwagandha is a root only extract and it does not contain any berries. In addition, three studies have been completed using KSM-66 Ashwagandha root extract in children, between the age group of 5-12 years (at a dose of 150mg twice daily). There were no adverse events reported in any of the studies, and it can be concluded that KSM-66 Ashwagandha is safe in children. Mention dose, duration and under publication

General Toxicity Studies: The Whole plant

Arseculeratne et al. 1985: *The DTU pointed out a study in which rats where ingested with approximately 5g plant/rat per day. There were no deaths, but 5/6 rats had centrilobular hydropic degeneration in the liver, 4/6 rats had peribronchial, and perivenous oedema in the lung and 5/6 rats displayed significant intertubular vascular congestion, tubular casts and tubular degeneration in the kidneys (5/6).*

Comment: This study model used to evaluate the toxicity had no control group used. Also, the 5g/rat which comes as a dose of 25 g/kg of the whole plant is way too high than all the safety studies undertaken up to 2g/kg in rats and NOAEL is considered as 2 gm/day which is clinically more relevant. The basis of this study itself is irrelevant in a clinical context. Hence it would be erroneous to question the safety of the root of Ashwagandha based on these findings and definitely not for a well-defined root extract like KSM-66 Ashwagandha.

Sharma 1986: *The DTU pointed out a study in which sexually matured rats were administered Ashwagandha whole plant with water for either 4 weeks or 8 months at a dose of 100mg/kg. The rats were divided into groups consisting of four females and two males.*

One animal died in the control group, none in the Withania somnifera groups. In the Ashwagandha group 6 rats became pregnant while 5 rats became pregnant in the control group. The litter size was eight in the dosed group and nine in the control group.

Comment: In the 8- month study it was mentioned that each group consisted of 4 females and 2 males in each group but the results mentioned that 6 rats were pregnant in the dosed group and 5 rats were pregnant in the control group. The study model is a weak and underlines the lack of quality.

The DTU also pointed out that in a 4-week study with 23 male rats in the control group and 35 in the active group that received 250mg/kg whole plant. There was no statistically significant difference in growth between the exposed group and the control group after four weeks. The relative liver weight was statistically significantly increased in the dosed group relative to the control group, but no histopathological changes in the liver were observed in the exposed group. The relative adrenal weight and cortisol content of blood plasma were both statistically significantly lower in the dosed group compared to the control group, and there were no histopathological changes in the adrenal glands. The dosed group had a lower body temperature of 1.5 ° C compared to the control group.

Comment: No increase in liver weight is reported in the well-designed 90 days toxicity study using KSM-66 Ashwagandha root extract as compared to this random study using the whole plant. Also, there are no associated histopathological changes. Hence this is difficult to attribute it to the safety of Ashwagandha. It is also difficult to draw any conclusions regarding the safety of the pure root extract of Ashwagandha based on this observation on whole plant and rather contradicted by other studies.

Data of preclinical studies on Ashwagandha root extract

Studies of Pregnancy and Fetal Development:

Prabhu & Panchapakesan, 2015: *The DTU pointed out a study in which the effect of a methanol extract of Withania somnifera root was investigated in pregnant female Wistar rats. The study included four groups of female rats (25 rats/group), each of which was mated with a male rat (day 0). From days 5-19, the females were orally dosed (through gastric tube) with the extract in doses of 0 (control), 500, 1000 or 2000 mg/kg body weight/day. The females were euthanised on day 20. The rats in the highest dose group had statistically significant higher body weights and weights of the pregnant uterus compared to the control group. The mean number of live-born pups was also higher (10.4 in the highest dose group versus 7.3 in the control group - the difference was not statistically significant).*

The DTU points out that the authors did not choose the option that the OECD guideline no 414 mentions where the dosing should take place throughout the gestation period. As the dosing in the experiment started on day 5 of pregnancy, the experiment is designed in a way where it is not possible to assess whether the extract has a detrimental effect on the fertilised egg/embryo earlier in fetal development.

Comment: The OECD Guideline point 13 on Dosing states the following: "Normally, the test chemical should be administered daily from implantation (e.g. day 5 post mating) to the day prior to scheduled caesarean section. If preliminary studies, when available, do not indicate a high potential for preimplantation loss, treatment may be extended to include the entire period of gestation, from mating to the day prior to scheduled humane killing. It is well known that inappropriate handling or stress during pregnancy can result in prenatal loss. To guard against fetal loss from factors which are not treatment-related, unnecessary handling of pregnant animals as well as stress from outside factors such as noise should be avoided"

There was no evidence of maternal or fetal toxicity. Also, *Withania somnifera* root extract caused no changes ($p < 0.05$) in body weight of parental females, number of corpora lutea, implantations, viable foetuses, external, skeletal and visceral malformations.

Also, reproductive and developmental toxicity using KSM-66 Ashwagandha is completed and the study suggests that KSM-66 Ashwagandha is safe and no toxicity was observed. The animals were assigned to four groups viz G1-Control, G2-500mg/kg, b.wt, G3-1000 mg/kg, b.wt G4 - 2000 mg/kg,b.wt. Dosing of both sexes started 2 weeks prior mating. Males were

dosed from pre-mating until the minimum dosing period of 28 days is completed and sacrificed. Females were dosed during pre-mating, mating, gestation, and at least thirteen days after delivery (PPD 13) or and up to a day before necropsy.

Specific Studies - Sex Hormones, Fertility and Mating Behaviour:

Effect of Roots on Adult Male Rats:

Ilayperuma 2002: *The DTU points out that a study by Ilayperuma et al 2002, examined the effect of methanol extract from the root of Withania somnifera on a number of parameters associated with the mating behaviour in rats at a dose of 0 mg/kg/body weight or 3000 mg/kg/body weight for seven days. The authors note that the dosed rats showed significant reduction in mating readiness and a few parameters were still reduced even 30 days after dosing was ceased. DTU notes that the article does not show data for the mentioned parameters.*

Comment: There are three critical points that need to be addressed in the study mentioned above, one the dose is very high even for a human dose. The dose used in this study is 3000mg/kg/body which is 300 times higher than the human dose. And also, despite such a high dose, there were no deaths and no changes in the genitals which is a positive sign. The extract used in the study is the methanolic extract and no specification of the extract is mentioned. Additionally, the author conclude that the reduced willingness to mate was not a result of decreased testosterone level or toxicity but probably due to the sedative effect of the extract used.

Although the animals demonstrated a reduced willingness to mate, it should be noted that there were no changes in the genitals. The unwillingness to mate can also be due to other external factors/reasons. One such reason can be that if a male rat has reached satiety, it does not copulate for 1 to 3 days (Hull EM 2007).

The result of this study is contradicted by several well-conducted human studies of KSM-66 which is mentioned above. Other authors have also shown the opposite result in animal studies where ashwagandha was found to improve spermatogenic activity which is proposed to be due to supporting hypothalamic-hypophysial-gonadal hormonal axis and testosterone balance in testes (Kumar A et al 2015, Nirupama M and Yajurvedi H, 2015).

Kiasalari et al. 2009: *The DTU points out that Kiasalari et al. (2009) investigated the effect of the root on various sex hormones in the blood of male rats. In this study, the effect of Withania somnifera on sexual function in diabetic male Wistar rats was assessed by measuring the serum levels of testosterone, progesterone, estrogen, FSH, and LH. Oral Withania somnifera root was given in pelleted food at a ratio of 6.25% for 4 weeks.*

The results indicate that Withania somnifera root was effective in lowering FSH serum level in somnifera-treated animals compared to controls in both diabetic and nondiabetic groups, whereas progesterone, testosterone and LH levels were significantly higher in non-diabetic treated animals. Oral somnifera root was also able to reverse the reductive effect of diabetes on the progesterone. The estrogen level did not show any significant difference in any of the groups.

Comment: The results in the study are **positive**, there is an i**ncrease in fertility parameters**, and the authors conclude that *Withania somnifera* is a good candidate in the treatment of reproductive hormones deficiency.

Ganu et al. 2010: *The DTU points out that in this study, the Ashwagandha root extract was dissolved in distilled water and various doses i.e. 100, 200 and 400 mg/ kg were used. Based on the results, it was concluded that W. somnifera possessed marked aphrodisiac activity complying many facets such as enhancement in libido, increase in the sexual performance, penile erection and anabolism, increased spermatogenesis as well as sperm validity. The DTU notes that the study did not adequately describe the extract production and experimental design and results.*

Comment: The full text article demonstrated the methods and results in a clear manner. Also, the results clearly indicate that Ashwagandha possessed marked aphrodisiac activity and increases sexual performance, libido and spermatogenesis.

Sahin et al 2016: *The DTU points out a study which examined the effects of Ashwagandha on organ weights, sex hormones, sperm quality and mating behaviours. The rats were administered 300mg/kg/ body weight Ashwagandha for 8 weeks. The results indicated a significant increase in sperm motility, mounting frequency, and an improvement in sexual function when compared to the untreated (negative) control group.*

Comment: The DTU does not comment on the results of the study but rather focuses on other things. The results suggest No significant change of the extracts was observed on final body weight, absolute and relative reproductive organ weights of the animals among the groups ($P > 0.05$), and serum testosterone was 1.58-fold higher in the Ashwagandha group as compared to the control group. Also, no significant change was observed neither in testes histopathology nor the sperm morphologies between the groups. The results of the study also suggest that Ashwagandha be effective as a sexual enhancer, indicating an improvement in sexual function while contradicting the claims by DTU.

It is highly important that we should understand that out of the 4 studies that DTU presents above, 3 demonstrate that Ashwagandha root is safe and enhance sexual functions. In the fourth study the author explains the reduced sexual drive by a possible sedative effect of the extract. These 4 references therefore contradict the conclusions of DTU.

Leaves: Sexual maturation in Rats

Abdel-Magied et al. 2001: *The DTU points out a study in which the aqueous extract of leaves was administered to 10 rats (20-day old) with distilled water at a dose of 470mg/kg body weight for 6 days. Spermatogenesis (sperm formation) was seen in the testes of the Withania. somnifera-dosed groups, but not in the control group. In addition, statistically, significantly lower levels of testosterone and FSH were measured in the extract group compared to the control group, while there was no difference in the levels of LH.*

Comment: Based on this small study on an unspecified leaf extract from Saudi Arabia it is impossible to draw any conclusions of the effect on well-defined root extract from India.

Al Qarawi 2000: *The DTU points out a study in which the aqueous extract of leaves was administered to immature female rats. Female Wistar rats (10 animals/group) that were either 17 or 25 days old at the beginning of the experiment were dosed orally (by gavage) with distilled water (control group) or extract (470 mg/kg body weight/day) for six days. After dosing, youngest rats weighed statistically significantly more than the control group. In rats dosed from day 25, the relative weight of the ovaries was statistically significantly higher in the dosed group compared to the control group (118 versus 78 mg / 100 g body weight) and the blood content of FSH was also statistically significantly higher in the dosed group (17, 6 IU / l) compared to the control group (13.9 IU / l). At the same time, the LH levels were not different between the groups. The higher weight of the ovaries in the oldest dosed animals was due to the development of follicles in this organ, which is a sign of early sexual maturation.*

Comment: Based on this small study on a unspecified leaf extract from Saudi Arabia it is impossible to draw any conclusions of the effect on well-defined root extract from India.

The stem: Sperm quality in male rats

Singh et al. 2013: *The purpose of the experiment was to investigate whether the stems had anti fertile properties. The authors prepared an ethanol extract (50% ethanol) from the dried stem. Male rats (6 animals per group) were orally administered (by gavage) distilled water (control group) or extract (dissolved in distilled water) in doses of 25 mg/kg bodyweight or 50 mg/kg bodyweight for 60 days daily. Five days before the end of the experiment, the male rats were put together with fertile female rats (2 females per male). The dosage did not affect the bodyweight of the male rats. The sperm motility was only statistically significantly reduced in the highest dose group compared to the control group. The histopathological examination showed that the formation of sperm had ceased in the dosed groups. Testosterone and FSH levels were statistically significantly reduced in the highest dose group. Fewer offspring was seen in the dosed group.*

DTU Food Institute notes that the number of animals (6/group) is small for such a type of study.

Comment: The results of the study are quite opposite to the results of the study conducted with the root extract. It should be noted that the stem was never used traditionally and it is only the

root that has been used for aphrodisiac purposes. Again, it is worth to underline how important it is to understand the different properties of different plant parts.

The fruit: Sperm quality in rats

Mali et al. 2008: *The DTU points out a study in which the ethanolic extract of the fruit of Withania somnifera is administered orally for 60 days at 50mg/kg body weight in male rats. In the dosed animals, the sperm quality was lower both in terms of sperm motility and number, weights of the testicles and seminal vesicles as well as other accessory genitals were lower and histopathological changes were seen in the areas of the testicles that are important for normal sperm formation.*

Comment: The number of rats is not specified in this preliminary abstract. This is a poorly described study and it should not have been included for the assessment and analysis. Also the results of the study are quite opposite to the results of the study conducted with the root extract. It should be understood that the fruit was never used traditionally and it is only the root that has been used for aphrodisiac purposes.

Evidence from clinical trials

Clinical trials in men - sex hormones

The root: effect on the sex hormone testosterone in men who also do strength training

Wankhede et al 2015: *This study is an 8-week randomized, double-blind, placebo-controlled study that included 50 healthy men undergoing resistance training. In this study, KSM-66 Ashwagandha root extract was administered at a dose of 300 mg twice daily. The study denotes a significant increase in testosterone levels in the KSM-66 group when compared to the placebo group. The DTU notes that the active group has a lower testosterone value at start-up, which means that if only absolute numbers are taken into account, there is no significant difference between the groups. Also, the DTU noted that the study reported no side effects.*

Comment: This study indicates that over the eight weeks, there was a significant increase in testosterone level in the ashwagandha treatment group relative to the placebo group and also all subjects rated tolerability as either “good” or “excellent” on the PGATT (Physician's Global Assessment of Tolerability to Therapy). This shows an increase in fertility parameters. Over the eight weeks, there was a significant increase in testosterone levels in the ashwagandha treatment group relative to the placebo group. The increase in testosterone level was significantly greater with ashwagandha supplementation than with the placebo. While the mean post-intervention level was notably higher in the ashwagandha group than in the placebo group (726 versus 693), the numbers are not detectable as statistically significantly different, very likely because the across-subject variance is high.

The root: effects on the level of sex hormones in infertile men

Ahmad et al. 2010, Mahdi et al. 2011, Gupta et al. 2013: *Three studies conducted by the same research group have been included in the DTU risk assessment report. A total of 315 infertile men were administered 5g of dried powdered root with milk daily for three months. After three months of Ashwagandha administration, the levels of testosterone, LH and FSH in the infertile men had normalized.*

Ambiye et al. 2013: *A fourth study was also pointed out in the report. This was also a randomized, double-blind, placebo-controlled study which included 46 men with lower sperm count and they were administered either 675mg Ashwagandha or placebo for 90 days. In this study, treatment with the Ashwagandha root extract resulted in an increase in sperm concentration, semen volume, sperm motility and higher level of testosterone and a concomitant increase in serum levels of LH. The authors concluded by suggesting the potential role of KSM-66 Ashwagandha root extract in treating male infertility, which needs further exploration.*

The DTU mentions that there is lack of information on the number of people who did not complete the study and also there is no mention of the side effects.

Comment: In Ambiye et al 2013, the PGATT and PGAET suggested that KSM-66 Ashwagandha root extract was tolerable and no adverse effects were seen. The studies by Ahmad et al. 2010, Mahdi et al. 2011 did not have any dropouts hence it can be concluded that all the participants have completed the study.

The DTU also notes that in the studies by Ahmad, Mahdi and Gupta the subjects ingested the active substance with milk which contains nutrients, vitamins and minerals.

Comment: According to Ayurveda, milk is considered the best vehicle (*Anupana*) for taking an herb. Ashwagandha administration in classical Ayurveda practice advocates 'medicated milk treated with Ashwagandha', which is a synergetic combination for augmenting safety and efficacy. The combination of herbs and milk increases the absorption of the herbs in the body, thereby promoting the effectiveness and potency of that herb. This is also the reason why KSM-66 full spectrum extract is extracted with both milk and water.

All four studies showed a significant improvement in the seminal profile and the fertility parameters which is a positive result. Also, another recent study published by Chauhan et al, 2022, investigated the aphrodisiac property of an ashwagandha root extract (KSM66) in adult males. 50 participants with lower sexual desire were randomly allocated to take 300 mg of ashwagandha root extract or placebo capsules twice daily. This clinical study clearly demonstrated that people who took the ashwagandha root extract had an 88.5% greater probability of improving the total DISF-M sexual health function score. The ashwagandha root extract also increased their abilities to perform better in all the five DISF-M domains, such as sexual cognition, sexual arousal, sexual behaviour, orgasm, and sexual desire. Also, an increase

in total testosterone levels was noted in the KSM-66 Ashwagandha-supplemented group. Except for a few instances of general discomfort, ashwagandha was well tolerated, with no serious side effects reported. In addition, there is another KSM-66 study under peer review. In this study, there were 80 participants that either received 300 mg KSM-66 twice a day or a placebo. Both free and total testosterone values increased significantly in the KSM-66 group.

Root and leaves: effects on sex hormones in obese men

Lopresti et al. 2019: *The DTU pointed out a study that examined the hormonal and vitality effects of ashwagandha (Withania somnifera) in aging, overweight males for 8 weeks. The tablets used had an identical appearance and contained either toasted rice flour (placebo) or an extract, "Shoden beads", made from root and leaf from W. somnifera by an Indian company Arjuna Natural Ltd. Of the 57 men included in the study, 43 completed. Intake of the extract gave rise to an increased content of DHEA-S (18%, P = 0.005) and an increased content of testosterone (14.7%, P = 0.01) in saliva compared to placebo with no statistically significant differences in the levels of cortisol and estradiol.*

DTU Food Institute assesses that hormone levels have been measured using a more unreliable method (in saliva not in blood plasma) and may further be affected by the fact that the circadian rhythm may be disturbed in participants who were shift workers.

Comment: KSM-66 Ashwagandha is a root-only extract and the studies conducted using KSM-66 Ashwagandha have assessed testosterone and cortisol levels using blood serum samples (Chandrasekhar et al 2012, Choudhary D et al 2017, Wankhede et al 2015, Ambiye et al 2013, and Chauhan et al 2022).

Specific Studies: The Thyroid Gland

Root: Thyroid gland, animal experiments

Panda & Kar 1998, 1999: *An aqueous extract of the powdered root of Indian Withania somnifera (concentration ratio root: extract, 5: 1) was given orally by gavage at a dose of 0 (distilled water) or 1.4 g/kg body weight/day (1400mg/kg) for 20 days for adult female mice (Swiss albino, 7 mice/group) and for adult male mice (Swiss albino, 10 mice/group). In female mice, when compared to the placebo, only a nonsignificant increase in T4 values was observed but not T3. In the male animals, the increase in T3 and T4 were identical and significant in both the ashwagandha group and in the placebo group.*

Panda & Kar 1997: *The same researchers conducted a similar study in chickens and it was found that daily administration of 20 mg of Ashwagandha root extract /animal extract for 30 days resulted in an increase in T4 but not T3.*

Abdel 2019: *An Egyptian study conducted using methanol extract of Ashwagandha root 500 mg/kg body weight. Normal animals that received ashwagandha methanolic extract (AME)*

recorded insignificant changes in levels of these hormones compared with that of the normal control. Both ashwagandha extract and the anti-hypothyroidism drug Eltroxin™ (thyroxine) significantly ameliorated the changes occurring in the levels of the above hormones but with different degrees. The authors conclude that animals treated with Ashwagandha extract or the reference drug (thyroxin) resulted in a significant improvement in thyroid gland tissue as compared to the group of hypothyroidism, as ashwagandha extract demonstrated the more improvement in pathological changes. The results from the current study indicate that ashwagandha methanolic extract treatment improves thyroid function by ameliorating thyroid hormones and by preventing oxidative stress.

Comment: Ashwagandha does not stimulate the thyroid gland directly, it basically tries to maintain the thyroid homeostasis by working on the hypothalamus and improving its activities. Also, in people undergoing stress, consistent release of cortisol occurs in the body and this can cause many damaging effects which includes its adverse effects on the thyroid gland. As an adaptogen, Ashwagandha can significantly reduce cortisol levels and reduce stress and in turn, bring the body to homeostasis.
A study conducted on Zebrafish embryos demonstrated that treatment with KSM-66 Ashwagandha showed similar levels to the vehicle control, and no statistically significant differences, indicating the safety of the extract to the thyroid gland in the body even if there is no treatment-related goitrogenic effect (Study under peer review).

Also, In the 90-Day Repeated Dose Oral Toxicity Study of KSM-66 Ashwagandha root extract in Wistar Rats (50 males and 50 females). The dose used was 500, 1000, and 2000 mg/kg. The result, there were no test item-related changes observed in T3, T4, and TSH of treated animals of both the sexes when compared to respective control animals.

The root: The thyroid gland - clinical trials in humans

Sharma et al 2018: *The DTU points out an 8 week study conducted on 50 subjects evaluated the effect of the 300mg KSM-66 Ashwagandha twice daily on thyroid indices. At the end of the study ashwagandha treatment effectively normalized the serum thyroid indices (TSH, T3 and T4) during the 8th week treatment period in a significant manner. Treatment with Ashwagandha was found safe and tolerable, with few mild and temporary adverse events.*

Comment: The DTU mentions that the treated group came closer to normal values, which must be considered positive. Also, the author calls for longer studies – which is ongoing. The effects of KSM-66 Ashwagandha on thyroid indices was evaluated in another published study which suggests that KSM-66 Ashwagandha root extract was safe and well tolerated and it also helped to maintain the thyroid indices under normal levels. It can be concluded that KSM-66 Ashwagandha does not affect normal thyroid functions (Verma et al, 2021). A conclusion that is also supported in the 90-Day Repeated Dose Oral Toxicity Study of KSM-66 described above.

Unspecified plant part - side effects humans

van der Hooft et al. 2005: *The DTU risk assessment report includes an article in which a healthy Dutch woman, aged 32 years developed thyrotoxicosis through the consumption of a nonspecific extract of Ashwagandha. The article describes that the symptoms disappeared after discontinuation of the extract.*

Comment: The Ashwagandha extract used in these capsules sold by Holisan Health products is an unspecified extract. The woman started Ashwagandha after a normal pregnancy and one reason for thyrotoxicosis can be the postpartum period. There are studies that portray the occurrence of thyroid imbalances in the postpartum period (Di Bari F et al 2017).

Unspecified plant part - thyroid gland - clinical trials in humans

Gannon et al. (2014): *The DTU risk assessment report includes Gannon et al. (2014) investigated a standardized root and leaf extract of Withania somnifera (Sensoril®) from manufacturer Natreon, USA. The purpose of the study was to investigate possible effects on mental skills (cognitive functions) in people with bipolar disorder. The current study demonstrates the elevated T4 levels, hence the authors suggest that Ashwagandha root leaf extract can be considered for treatment of sub-clinical hypothyroidism in mood disorders.*

Comment: The DTU does not comment on that the three patients in the ashwagandha group with elevated T4 values and declining T3 values actually come closer to normal values after administration of Sensoril which indicates a normalization of T4 and T3. We also find it remarkable that DTU does not comment on the fact that 6 (out of 19) in the placebo group are having significant falling T4 values, of which 3 are falling below normal values. This should have led to the study being questioned.

What is also worth noting, which DTU did not comment on, is that the main study was not designed to primarily examine thyroid function in bipolar patients treated with ASW and that the sample size is small and unequally divided between patients who received placebo. It should also be considered that all patients were bipolar which creates stress and ashwagandha increases stress resistance which is the most credible explanation to why the ashwagandha group has a tendency of normalizing the T4 and T3 values.

Specific Studies - The Central Nervous System

The root: Animal experiments:

Visweswari et al 2014: *The DTU risk assessment report points out a study which showed dose-dependent inhibitory effects on acetylcholinesterase of both the aqueous extract and methanolic extract of Ashwagandha root when studied in different brain regions. The DTU states that reduction in Acetylcholine below a certain threshold can be detrimental and harmful.*

Comment: Acetylcholine is a neurotransmitter and significant reduction can cause problems in the areas of memory, learning and concentration etc. A study conducted with KSM-66 Ashwagandha root extract demonstrated an increase in memory and cognition and improvement in attention processing speed and executive function. Also, in our literature search, we have found 32 studies published till date with a possible neuroprotective activity of Ashwagandha (Wongtrakul, J et al 2021, Khalil, H et al, 2021, Singh, S. K. et al 2021, Siddiqui, M. A et al 2021) **(*Appendix III)***

Gupta & Rana (2007): *The DTU risk assessment report also includes behavioural studies on animal models. Gupta & Rana (2007) compared the anxiolytic effect of a commercial extract extracted from the root of Withania somnifera (from Dabur, India) with the drug diazepam. The sub effective dose of WS (50 mg/kg, oral) potentiated the anxiolytic action of diazepam (0.5, 1 or 2 mg/kg, ip). WS (100, 200 or 500 mg/kg, oral) also reduced the immobility time in the Forced Swimming Test, showing antidepressant effect in both groups. The investigations support the use of WS as a mood stabilizer.*

Kumar & Kalonia, 2007: *Pretreatment with W. somnifera root extract (100. 200 mg/kg) and diazepam (0.5 mg/kg) significantly protected reduction in body weight, and improved the reduced locomotor activity and anxiety levels in animals. The present study suggests that Withania root extract can be used in the management of sleep loss and related behavioral and biochemical alterations.*

Comment: As an adaptogen, Ashwagandha is known to increase the resistance to stress and normalize cortisol levels and reduce anxiety. Studies conducted in humans demonstrate that KSM-66 Ashwagandha root extract can reduce stress and anxiety and also enhance happiness, and sleep and thus stabilize the mood (Chandrasekhar et al 2012, Choudhary D et al 2017, Salve et al 2019, Langade et al 2019, and Langade et al 2020).

Specific Studies - The Immune System

The root:

Malik et al 2007: *The DTU risk assessment report includes studies that demonstrate the immunostimulant effects of Ashwagandha. In a study by Malik et al 2007, an ethanol extract extracted from the root was administered orally daily to groups of 6 female mice at doses of 0, 10, 30, and 100 mg/kg body weight for 15 days. In all dosed groups, there was an increased incidence of cells in lymphoid organs such as the spleen, thymus, and bone marrow. In animals dosed with the two lowest doses of the extract, there was increased cell proliferation of T and B lymphocytes when stimulated with mitogen. In all dose groups, an increased type IV immunological response (delayed allergic response) associated with T lymphocytes was seen.*

Siddiqui et al. 2012, Kushwaha et al. 2012; Rasool & Varalakshmi 2006: *Other studies in mice suggested that extracts of the root may affect the immune system, e.g. shown with ethanol*

extracts of several samples just as there has also been an effect on the immune system in rats dosed with an aqueous suspension of the powdered root.

Comment: As an adaptogen, Ashwagandha acts as an immunomodulator and not as an immunostimulant. Several animal studies have been conducted that suggest Ashwagandha's ability to impact multiple layers of immune function which span both innate and adaptive immunity.

Two references (Priyanka G et al, 2020; Puri et al 2011) clearly exemplifies how ashwagandha is modulating the immune system through its anti-stress properties. In these two studies ARE is balancing an overactive immune system. In the first study conducted on stressed equines with an overactive immune system, it was seen that Ashwagandha root extract decreased white blood cell count and lymphocyte percentage while IL-6 levels increased. In the second study conducted on rodents with an overactive immune defense system due to cold restraint stress and forced swimming, a decrease in white blood cell counts, neutrophils, lymphocytes, and eosinophils were observed. These two studies clearly indicate a healthy, immune-modulating effect of ARE, not an immunostimulant effect that the DTU claims.

Adverse Reaction Reports

Unspecified plant part: Liver

Björnsson et al. 2020: *A recently published study described five cases of liver damage in three men and two women (21-62 years) from Iceland and the USA after taking supplements with W. somnifera (plant part and preparation were not specified) occurred in the period 2016-2018. In the three cases from Iceland, the supplements came from the same manufacturer and they had ingested from 450-1350 mg/day. Symptoms of liver damage (nausea, fatigue, itching, and jaundice) occurred 2-12 weeks after ingestion. The patient's liver numbers fell to normal levels 1-6 months after cessation of intake.*

Comment: According to the traditional system of Ayurvedic medicine, Ashwagandha has a free radical scavenging activity and is used for the prevention of liver damage. Many preclinical studies conducted on different animal models demonstrate the hepatoprotective role of Ashwagandha (Khalil, H et al 2021; Ebtihal Y et al, 2020; Dhenge SA et al, 2018; Jamuna G et al, 2018; Sharma A et al, 2017; Shahraki, MR et al 2016). In gentamicin-intoxicated experimental Wister albino rats, Sultana et al. suggested restoration of serum aspartate aminotransferase and alanine aminotransferase level, due to the probable free radical scavenging activity of Ashwagandha root extract (Sultana et al.,2012).

The safety study conducted by a group of investigators suggested that no significant changes were observed in Alanine Transaminase (ALT/SGPT), Aspartate Transaminase (AST/SGOT), and Alkaline Phosphatase upon supplementation with KSM-66 Ashwagandha (Verma et al, 2021).

An exploratory clinical study on healthy human beings was conducted to evaluate the safety and tolerability of Ashwagandha related to muscle activity, exercise tolerance, cholesterol level (LDL), and body fat percentage. The results suggested that no significant change was observed in Serum Bilirubin, Proteins, Albumin, Alanine Transaminase, Aspartate Transaminase, and Alkaline Phosphatase at all the visits in each of the volunteers and it remained within normal range (Raut et al, 2012).

The total number of participants in all these 19 published studies was 1114 out of which 577 received KSM-66 Ashwagandha and 537 received placebo. Only about 3.98% (23/577) participants receiving KSM-66 Ashwagandha reported adverse events (mild severity), whereas 5.02% (27/537) participants receiving placebo reported mild adverse events. According to the investigators, no serious adverse events were reported in the studies and it did not raise any suspicion of toxicity with daily consumption of ashwagandha. Thus, it may be mentioned that KSM66 Ashwagandha was well-tolerated among the participants of the studies published.

In addition to the human clinical studies, animal studies have also been compiled where the possible hepatoprotective effect of ashwagandha has been studied. A total of 31 studies have been published, all of which report a hepatoprotective effect (***Annexure-III***).

International Institutions' Assessments of the Root *W. Somnifera* As A Medicinal Product

Germany authority 2012: *The German Risk Assessment Institute carried out an assessment of the root of Withania somnifera and its extracts in 2012. The authors estimate that the root has been used in Ayurvedic medicine and there is little information upon its use in Europe. Also, only a few human studies and no toxicological studies were conducted; they expressed safety concerns. It was also mentioned that EFSA (European Food Safety Authority) has suggested that some plants may have a "presumption of safety" if comprehensive intake data is available. There were no historical data to suggest that the plant was safe to ingest*

Comment: The assessment by the German authorities was conducted in 2012 and at that time Ashwagandha was mostly used in India. It should be understood that a lot of research has been conducted and published in recent years. Studies that confirms both safety and efficacy of ARE. As a result of this, the assessments from Germany and EFSA from 2012 are not based on the current knowledge of ARE.

European Medicines Agency: *The European Medicines Agency (EMA) concluded that the conditions under which the Agency could draw up a monograph on the root of W. somnifera (L.) Dunal were not met and that the root could not be assessed and accepted as a traditional herbal medicinal product in the EU.*

Comment: The European Medicines Agency (EMA) has an ongoing case making a monograph for Withania somnifera. The EMA launched an investigation in 2011 that was completed in

2013 with the conclusion that, until then, 15 years of widespread use could not be shown in the EU. Due to the merging numbers of clinical studies conducted during recent years, the new investigating will most certainly come to a different conclusion.

World Health Organization (WHO)

In a monograph from 2009, the WHO assessed the root as a medicine. Here it was mentioned that the root could cause side effects such as nausea, vomiting, and diarrhoea. The WHO also mentioned that the root is contraindicated during pregnancy and lactation due to a lack of data on safety as well as information that the root has been used to induce abortion in traditional medicine

Comment: This monograph was from 2009, and a lot of scientific evidence has been published to support the safety and efficacy of Ashwagandha root. Today, KSM66 is available in over 1600 different brands in the US and Europe. KSM-66 has 30 completed, randomized, and double-blind studies (of which 27 are conducted in humans) and various toxicology studies.

Conclusion:

Ashwagandha (Withania somnifera) root extract is a clinically effective and safe herbal supplement. As we have shown in this assessment, there are many studies conducted in both *invitro* and *invivo* models that support the safety of ARE. This report also clearly shows that the phytochemistry of the aerial parts and the root of Ashwagandha is different, hence the part of the plant used and its extraction methods are important considerations when assessing safety.

Since the conclusion in the DTU report is that there can be a safety concern with root, the safety evaluation should therefore focus on root extracts only. An important aspect since studies on other parts of the plants will most certainly lead to different conclusions on safety, which may be incorrect and seriously misleading.

The majority of the comments in the DTU report do not consider this difference. Some of the studies mentioned in the DTU report also have limited validity and are with serious lacunae. The part of the plant (root) and validated extraction methods are the most important factors for the safety of Ashwagandha. Factors that the DTU report has not taken into consideration which makes the report lose credibility.

Contradictory to the conclusion of DTU, our assessment has demonstrated that a well-defined root extract like KSM-66 is safe and promotes a healthy and normal function of both thyroid and sex hormones through multiple human and animal studies.

Since the DTU report was published in May 2020 a large number of well-done studies have been published regarding both efficacy and safety. This circumstance together with the fact that DTU has ignored the evidence that there are significant differences between the root and other plant parts both in terms of both effect and safety indicates that there are good reasons to redo the report.

We understand that the spirit of the DTU report is to ensure the safety of people, and we agree with DTU that there can be safety concerns when aerial parts of Ashwagandha are used internally and that it is important that root extracts should be well-defined and free from withaferin A, withanone, and alkaloids.

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List of Annexures

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ANNEXURE - I

OPINION: SAFETY EVALUATION ON THE ROOT PART VS. THE ENTIRE PLANT/ AERIAL PARTS

To understand why it is inappropriate to conduct a safety assessment on the entire plant when only a specific plant part, such as the root, is used in a food supplement, one must first be aware that the reason why the root and above-ground parts (aerial) are not comparable to each other is because they have different functions and structures, making it difficult to compare them meaningfully. The above-ground part of a plant, such as stems, leaves, and flowers, is responsible for photosynthesis, reproduction, and giving the plant its form and structure. The root part, on the other hand, is responsible for absorbing water and nutrients from the soil and stabilizing the plant in the ground.

Because of their unique functions and structures, the chemical composition of a plant's above-ground parts differs from that of its roots. Typically, the above-ground part contains high levels of carbohydrates and chlorophyll, while the root contains high levels of minerals and water. Comparing the above-ground and root parts of a plant meaningfully can be challenging as they have different functions and structures. Comparing them is like comparing apples to oranges - they possess entirely different characteristics and contents that cannot be judged similarly.

Therefore, conducting a safety assessment on an entire plant when only the specific part, e.g. the root part, is used in a food supplement is inappropriate for several reasons. It is crucial to analyze the specific part of the plant that is used in the supplement to evaluate its safety, as different parts of the plant may have varying chemical compositions, as shown for *Withania somnifera* (L.) Dunal in Tables 1 and 2, that may have different effects on the body. This is particularly important to identify potential risks or side effects related to the specific part of the plant used in the supplement. For instance, root nodules may contain higher concentrations of specific nutrients or compounds that could affect health differently than the stem or leaves, and the opposite may also be true. Assessing the safety of the entire plant or other parts of the plant may produce misleading results since it's possible that other parts of the plant contain chemical compounds that are absent in the specific part, which can affect the outcome of the safety evaluation. Therefore, it is critical to ensure that the safety evaluation is focused on the specific part of the plant used in the food supplement to guarantee its efficacy and safety for consumers and prevent potential health risks and misleading outcomes.

If a plant is contaminated with pollutants such as heavy metals or pesticides, different parts of the plant may contain varying amounts of these impurities. A safety assessment that is carried out on the whole plant, including the parts that are not used in the supplement, may not accurately represent the safety of the specific part of the plant used in the supplement. To ensure that the evaluation is focused on the specific part of the plant used in the food supplement, it is necessary to investigate and control any impurities that may affect the safety of consumers. This is particularly important because some parts of the plant may have a higher risk of containing contaminants than others.

To avoid exposing consumers to potentially hazardous substances, it is crucial that safety evaluations concentrate on the specific part of the plant used in the food supplement. Some parts of a plant can be toxic or have negative health effects when consumed, which can be overlooked if a safety evaluation is done on the whole plant. It is therefore essential to identify any possible risks associated with the specific part of the plant used in the food supplement and take necessary measures to minimize any harm.

For a reliable detection of any side effects or risks, safety evaluations must concentrate on the specific part of the plant used in the food supplement. The chemical composition of a plant can be affected differently by various

environmental factors and cultivation methods, and examining the entire plant can lead to false conclusions and misleading results about the safety of the supplement. Hence, it is necessary to focus on the specific part of the plant used in the supplement to ensure its safety, and also investigate the plant's source and cultivation methods.

Another important aspect to consider is the bioavailability of nutrients or compounds in the specific part of the plant used in the supplement. Therefore, a safety evaluation should concentrate on the specific part of the plant to obtain a more accurate understanding of its effectiveness and safety since different parts of a plant may have varying degrees of bioavailability. Examining the entire plant may provide misleading results because bioavailability can differ among different parts of the plant. By focusing on the specific part of the plant used in the food supplement, it is possible to avoid misleading results and guarantee a higher degree of safety for consumers.

Choosing the right extraction agent is crucial to ensure the safety of food supplements containing plant extracts. Different extraction solvents can have a significant impact on the effectiveness and safety of the supplement. Organic solvents like ethanol or hexane are commonly used for extracting plant-based compounds. However, if not used correctly, these solvents can lead to negative health effects and contamination of the supplement. Harmful chemicals like pesticide residues, pollutants, and heavy metals can also be extracted if the process is not done correctly, posing health risks for consumers. To minimize the risk of contamination and side effects, non-organic solvents like water or carbon dioxide can be used as they are usually less toxic and less likely to extract harmful substances from the plants.

CONCLUSION

In conclusion, to ensure the safety and effectiveness of food supplements that contain plant extracts, it is crucial to conduct a comprehensive safety evaluation on the specific part of the plant used in the supplement, such as the root extract. The content of several substances has been shown to varies significantly between the root and aerial parts of *Withania somnifera* (L.) Dunal, as indicated in Table 1 and 2. This emphasizes the importance of conducting a safety evaluation on the specific plant part used in the food supplement. Evaluating the entire plant, as was done in the DTU assessment, can lead to misleading results since different parts of a plant have unique functions, structures, and chemical compositions that can affect the supplement's safety and efficacy, making it difficult to compare them and draw meaningful conclusions. Concluding the safety of Ashwagandha root (root from *Withania somnifera* (L.) Dunal based on studies of leaves or the entire plant can also be deceptive because other parts of the plant, such as the leaves, contain different bioactive compounds, including high amounts of withaferin A and withanone that are present in much lower concentrations in the root. Thus, the safety and efficacy of a supplement with Ashwagandha root extract is likely to differ from one containing extracts from other plant parts. Consequently, it is crucial to perform a safety assessment of the exact plant part utilized in the supplement to guarantee its safety and effectiveness for consumers.

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Table 1 – Total metabolite content in leaf and root

Studies have shown for different extracts of *Withania somnifera* L. that the quantity of metabolite in leaves is much higher than that in roots, particularly in the aqueous methanolic fraction (Table 1).

Extract partition	**Total metabolite content mg/gm of DW**	
	Leaf	Root
Hexane	34.29 ± 2.0	4.44 ± 0.8
CHCl3	35.71 ± 1.5	10.00 ± 1.0
n-BuOH	28.57 ± 1.6	11.11 ± 1.2
Methanolic water	228.57 ± 5.2	15.00 ± 1.6

Ref: Chatterjee S, Srivastava S, Khalid A, Singh N, Sangwan RS, Sidhu OP, Roy R, Khetrapal CL, Tuli R. Comprehensive metabolic fingerprinting of Withania somnifera leaf and root extracts. Phytochemistry. 2010 Jul;71(10):1085-94.

Table 2 – The amount of different constituents

The different parts of *Withania somnifera* L. tends to include:

Substance	**Root**	**Leaves**	**Root & leaves**	**Fruit**	**Ref.**
Triterpenoids					
Withanone	5.54 ± 0.4mg/g (DW)	18.42 ± 0.8mg/g (DW)			[1]
27-deoxywithanone	3.94 ± 0.4mg/g (DW)	1.63 ± 0.2mg/g (DW)			[1]
27-hydroxywithanone			0.50 ± 0.1mg/g (DW)		[1]
Steroids					
Withaferin A	0.92 ± 0.4mg/g (DW)	22.31 ± 1mg/g (DW)			[2,3]
17-hydroxy-27-deoxy-Withaferin A	0.66 ± 0.2mg/g (DW)	3.61 ± 0.5mg/g (DW)			[1]
Withanolide A	3.88 ± 0.7mg/g (DW)	2.11 ± 0.5mg/g (DW)			[1,2]
Withanolide B-D					[4]
27-hydroxy Withanolide B	0.55 ± 0.2mg/g (DW)	2.78 ± 0.5mg/g (DW)			[1]
Withanoside IV	0.44 ± 0.1mg/g (DW)	1.60 ± 0.2mg/g (DW)			[1]
Withanoside VI	3.74 ± 0.2mg/g (DW)	1.90 ± 0.2mg/g (DW)			[1]
12-deoxywithastromonolide	1.90+/-0.5mg/g (DW)	2.15+/-0.5mg/g (DW)			[1]
Physagulin	Not detected	3.46 ± 0.4mg/g (DW)			[1]
Flavonoids					
Kaempferol	Not detected	Not detected		0.06mg/g (DW)	[5]
Naringenin	Not detected	Not detected		0.50mg/g (DW)	[5]
(+)-Catechin	12.82mg/g (DW)	28.38mg/g (DW)		19.48mg/g (DW)	[5]
Phenols					
Gallic acid	Not detected	0.18mg/g (DW)		Not detected	[5]
Syringic acid		0.30mg/g			[5]
p-coumaric acid		0.80mg/g			[5]

[1] Chatterjee S, Srivastava S, Khalid A, Singh N, Sangwan RS, Sidhu OP, Roy R, Khetrapal CL, Tuli R. Comprehensive metabolic fingerprinting of Withania somnifera leaf and root extracts. Phytochemistry. 2010 Jul;71(10):1085-94.

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vanillic acid		0.15mg/g (DW)			5
benzoic acid		0.80mg/g			5
Alkaloids					
Trigonelline		1.33 ± 0.3mg/g (DW)			1
Choline			3.53 ± 0.5mg/g (DW)		1
Uracil		3.90 ± 0.2mg/g (DW)			3
Fatty acids					
Palmitic acid	1.18 ± 0.2mg/g (DW)	3.55 ± 0.5mg/g (DW)			1
Oleic acid	0.39+/-0.1mg/g (DW)	0.71 ± 0.1mg/g (DW)			1
Linoleic acid	1.31+/-0.2mg/g (DW)	1.52 ± 0.2mg/g (DW)			1
Linolenic acid	0.15 ± 0.1mg/g (DW)	4.38 ± 0.5mg/g (DW)			1
Amino acids					
Alanine		Detected			1
Aspartate		Detected			1
Asparagine			Detected		1
Glutamine			Detected		1
Isoleucine		19.83 ± 0.8mg/g (DW)			1
Lysine		Detected			1
Leucine		Detected			1
Phenyl alanine		Detected			1
Tyrosine		Detected			3
Threonine		Detected			3
Ornithine		21.5 ± 0.8mg/g (DW)			1
Valine		5.60 ± 0.5mg/g (DW)			3
Monosaccharide					
Galactose			Detected		1
α -Glucose		6.11 ± 0.5mg/g (DW)			1
B-Glucose		10.22 ± 0.9mg/g (DW)			1
Alcohol					
Glycerol	Detected				1
Organic acid					
Lactic acid		Detected			1
Tartaric acid		4.10 ± 0.4mg/g (DW)			3
Other					
Citric acid		Detected			1
Fructose-5	Detected				1
Fumaric acid		0.6 ± 0.2mg/g (DW)			1
GABA	Detected	16.74 ± 0.8mg/g (DW)			1
Glutamate			Detected		1
Succinate		12.75 ± 0.5mg/g (DW)			3

DW= Dry weight

Annexure - II - Human Clinical Studies

	Title	Journal Name	Total no of subjects in the study	No. of subjects in Ashwagandha group	No. of subjects in Placebo / control / comparator group	No of Adverse events reported in Ashwagandha group	No of Adverse events reported in Placebo group	Adverse events reported in Ashwagandha group	Adverse events reported in Placebo group	Extract used	Dose	Duration	Country	Citation	Link
1	Effects of Ashwagandha (Withania somnifera) standardized root extract on physical endurance and VO2max in healthy adults preforming resistance training: An eight-week, prospective, randomized, double-blind, placebo-controlled study	F1000	73	37	36	0	0	N/A	N/A	Ashwagandha Root Extract (KSM-66)	300 mg capsule twice daily (600mg/d)	8 Weeks	India	Verma N, Gupta SK, Patil S et al. Effects of Ashwagandha (Withania somnifera) standardized root extract on physical endurance and VO2max in healthy adults preforming resistance training: An eight-week, prospective, randomized, double-blind, placebo-controlled study. F1000Research 2023, 12:335	https://f1000research.com/articles/12-335/v1
2	A Study of Efficacy and Safety of Ashwagandha (Withania somnifera) Lotion on Facial Skin in Photoaged Healthy Adults	Cureus	53	27	26	4	5	Local swelling, Erythema	Local swelling, Erythema, Local irritation	Ashwagandha Root Extract (KSM-66)	1ml of skin lotion	8 Weeks	India	Narra K, Naik S K, Ghatge A S (March 15, 2023) A Study of Efficacy and Safety of Ashwagandha (Withania somnifera) Lotion on Facial Skin in Photoaged Healthy Adults. Cureus 15(3): e36168. DOI 10.7759/cureus.36168	https://www.cureus.com/articles/141363-a-study-of-efficacy-and-safety-of-ashwagandha-withania-somnifera-lotion-on-facial-skin-in-photoaged-healthy-adults#!/
3	Efficacy and Safety of Ashwagandha (Withania somnifera) Root Extract for Improvement of Sexual Health in Healthy Women: A Prospective, Randomized, Placebo-Controlled Study	Cureus	80	40	40	3	3	Nausea, Drowsiness		Ashwagandha Root Extract (KSM-66)	300 mg capsule twice daily (600mg/d)	8 Weeks	India	Ajgaonkar A, Jain M, Debnath K (October 28, 2022) Efficacy and Safety of Ashwagandha (Withania somnifera) Root Extract for Improvement of Sexual Health in Healthy Women: A Prospective, Randomized, Placebo-Controlled Study. Cureus 14(10): e30787. DOI 10.7759/cureus.30787	https://www.cureus.com/articles/118730-efficacy-and-safety-of-ashwagandha-withania-somnifera-root-extract-for-improvement-of-sexual-health-in-healthy-women-a-prospective-randomized-placebo-controlled-study
4	Effect of standardized root extract of ashwagandha (Withania somnifera) on well-being and sexual performance in adult males: A randomized controlled trial	Health Science Reports	50	25	25	4	3	Sleepiness, Mild abdominal pain, Low-grade joint pain	Abdominal Pain, Mild diarrhea	Ashwagandha Root Extract (KSM-66)	300 mg capsule twice daily (600mg/d)	8 Weeks	India	Chauhan, S., Srivastava, M. K., & Pathak, A. K. (2022). Effect of standardized root extract of ashwagandha (Withania somnifera) on well-being and sexual performance in adult males: A randomized controlled trial. Health science reports, 5(4), e741. https://doi.org/10.1002/hsr2.741	https://pubmed.ncbi.nlm.nih.gov/35873404/
5	Effect of an ashwagandha (Withania Somnifera) root extract on climacteric symptoms in women during perimenopause: A randomized, double-blind, placebo-controlled study	Journal of Obstetrics and Gynecology Research	91	46	45	3	4	Abdominal Discomfort, Abdominal Pain, Nausea	Abdominal Discomfort, Abdominal Pain, Insomnia, Nausea	Ashwagandha Root Extract (KSM-66)	300 mg capsule twice daily (600mg/d)	8 Weeks	India	Gopal, S., Ajgaonkar, A., Kanchi, P., Kaundinya, A., Thakare, V., Chauhan, S. and Langade, D. (2021), Effect of an ashwagandha (Withania Somnifera) root extract on climacteric symptoms in women during perimenopause: A randomized, double-blind, placebo-controlled study. J. Obstet. Gynaecol. Research https://doi.org/10.1111/jog.15030	https://pubmed.ncbi.nlm.nih.gov/34553463/
6	A double-blind, randomized, placebo-controlled trial on the effect of Ashwagandha (Withania somnifera Dunal.) root extract in improving cardiorespiratory endurance and recovery in healthy athletic adults	Journal of Ethnopharmacology	50	25	25	1	3	Mild ear pain	Diarrhea, Low grade fever	Ashwagandha Root Extract (KSM-66)	300 mg capsule twice daily (600mg/d)	8 Weeks	India	Tiwari, S., Gupta, S. K., & Pathak, A. K. (2021). A double-blind, randomized, placebo-controlled trial on the effect of Ashwagandha (*Withania somnifera Dunal.*) root extract in improving cardiorespiratory endurance and recovery in healthy athletic adults. Journal of ethnopharmacology, 113929. DOI: 10.1016/j.jep.2021.113929	https://pubmed.ncbi.nlm.nih.gov/33600918/
7	Safety of Ashwagandha Root Extract: A Randomized, Placebo-Controlled, study in Healthy Volunteers.	Complementary therapies in medicine	80	40	40	0	0	N/A	N/A	Ashwagandha Root Extract (KSM-66)	300 mg capsule twice daily (600mg/d)	8 Weeks	India	Verma, N., Gupta, S. K., Tiwari, S., & Mishra, A. K. (2021). Safety of Ashwagandha Root Extract: A Randomized, Placebo-Controlled, study in Healthy Volunteers. Complementary therapies in medicine. 57, 102642.	https://pubmed.ncbi.nlm.nih.gov/33338583/
8	Clinical evaluation of the pharmacological impact of ashwagandha root extract on sleep in healthy volunteers and insomnia patients: A double-blind, randomized, parallel-group, placebo-controlled study	Journal of Ethnopharmacology	73	38	35	0	0	N/A	N/A	Ashwagandha Root Extract (KSM-66)	300 mg capsule twice daily (600mg/d)	8 Weeks	India	Langade, D., Thakare, V., Kanchi, S., & Kelgane, S., (2020). Clinical evaluation of the pharmacological impact of ashwagandha root extract on sleep in healthy volunteers and insomnia patients: A double-blind, randomized, parallel-group, placebo-controlled study. Journal of Ethnopharmacology. DOI: 10.1016/j.jep.2020.113276	https://pubmed.ncbi.nlm.nih.gov/32818573/
9	Efficacy and Tolerability of Ashwagandha root extract in the Elderly for Improvement of General Wellbeing and Sleep: A Prospective Randomized, Double Blind, Placebo Controlled Study	Cureus	39	19	20	0	0	N/A	N/A	Ashwagandha Root Extract (KSM-66)	300 mg capsule twice daily (600mg/d)	12 Weeks	India	Kelgane S B, Salve J, Sampara P, et al. (February 23, 2020) Efficacy and Tolerability of Ashwagandha Root Extract in the Elderly for Improvement of General Well-being and Sleep: A Prospective, Randomized, Double-blind, Placebo-controlled Study. Cureus 12(2): e7083. DOI 10.7759/cureus.7083	https://pubmed.ncbi.nlm.nih.gov/32226684/
10	Adaptogenic and Anxiolytic Effects of Ashwagandha Root Extract in Healthy Adults: A Double-blind, Randomized, Placebo-controlled Clinical Study	Cureus	58	39	19	0	0	N/A	N/A	Ashwagandha Root Extract (KSM-66)	125mg capsule twice daily (250mg/d). 300 mg capsule twice daily (600mg/d)	8 Weeks	India	Salve J, Pate S, Debnath K, et al. (December 25, 2019) Adaptogenic and Anxiolytic Effects of Ashwagandha Root Extract in Healthy Adults: A Double-blind, Randomized, Placebo-controlled Clinical Study. Cureus 11(12): e6466. doi:10.7759/cureus.6466	https://www.ncbi.nlm.nih.gov/pubmed/32021735
11	Efficacy and Safety of Ashwagandha (Withania somnifera) Root Extract in Insomnia and Anxiety: A Double-blind, Randomized, Placebo-controlled Study	Cureus	58	39	19	0	0	N/A	N/A	Ashwagandha Root Extract (KSM-66)	300 mg capsule twice daily (600mg/d)	10 Weeks	India	Langade, D., Kanchi, S., Salve, J., Debnath, K., Ambegaokar, D., 2019. Efficacy and safety of ashwagandha (Withania somnifera) root extract in insomnia and anxiety: a double-blind, randomized, placebo-controlled study. Cureus 11, E5797.	https://www.ncbi.nlm.nih.gov/pubmed/31728244
12	Efficacy and Safety of Ashwagandha Root Extract in Subclinical Hypothyroid Patients: A Double-Blind, Randomized Placebo-Controlled Trial	Journal of Alternative and complementary medicine	50	25	25	1	3	Fever, Asthenia, cough and headache		Ashwagandha Root Extract (KSM-66)	300 mg capsule twice daily (600mg/d)	8 Weeks	India	Sharma, A.K., Basu, I., Singh, S., 2018. Efficacy and Safety of Ashwagandha Root Extract in Subclinical Hypothyroid Patients: A Double-Blind, Randomized Placebo-Controlled Trial. J Altern Complement Med 24, 243-248.	https://www.ncbi.nlm.nih.gov/pubmed/28829155
13	Body Weight Management in Adults Under Chronic Stress Through Treatment With Ashwagandha Root Extract: A Double-Blind, Randomized, Placebo-Controlled Trial	Journal of Evidence Based Complementary Alternative Medicine	52	26	26	1	1	Giddiness, Heaviness of head, Blurring of vision, hyperacidity		Ashwagandha Root Extract (KSM-66)	300 mg capsule twice daily (600mg/d)	8 Weeks	India	Choudhary, D., Bhattacharyya, S., Joshi, K., 2017b. Body Weight Management in Adults Under Chronic Stress Through Treatment with Ashwagandha Root Extract: A Double-Blind, Randomized, Placebo-Controlled Trial. J Evid Based Complementary Altern Med 22, 96-106	https://www.ncbi.nlm.nih.gov/pubmed/27055824
14	Efficacy and Safety of Ashwagandha (Withania somnifera (L.) Dunal) Root Extract in Improving Memory and Cognitive Functions	Journal of Dietary Supplements	50	25	25	0	0	N/A	N/A	Ashwagandha Root Extract (KSM-66)	300 mg capsule twice daily (600mg/d)	8 Weeks	India	Choudhary, D., Bhattacharyya, S., Bose, S., 2017a. Efficacy and Safety of Ashwagandha (Withania somnifera (L.) Dunal) Root Extract in Improving Memory and Cognitive Functions. J Diet Suppl 14, 599-612	https://www.ncbi.nlm.nih.gov/pubmed/28471731
15	Examining the effect of Withania somnifera supplementation on muscle strength and recovery: a randomized controlled trial	Journal of International society of sports nutrition (JISSN)	50	25	25	0	0	N/A	N/A	Ashwagandha Root Extract (KSM-66)	300 mg capsule twice daily (600mg/d)	8 Weeks	India	Wankhede, S., Langade, D., Joshi, K., Sinha, S.R., Bhattacharyya, S., 2015. Examining the effect of Withania somnifera supplementation on muscle strength and recovery: a randomized controlled trial. J Int Soc Sports Nutr 12, 43.	https://www.ncbi.nlm.nih.gov/pubmed/26609282
16	Efficacy of Ashwagandha (Withania somnifera [L.] Dunal) in improving cardiorespiratory endurance in healthy athletic adults	AYU	50	25	25	0	0	N/A	N/A	Ashwagandha Root Extract (KSM-66)	300 mg capsule twice daily (600mg/d)	12 Weeks	India	Choudhary, B., Shetty, A., Langade, D.G., 2015. Efficacy of Ashwagandha (Withania somnifera [L.] Dunal) in improving cardiorespiratory endurance in healthy athletic adults. Ayu 36, 63-68.	https://www.ncbi.nlm.nih.gov/pubmed/26730141
17	Efficacy and Safety of Ashwagandha (Withania somnifera) Root Extract in Improving Sexual Function in Women: A Pilot Study	BioMed Research International	50	25	25	0	0	N/A	N/A	Ashwagandha Root Extract (KSM-66)	300 mg capsule twice daily (600mg/d)	8 Weeks	India	Dongre, S., Langade, D., & Bhattacharyya, S. (2015). Efficacy and Safety of Ashwagandha (Withania somnifera) Root Extract in Improving Sexual Function in Women: A Pilot Study. BioMed research international, 2015, 284154. https://doi.org/10.1155/2015/284154	https://www.ncbi.nlm.nih.gov/pubmed/26504795
18	Clinical Evaluation of the Spermatogenic Activity of the Root Extract of Ashwagandha (Withania somnifera) in Oligospermic Males: A Pilot Study	Evidence based complementary and alternative medicine	46	21	25	0	0	N/A	N/A	Ashwagandha Root Extract (KSM-66)	225mg capsule thrice dailu (675 mg/d)	3 months	India	Ambiye, V.R., Langade, D., Dongre, S., Aptikar, P., Kulkarni, M., Dongre, A., 2013. Clinical Evaluation of the Spermatogenic Activity of the Root Extract of Ashwagandha (Withania somnifera) in Oligospermic Males: A Pilot Study. Evid Based Complement Alternat Med 2013, 571420	https://www.ncbi.nlm.nih.gov/pubmed/24371462
19	A Prospective, Randomized Double-Blind, Placebo-Controlled Study of Safety and Efficacy of a High-Concentration Full-Spectrum Extract of Ashwagandha Root in Reducing Stress and Anxiety in Adults	Indian Journal of Psychological Medicine	61	30	31	6	5	Nasal congestion, Constipation, Cough and cold, Drowsiness, Decreased appetite	Dryness of mouth, tiredness, fever, headache, abdominal pain, diarrhea, tremor in legs	Ashwagandha Root Extract (KSM-66)	300 mg capsule twice daily (600mg/d)	8 Weeks	India	Chandrasekhar, K., Kapoor, J., Anishetty, S., 2012. A prospective, randomized double-blind, placebo-controlled study of safety and efficacy of a high-concentration full-spectrum extract of ashwagandha root in reducing stress and anxiety in adults. Indian J Psychol Med 34, 255-262	https://www.ncbi.nlm.nih.gov/pubmed/23439798

20	Withania somnifera (L.) Dunal as Add-On Therapy for COPD Patients: A Randomized, Placebo-Controlled, Double-Blind Study	Frontiers in pharmacology	150	50	100	0	0	N/A	N/A	Withania somnifera root powder	250 mg of WS root capsules twice daily	12 Weeks	India	Singh, P., Salman, K. A., Shameem, M., & Warsi, M. S. (2022). Withania somnifera (L.) Dunal as Add-On Therapy for COPD Patients: A Randomized, Placebo-Controlled, Double-Blind Study. Frontiers in pharmacology, 13, 901710. https://doi.org/10.3389/fphar.2022.901710	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9243480/
21	Withania somnifera as a safer option to hydroxychloroquine in the chemoprophylaxis of COVID-19: Results of interim analysis	Complementary therapies in medicine	160	80	80	26	40	N/A	N/A	Standardized Withania somnifera root Extract	2 tablets of 250 mg standardized extract of WS twice daily .	16 Weeks	India	Chopra, A., Srikanth, N., Patwardhan, B., & AYUSH CCRAS Research Group (2021). Withania somnifera as a safer option to hydroxychloroquine in the chemoprophylaxis of COVID-19: Results of interim analysis. Complementary therapies in medicine, 62, 102768.	https://pubmed.ncbi.nlm.nih.gov/34418550/
22	Efficacy and Safety of Ashwagandha Root Extract on Cognitive Functions in Healthy, Stressed Adults: A Randomized, Double-Blind, Placebo-Controlled Study	Evidence-based complementary and alternative medicine	130	65	65	0	0	N/A	N/A	Withania somnifera root extract	One 300 mg capsule capsule daily after breakfast	90 Days	India	Gopukumar, K., Thanawala, S., Somepalli, V., Rao, T., Thamatam, V. B., & Chauhan, S. (2021). Efficacy and Safety of Ashwagandha Root Extract on Cognitive Functions in Healthy, Stressed Adults: A Randomized, Double-Blind, Placebo-Controlled Study. Evidence-based complementary and alternative medicine : eCAM, 2021, 8254344. https://doi.org/10.1155/2021/8254344	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8632422/
23	Efficacy of Brimhana Nasya and Ashwagandha (Withania somnifera (L.) Dunal) root powder in primary insomnia in elderly male: A randomized open-label clinical study	AYU	60	30	30	0	0	N/A	N/A	Ashwagandha root powder	6 g Ashwagandha root powder orally with 100 ml of milk	30 Days	India	Atul, U., Charu, B., & Umesh, S. (2020). Efficacy of Brimhana Nasya and Ashwagandha (Withania somnifera (L.) Dunal) root powder in primary insomnia in elderly male: A randomized open-label clinical study. Ayu, 41(3), 159–165. https://doi.org/10.4103/ayu.AYU_177_19	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8966760/
24	Assessment of the Efficacy of Withania somnifera Root Extract in Patients with Generalized Anxiety Disorder: A Randomized Double-blind Placebo- Controlled Trial	Current reviews in clinical and experimental pharmacology	40	22	18	0	0	N/A	N/A	Withania somnifera root extract	1g capsule of Withania somnifera extract once daily (1g/d)	6 weeks	N/A	Fuladi, S., Emami, S. A., Mohammadpour, A. H., Karimani, A., Manteghi, A. A., & Sahebkar, A. (2020). Assessment of Withania somnifera root extract efficacy in patients with generalized anxiety disorder: A randomized double-blind placebo-controlled trial. Current clinical pharmacology, 10.2174/1574884715666200413120413.	https://pubmed.ncbi.nlm.nih.gov/32282308/
25	Evaluation of the Effectiveness of Withania Somnifera Root Extract on the Anxiety Symptoms among children with ADHD in Mashhad	Journal of Medical and Health Sciences	28	14	14	0	0	N/A	N/A	Withania somnifera root extract	10 mg Withania somnifera once daily	6 weeks	Iran	Narges Hosseini, Fatemeh Moharari, Atefeh Soltanifar, Amirhoshang M Poor, Ahmadreza Hosseini, Fatemeh Zahedian. Evaluation of the Effectiveness of Withania Somnifera Root Extract on the Anxiety Symptoms among children with ADHD in Mashhad. Journal of Medical and Health Sciences Vol. 13, Issue 1, JAN – MAR 2019	http://www.pjmhsonline.com/2019/jan_march/pdf/210.pdf
26	Comparative evaluation of the effects of Withania somnifera with pentoxifylline on the sperm parameters in idiopathic male infertility: A triple-blind randomised clinical trial.	Andrologia	100	50	50	0	0	N/A	N/A	Methanolic root extract of Withania somnifera	Six capsules containing 5 g/daily of W. somnifera root	12 Weeks	Iran	Nasimi Doost Azgomi, R., Nazemiyeh, H., Sadeghi Bazargani, H., Fazljou, S.M.B., Nejatbakhsh, F., Moini Jazani, A., Ahmadi AsrBadr, Y., Zomorrodi, A., 2018a. Comparative evaluation of the effects of Withania somnifera with pentoxifylline on the sperm parameters in idiopathic male infertility: A triple-blind randomised clinical trial. Andrologia 50, e13041.	https://www.ncbi.nlm.nih.gov/pubmed/29770466
27	Comparative study of effect of Withania somnifera as an adjuvant to DOTS in patients of newly diagnosed sputum smear positive pulmonary tuberculosis.	The Indian journal of tuberculosis	79	39	40	0	0	N/A	N/A	Withania somnifera root extract	Withania somnifera root extract capsules	12 Weeks	India	Kumar, R., Rai, J., Kajal, N.C., Devi, P., 2018. Comparative study of effect of Withania somnifera as an adjuvant to DOTS in patients of newly diagnosed sputum smear positive pulmonary tuberculosis. Indian J Tuberc 65, 246-251.	https://www.ncbi.nlm.nih.gov/pubmed/29933868
28	A clinical trial for the efficacy of Ashwagandha powder in obsessive-compulsive disorder	Current Medical and Drug Research	30	30	0	0	0	N/A	N/A	Ashwagandha powder	4.5 gm of Ashwagandha powder two times per day (i.e. 9 gm/day)	60 days	India	Saini N (2018). A clinical trial for the efficacy of Ashwagandha powder in obsessive compulsive disorder. Current Medical and Drug Research, 2 (2), Article ID 188.	http://globalscitechocean.com/ReportFile/6c704c5e07e14db0886986d3df51c655.pdf
29	A randomized clinical study to evaluate the efficacy of ashwagandha rasayana on karshya w.s.r. to underweight.	World Journal of Pharmaceutical Research	30	30	0	0	0	N/A	N/A	Ashwagandha Rasayana	12 g in two divided dose morning and evening.	90 days	India	Sharma Sudhanshu Dutt, Masand Sameet, Sharma Suryanshu Dutt,Sharma Gopal Dutt and Thakur Jyotsna. A randomized clinical study to evaluate the efficacy of ashwagandha rasayana on karshya w.s.r. to underweight. World Journal of Pharmaceutical Research, Volume 6, Issue 15, 743-755. Research Article ISSN 2277–7105	https://wjpr.net/dashboard/abstract_id/8377
30	Evaluation of the efficacy of Withania somnifera (Ashwagandha) root extract in patients with obsessive-compulsive disorder: A randomized double-blind placebo-controlled trial	Complementary therapies in medicine	30	15	15	0	0	N/A	N/A	Ethanolic Ashwagandha root extract	4 capsules (250mg each) day (1000mg/d)	6 Weeks	Iran	Jahanbakhsh, S.P., Manteghi, A.A., Emami, S.A., Mahyari, S., Gholampour, B., Mohammadpour, A.H., Sahebkar, A., 2016. Evaluation of the efficacy of Withania somnifera (Ashwagandha) root extract in patients with obsessive-compulsive disorder: A randomized double-blind placebo-controlled trial. Complement Ther Med 27, 25-29.	https://www.ncbi.nlm.nih.gov/pubmed/27515872
31	A Clinical Evaluation of Antistress Activity of Ashwagandha (Withania Somnifera Dunal) on Employees Experiencing Mental Stress at Work Place.	International Journal of Ayurveda and Pharma Research	40	40	0	0	0	N/A	N/A	Ashwagandha granules	10gms twice a day	60 Days	India	Gajarmal Amit Ashok, Shende M.B. A Clinical Evaluation of Antistress Activity of Ashwagandha (Withania Somnifera Dunal) on Employees Experiencing Mental Stress at Work Place. Int. J. Ayur. Pharma Research. 2015;3(1):37-45.	https://ijapr.in/index.php/ijapr/article/view/173/159
32	Efficacy & safety evaluation of Ayurvedic treatment (Ashwagandha powder & Sidh Makardhwaj) in rheumatoid arthritis patients: a pilot prospective study	The Indian Journal of medical research	86	86	0	0	0	N/A	N/A	Ashwagandha powder	5g of Ashwagandha powder twice a day	3 Weeks	India	Kumar, G., Srivastava, A., Sharma, S. K., Rao, T. D., & Gupta, Y. K. (2015). Efficacy & safety evaluation of Ayurvedic treatment (Ashwagandha powder & Sidh Makardhwaj) in rheumatoid arthritis patients: a pilot prospective study. The Indian journal of medical research, 141(1), 100–106. doi:10.4103/0971-5916.154510	https://pubmed.ncbi.nlm.nih.gov/25857501/
33	A Clinical Study on Management of Stress in Type-2 Diabetes Mellitus (Madhumeha) with Ashwagandha (Withania Somnifera).	Ayushdhara	55	28	27	0	0	N/A	N/A	Aqueous extract of Withania somnifera (root)	300 mg capsules of root extract twice daily	6 weeks	India	Shobha Nayak, Saurabha Nayak, Binod Kumar Panda, Sambit Das. A Clinical Study on Management of Stress in Type-2 Diabetes Mellitus (Madhumeha) with Ashwagandha (Withania Somnifera). AYUSHDHARA, 2015;2(6):413-417	https://ayushdhara.in/research/pdf/ayush_261591.pdf
34	Effect of Ashwagandha (Withania somnifera) root powder supplementation on the core muscle strength and stability in hockey players.	International Journal of Behavioural Social and Movement Sciences	48	24	24	0	0	N/A	N/A	Withania somnifera root powder	500 mg capsules of aqueous roots of Ashwagandha twice daily	8 Week	India	Arvind Malik; Vikas Mehta; Sonia Malik; Pradeep Sharma (2014). Effect of Ashwagandha (Withania somnifera) root powder supplementation on the core muscle strength and stability in hockey players. International Journal of Behavioural Social and Movement Sciences, Vol. 3 No. 3 pp. 83-91	http://www.ijobsms.in/6cvol03ssue4p10.html
35	Effect of Ashwagandha (Withania somnifera) root powder supplementation on VO2 max and hemoglobin in hockey players	International Journal of Behavioural Social and Movement Sciences	32	16	16	0	0	N/A	N/A	Ashwagandha root powder	500 mg capsules of aqueous roots of Ashwagandha twice daily	8 Weeks	India	Arvind Malik; Vikas Mehta; Sonia Malik; Pradeep Sharma (2014). Effect of Ashwagandha (Withania somnifera) root powder supplementation on VO2 max and hemoglobin in hockey players. International Journal of Behavioural Social and Movement Sciences, Vol. 2 No. 3 pp. 91-99	http://ijobsms.in/A12%20JULY%20%20p12%20%20%201Dr.%20Arvind%20Malik%20%20%202Vikas%20Mehta%20%20%203%20Vishal%20Dahiya.pdf
36	Effect of ashwagandha (Withania Somnifera) consumption on the selected physical fitness variables of male sprinters	Turkish Journal of Sport and Exercise	20	10	10	0	0	N/A	N/A	Ashwagandha	2.5g to 3g Ashwagandha thrice a week (alternative days)	12 Weeks	India	YADAV, Satpal (2014). Effect of ashwagandha (Withania Somnifera) consumption on the selected physical fitness variables of male sprinters. Turkish Journal of Sport and Exercise 16.3 (2014): 45-47.	https://dergipark.org.tr/en/pub/tsed/issue/21505/230691
37	Effects of Withania somnifera in patients of schizophrenia: a randomized, double blind, placebo-controlled pilot trial study.	Indian Journal of Pharmacology	30	15	15	0	0	N/A	N/A	Withania somnifera root extract	400 mg of WSE thrice daily	4 Weeks	India	Agnihotri, A.P., Sontakke, S.D., Thawani, V.R., Saoji, A., Goswami, V.S., 2013. Effects of Withania somnifera in patients of schizophrenia: a randomized, double blind, placebo-controlled pilot trial study. Indian J Pharmacol 45, 417-418.	https://www.ncbi.nlm.nih.gov/pubmed/24014929
38	Effect of Withania somnifera (Ashwagandha) on the development of chemotherapy-induced fatigue and quality of life in breast cancer patients.	Integrative Cancer Therapies	100	50	50	0	0	N/A	N/A	Withania somnifera root extract	4 capsules 500mg each 3 times daily	15 - 18 Weeks	India	Biswal, B.M., Sulaiman, S.A., Ismail, H.C., Zakaria, H., Musa, K.I., 2013. Effect of Withania somnifera (Ashwagandha) on the development of chemotherapy-induced fatigue and quality of life in breast cancer patients. Integr Cancer Ther 12, 312-322.	https://www.ncbi.nlm.nih.gov/pubmed/23142798
39	Efficacy of Withania somnifera on seminal plasma metabolites of infertile males: a proton NMR study at 800 MHz.	Journal of Ethnopharmacology	230	180	50	0	0	N/A	N/A	Withania somnifera root powder	Withania somnifera (5 g/d)	3 months	India	Gupta, A., Mahdi, A.A., Shukla, K.K., Ahmad, M.K., Bansal, N., Sankhwar, P., Sankhwar, S.N., 2013. Efficacy of Withania somnifera on seminal plasma metabolites of infertile males: a proton NMR study at 800 MHz. J Ethnopharmacol 149, 208-214.	https://www.ncbi.nlm.nih.gov/pubmed/23796876
40	A randomized double-blind placebo-controlled study of ashwagandha on generalized anxiety disorder	International Ayurvedic Medicine Journal	86	44	42	0	0	N/A	N/A	Granules made with dried root powder of WS; 4-g granules	One 4-g granule 3 times a day (12,000 mg/day)	8 Weeks	India	Khyati, S., Anup, B., 2013. A randomized double-blind placebo-controlled study of ashwagandha on generalized anxiety disorder. Int Ayurvedic Med J 1, 1-7.	http://www.iamj.in/posts/images/upload/01.05.14_IAMJ.pdf

41	Effects of eight-week supplementation of Ashwagandha on cardiorespiratory endurance in elite Indian cyclists.	Journal of Ayurveda and Integrative Medicine	40	20	20	0	0	N/A	N/A	Aqueous extract of Withania somnifera (root)	500 mg capsules twice daily	8 Weeks	India	Shenoy, S., Chaskar, U., Sandhu, J.S., Paadhi, M.M., 2012. Effects of eight-week supplementation of Ashwagandha on cardiorespiratory endurance in elite Indian cyclists. J Ayurveda Integr Med 3, 209-214.	https://www.ncbi.nlm.nih.gov/pubmed/?term=Effects+of+eight-week+supplementation+of+Ashwagandha+on+cardiorespiratory+endurance+in+elite+Indian+cyclists
42	A clinical trial with Withania somnifera (Solanaceae) extract in the management of sarcopenia (muscle aging)	Organic & Biomolecular Chemistry Journal	35	35	0	0	0	N/A	N/A	Withania somnifera whole root extract	500mg W. somnifera (in capsule) twice daily	N/A	N/A	Mishra S. K., and Trikamji B. (2013) Signpost Open. A clinical trial with Withania somnifera (Solanaceae) extract in the management of sarcopenia (muscle aging) . Access J. Org. Biomol. Chem. 1, 187-194	https://pdfslide.net/documents/a-clinical-trial-with-withania-somnifera-solanaceae-extract-in-the-.html
43	Exploratory study to evaluate tolerability, safety, and activity of Ashwagandha (Withania somnifera) in healthy volunteers.	Journal of Ayurveda and Integrative Medicine	18	18	0	1	0	Increase in appetite, libido, and hallucinogenic effects with vertigo	N/A	Withania somnifera root extract	750 mg/day x10 days, 1000 mg/day x 10 days, 1250 mg/day x 10 days	30 Days	India	Raut, A.A., Rege, N.N., Tadvi, F.M., Solanki, P.V., Kene, K.R., Shirolkar, S.G., Pandey, S.N., Vaidya, R.A., Vaidya, A.B., 2012. Exploratory study to evaluate tolerability, safety, and activity of Ashwagandha (Withania somnifera) in healthy volunteers. J Ayurveda Integr Med 3, 111-114.	https://www.ncbi.nlm.nih.gov/pubmed/?term=Exploratory+study+to+evaluate+tolerability%2C+safety%2C+and+activity+of+Ashwagandha+(Withania+somnifera)+in+healthy+volunteers.
44	Efficacy of Ashwagandha (Withania somnifera Dunal. Linn.) in the management of psychogenic erectile dysfunction	Ayu	95	46	49	0	0	N/A	N/A	Ashwagandha root powder	4 tablets (500 mg each) thrice a day	60 Days	India	Mamidi, P., Thakar, A.B., 2011. Efficacy of Ashwagandha (Withania somnifera Dunal. Linn.) in the management of psychogenic erectile dysfunction. Ayu 32, 322-328.	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3326875/
45	Withania somnifera improves semen quality by combating oxidative stress and cell death and improving essential metal concentrations.	Reproductive biomedicine online	150	75	75	0	0	N/A	N/A	Ashwagandha root powder	5 g/day in a single dose with milk	3 months	India	Shukla, K. K., Mahdi, A. A., Mishra, V., Rajender, S., Sankhwar, S. N., Patel, D., & Das, M. (2011). Withania somnifera improves semen quality by combating oxidative stress and cell death and improving essential metal concentrations. Reproductive biomedicine online, 22(5), 421–427. doi:10.1016/j.rbmo.2011.01.010	https://www.ncbi.nlm.nih.gov/pubmed/21388887
46	A clinical study of Ashwagandha ghrita and Ashwagandha granules for its Brumhana and Balya effect.	Ayu	121	87	34	0	0	N/A	N/A	Ashwagandha Ghrita and granules	2.5 – 4 gm of Ashwagandha Ghrita and granules 6 – 8 gm of Ashwagandha Ghrita and granules	1 1/2 months	India	Mishra, R. K., Trivedi, R., & Pandya, M. A. (2010). A clinical study of Ashwagandha ghrita and Ashwagandha granules for its Brumhana and Balya effect. Ayu, 31(3), 355–360. doi:10.4103/0974-8520.77164	https://www.ncbi.nlm.nih.gov/pubmed/22131739
47	Effects of Withania somnifera (Ashwagandha) and Terminalia arjuna (Arjuna) on physical performance and cardiorespiratory endurance in healthy young adults	International Journal of Ayurveda Research	30	20	10	0	0	N/A	N/A	Aqueous extract of Withania somnifera (root)	One 500mg capsule/day	8 Weeks	India	Sandhu, J.S., Shah, B., Shenoy, S., Chauhan, S., Lavekar, G.S., Padhi, M.M., 2010. Effects of Withania somnifera (Ashwagandha) and Terminalia arjuna (Arjuna) on physical performance and cardiorespiratory endurance in healthy young adults. Int J Ayurveda Res 1, 144-149.	https://www.ncbi.nlm.nih.gov/pubmed/21170205
48	Withania somnifera improves semen quality by regulating reproductive hormone levels and oxidative stress in seminal plasma of infertile males.	Fertility Sterility	150	75	75	0	0	N/A	N/A	Withania somnifera root powder	W. somnifera root powder (5 g/day) orally	3 months	India	Ahmad, M.K., Mahdi, A.A., Shukla, K.K., Islam, N., Rajender, S., Madhukar, D., Shankhwar, S.N., Ahmad, S., 2010. Withania somnifera improves semen quality by regulating reproductive hormone levels and oxidative stress in seminal plasma of infertile males. Fertil Steril 94, 989-996.	https://www.ncbi.nlm.nih.gov/pubmed/19501822
49	In Vivo Effects of Ashwagandha (Withania somnifera) Extract on the Activation of Lymphocytes	The Journal of Alternative and Complementary Medicine	5	5	0	0	0	N/A	N/A	Withania somnifera root extract	6 mL of an Ashwagandha root extract twice daily	96 hours (4 days)	USA	Mikolai, J., Erlandsen, A., Murison, A., Brown, K. A., Gregory, W. L., Raman-Caplan, P., & Zwickey, H. L. (2009). In Vivo Effects of Ashwagandha (Withania somnifera) Extract on the Activation of Lymphocytes. The Journal of Alternative and Complementary Medicine, 15(4), 423–430. doi:10.1089/acm.2008.0215	https://www.liebertpub.com/doi/abs/10.1089/acm.2008.0215
50	Withania somnifera Improves Semen Quality in Stress-Related Male Fertility	Evidence based complementary and alternative medicine	120	60	60	0	0	N/A	N/A	Withania somnifera root powder	W. somnifera root powder (5 g/day) orally	3 months	India	Mahdi, A.A., Shukla, K.K., Ahmad, M.K., Rajender, S., Shankhwar, S.N., Singh, V., Dalela, D., 2009. Withania somnifera Improves Semen Quality in Stress-Related Male Fertility. Evid Based Complement Alternat Med.	https://www.ncbi.nlm.nih.gov/pubmed/19789214
51	Naturopathic Care for Anxiety: A Randomized Controlled Trial ISRCTN78958974	PloS one	75	36	39	0	0	N/A	N/A	Withania somnifera root (Swiss ashwagandha); 300-mg supplements	One 300-mg supplement twice a day (600 mg/d)	12 Weeks	Canada	Cooley, K., Szczurko, O., Perri, D., Mills, E. J., Bernhardt, B., Zhou, Q., & Seely, D. (2009). Naturopathic care for anxiety: a randomized controlled trial ISRCTN78958974. PloS one, 4(8), e6628. https://doi.org/10.1371/journal.pone.0006628	https://www.ncbi.nlm.nih.gov/pubmed/19718255
52	Hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry (Withania somnifera, Dunal) root.	India Journal of Experimental Biology	12	12	0	0	0	N/A	N/A	Withania somnifera root powder	6 capsules of 500mg each per day (3g/d)	30 days	India	Andallu, B., Radhika, B., 2000. Hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry (Withania somnifera, Dunal) root. Indian J Exp Biol 38, 607-609.	https://www.ncbi.nlm.nih.gov/pubmed/11116534
53	A Clinical Study to Evaluate Efficacy of Agnikarma (Locally) and Ashwagandha Ghanavati (Internally) in the Management of Manyagata Vata w.s.r Cervical Spondylosis	International Journal of Ayurvedic Medicine	50	50	0	0	0	N/A	N/A	Ashwagandha Ghanavati	Two 250-mg tablets, twice a day	60 Days	India	Amarprakash, D., Anaya, P., Shubhangi, K., & Anjna, K. (2018). A Clinical Study to Evaluate Efficacy of Agnikarma (Locally) and Ashwagandha Ghanavati (Internally) in the Management of Manyagata Vata w.s.r Cervical Spondylosis. International Journal of Ayurvedic Medicine, 9(3), 208–213. https://doi.org/10.47552/ijam.v9i3.1109	https://ijam.co.in/index.php/ijam/article/view/1109/451
54	EFFICACY STUDY OF ASHWAGANDHA AND YASHTIMADHU CHOORNA IN THE MANAGEMENT OF SHUKRAKSHAYA (OLIGOSPERMIA)	International Ayurvedic Medical Journal	30	15	15	0	0	N/A	N/A	Ashwagandha choorna	3g Ashwagandha choorna with 150 ml twice a day	30 Days	India	Amit, C., & Rachna, G. (2013). Chowdhary Amit et al: Efficacy Study of Ashwagandha and Yashtimadhu Choorna in Shukra Kshaya. International Ayurvedic Medical Journal | Year : 2013 | Volume : 1 | Issue : 3 | Page : 1-6	http://www.iamj.in/images/upload/01.03.12.pdf
55	Management Of Pre Menstrual Syndrome With Combined Ayurveda Interventions (Ashwagandha Vati And Satvavajaya Chikitsa) - An Open Label Single Arm Clinical Study	Annals of Ayurvedic Medicine	30	30	0	**0**	**0**	**N/A**	**N/A**	Ashwagandha Root powder	Two 500mg Ashwagandha pills twice daily	30 Days	India	Shrilata, Adiga M, T SK. Management Of Pre Menstrual Syndrome With Combined Ayurveda Interventions (Ashwagandha Vati And Satvavajaya Chikitsa) - An Open Label Single Arm Clinical Study. AAM. 2022; 11(1): 48-63. doi:10.5455/AAM.80852	https://www.aamjournal.in/?mno=80852#cite
56	An approach of Ashwagandha + Guggulu in Atheromatous CHD associated with Obesity	Ayu	20	20	0	**0**	**0**	**N/A**	**N/A**	Ashwagandha powder 500 mg	Ashwagandha powder 500 mg + Shuddha Guggulu 500 mg twice a day	12 Weeks	India	Mehra R, Prasad Mahadeo, Lavekar G S (2009). An approach of Ashwagandha + Guggulu in Atheromatous CHD associated with Obesity. Ayu Volume : 30 | Issue : 2 | Page : 121-125	http://www.ayujournal.org/downloadpdf.asp?issn=0974-8520;year=2009;volume=30;issue=2;spage=121;epage=125;aulast=Mehra;type=2
	Polyherbal Studies														
57	Randomized placebo-controlled pilot clinical trial on the efficacy of ayurvedic treatment regime on COVID-19 positive patients	Phytomedicine : international journal of phytotherapy and phytopharmacology	95	45	50	0	0	N/A	N/A	1 g of Giloy Ghanvati (Tinospora cordifolia) and 2 g of Swasari Ras (traditional herbo-mineral formulation) and 0.5 g each of Ashwagandha (Withania somnifera) and Tulsi Ghanvati (Ocimum sanctum) were given orally to the patients in treatment group twice per day for 7 days.	500mg tablet twice a day	7 days	India	Devpura, G., Tomar, B. S., Nathiya, D., Sharma, A., Bhandari, D., Haldar, S., Balkrishna, A., & Varshney, A. (2021). Randomized placebo-controlled pilot clinical trial on the efficacy of ayurvedic treatment regime on COVID-19 positive patients. Phytomedicine : international journal of phytotherapy and phytopharmacology, 84, 153494. https://doi.org/10.1016/j.phymed.2021.153494	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7857981/
58	Effects of an Adaptogenic Extract on Electrical Activity of the Brain in Elderly Subjects with Mild Cognitive Impairment: A Randomized, Double-Blind, Placebo-Controlled, Two-Armed Cross-Over Study	Pharmaceuticals (Basel, Switzerland)	30	15	15	3	0	Allergic reaction and hypertension due to concomitant medications	N/A	550 mg Adaptra® Forte capsule containing 400 mg of A. paniculata herb dry native extract and 150 mg of Withania somnifera extract	One 550 mg Adaptra® Forte capsule twice daily	4 Weeks	Germany	Dimpfel, W., Schombert, L., Keplinger-Dimpfel, I. K., & Panossian, A. (2020). Effects of an Adaptogenic Extract on Electrical Activity of the Brain in Elderly Subjects with Mild Cognitive Impairment: A Randomized, Double-Blind, Placebo-Controlled, Two-Armed Cross-Over Study. Pharmaceuticals (Basel, Switzerland), 13(3), 45. https://doi.org/10.3390/ph13030045	https://pubmed.ncbi.nlm.nih.gov/32183355/
59	Clinical Evaluation of Ashwagandha and Mandookaparni in the Management of Manodwega (Generalized Anxiety Disorder)	J Res Ayurvedic Sci	86	40	46	0	0	N/A	N/A	Ashwagandha churna	3 tablets of 500 mg twice daily	12 weeks	India	Ramana GV, Gupta H, Sudhakar D, Singh R, Rana R, Singhal R. Clinical Evaluation of Ashwagandha and Mandookaparni in the Management of Manodwega (Generalized Anxiety Disorder). J Res Ayurvedic Sci 2018;2(2):70-79.	https://www.jaypeedigital.com/doi/JRAS/pdf/10.5005/jp-journals-10064-0042

60	A clinical study to evaluate role of ayurvedic treatment for management of cerebral palsy in children w.s.r to gross mo-tor function classification system.	International Ayurvedic Medical Journal	42	14	28	0	0	N/A	N/A	Withania somnifera	(Ayurvedic Compound Syrup with Ashwagandha) - 1ml/kg/day in three divided doses	6 months	India	Kuldeep, C., & Anjali, S. (2014). A clinical study to evaluate role of ayurvedic treatment for management of cerebral palsy in children w.s.r to gross mo- tor function classification system. International Ayurvedic Medical Journal IAMJ: Volume 2; Issue 3; May -June 2014	http://www.iamj.in/posts/2014/images/upload/328_336.pdf
61	Herbo-mineral supplementation in men with idiopathic oligoasthenoteratospermia : A double blind randomized placebo-controlled trial.	Indian journal of urology	44	21	23	0	0	N/A	N/A	Withania somnifera 150 mg	2 capsules, twice daily	3 months	India	Kumar, R., Saxena, V., Shamsi, M. B., Venkatesh, S., & Dada, R. (2011). Herbo-mineral supplementation in men with idiopathic oligoasthenoteratospermia : A double blind randomized placebo-controlled trial. Indian journal of urology : IJU : journal of the Urological Society of India, 27(3), 357–362.	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3193736/pdf/IJU-27-357.pdf
62	A double-blind randomized placebo-controlled clinical study to evaluate the efficacy and safety of a polyherbal formulation in geriatric age group: A phase IV clinical report.	Journal of Ethnopharmacology	111	89	22	0	0	N/A	N/A	Withania somnifera 30 mg	2 tablets, twice daily	6 months	India	Banerjee, P., Maity, S., Das, T., & Mazumder, S. (2011). A double-blind randomized placebo-controlled clinical study to evaluate the efficacy and safety of a polyherbal formulation in geriatric age group: A phase IV clinical report. Journal of Ethnopharmacology, 134(2), 429–433. doi:10.1016/j.jep.2010.12.044	https://www.sciencedirect.com/science/article/abs/pii/S0378874110009207?via%3Dihub
63	Efficacy and safety of livwin (polyherbal formulation) in patients with acute viral hepatitis: A randomized double-blind placebo-controlled clinical trial.	International journal of Ayurveda research	58	29	29	N/A	N/A	Epigastric pain (2) ; Diarrhoea (1)	Epigastric pain (2)	**Livwin** - Ashwagandha 100 mg	2 capsules, twice daily	8 weeks	India	Keche, Y., Badar, V., & Hardas, M. (2010). Efficacy and safety of livwin (polyherbal formulation) in patients with acute viral hepatitis: A randomized double-blind placebo-controlled clinical trial. International journal of Ayurveda research, 1(4), 216–219. https://doi.org/10.4103/0974-7788.76784	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3059443/pdf/IJAR-1-216.pdf
64	In Vivo Enhancement of Natural Killer Cell Activity through Tea Fortifi ed with Ayurvedic Herbs - Study I	Phytotherapy Research	30	15	15	0	0	N/A	N/A	**NC Tea** - Withania somnifera	3 Cups tea/day	2 months	India	Bhat, J., Damle, A., Vaishnav, P. P., Albers, R., Joshi, M., & Banerjee, G. (2010). In vivoenhancement of natural killer cell activity through tea fortified with Ayurvedic herbs. Phytotherapy Research, 24(1), 129–135. doi:10.1002/ptr.2889	https://onlinelibrary.wiley.com/doi/10.1002/ptr.2889
65	In Vivo Enhancement of Natural Killer Cell Activity through Tea Fortifi ed with Ayurvedic Herbs - Study II	Phytotherapy Research	110	55	55	0	0	N/A	N/A	**NC Tea** - Withania somnifera	3 Cups tea/day	2 months	India	Bhat, J., Damle, A., Vaishnav, P. P., Albers, R., Joshi, M., & Banerjee, G. (2010). In vivoenhancement of natural killer cell activity through tea fortified with Ayurvedic herbs. Phytotherapy Research, 24(1), 129–135. doi:10.1002/ptr.2889	https://www.researchgate.net/publication/26274207_In_Vivo_Enhancement_of_Natural_Killer_Cell_Activity_through_Tea_Fortified_with_Ayurvedic_Herbs
66	Influence of Yoga and Ayurveda on self-rated sleep in a geriatric population	The Indian journal of medical research	50	12	38	0	0	N/A	N/A	Rasayana Kalpa with Withania somnifera roots 2 g; Emblica officinalis (amalaki, 1 g), Sida cordifolia (bala, 0.25 g), Terminalia arjuna (arjuna, 0.25 g), Piper longum (pippali, 0.5 g)	10 g (1 tablespoon, approximately) of Rasayana Kalpa, twice a day	6 months	India	Manjunath, N.K., & Telles, S. (2005). Influence of Yoga and Ayurveda on self-rated sleep in a geriatric population. The Indian journal of medical research, 121 5, 683-90.	https://pubmed.ncbi.nlm.nih.gov/15937373/
67	Evaluation of the efficacy and safety of Reosto in postmenopausal osteoporosis: A prospective, randomized, placebo-controlled, double blind, phase III clinical trial	Obstetrics and Gynaecology Today	78	39	39	0	0	N/A	N/A	**Reosto** - Withania somnifera	2 tablets, twice daily	6 months	India	Deepti Dongaonkar, Rajeev Mehta, Kolhapure, S.A. Evaluation of the efficacy and safety of Reosto in postmenopausal osteoporosis: A prospective, randomized, placebo-controlled, double blind, phase III clinical trial. Obst. & Gynae. Today 2005; X(7), 362-367	https://citeseerx.ist.psu.edu/viewdoc/download;jsessionid=90081EDA110EEA2F497C3F39D78667BB?doi=10.1.1.424.4310&rep=rep1&type=pdf
68	Evaluation of the Efficacy and Safety of Reosto in Postmenopausal osteoporosis: A prospective, randomized, placebo-controlled, double blind, phase III clinical trial Blind, Phase III Clinical Trial	MEDICINE UPDATE	72	36	36	0	0	N/A	N/A	**Reosto** - Withania somnifera	2 tablets, twice daily	6 months	India	Jayashree Reddy, S.A. Kolhapure. Evaluation of the Efficacy and Safety of Reosto in Postmenopausal osteoporosis: A prospective, randomized, placebo-controlled, double blind, Blind, Phase III Clinical Trial.MEDICINE UPDATE 2005 13 (2)	https://pubmed.ncbi.nlm.nih.gov/25593843/
69	A 32-Week Randomized, Placebo-Controlled Clinical Evaluation of RA-11, an Ayurvedic Drug, on Osteoarthritis of the Knees	Journal of Clinical Rheumatology	90	45	45	N/A	N/A	Anorexia (9); Nausea (10); Abdominal pain (9); Skin rash (11); Stromatitis (6); Insomnia(7); others (3).	Anorexia (7); Nausea (8); Abdominal pain (10) ; Skin rash (6); Stromatitis (8); Insomnia (8); others (9).	**RA-11** - Withania somnifera	2 capsules, twice daily	32 Weeks	India	Chopra, A., Lavin, P., Patwardhan, B., & Chitre, D. (2004). A 32-Week Randomized, Placebo-Controlled Clinical Evaluation of RA-11, an Ayurvedic Drug, on Osteoarthritis of the Knees. JCR: Journal of Clinical Rheumatology, 10(5), 236–245. doi:10.1097/01.rhu.0000138087.47382.6d	https://journals.lww.com/jclinrheum/Abstract/2004/10000/A_32_Week_Randomized,_Placebo_Controlled_Clinical.3.aspx
70	Evaluation of efficacy and safety of Reosto in senile osteoporosis: A randomized, double-blind placebo-controlled clinical trial.	Indian Journal of Clinical Practice	90	47	43	0	0	N/A	N/A	**Reosto** - Withania somnifera	2 tablets, twice daily	12 months	India	Shah, A. (2004). Evaluation of efficacy and safety of Reosto in senile osteoporosis: A randomized, double-blind placebo-controlled clinical trial. ." Indian J. Clin. Pract 15 (2004): 25-36	https://citeseerx.ist.psu.edu/viewdoc/download;jsessionid=90081EDA110EEA2F497C3F39D78667BB?doi=10.1.1.424.4310&rep=rep1&type=pdf
71	Evaluation of the efficacy and safety of OST-6* in Senile Osteoporosis	Indian Journal of Clinical Practice	36	18	18	0	0	N/A	N/A	**OST-6** - Withania somnifera	2 tablets, twice daily	6 months	India	Nigam, D.K., Reshma Agarwal. Evaluation of the efficacy and safety of OST-6* in Senile Osteoporosis. Indian Journal of Clinical Practice (2004): 14(9), 63-70	https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.582.6187&rep=rep1&type=pdf
72	Evaluation of the antiretroviral activity of a new polyherbal drug (Immu-25) in patients with HIV infection.	Drugs in R&D	36	36	0	23	N/A	Nausea (7), Gastritis (5), Dyspepsia (4), Vomiting (4), Abdominal pain (3)	N/A	Immu-25 - Withania somnifera 100mg	2 capsules, twice daily	18 months	India	Usha, P. R., Naidu, M. U., & Raju, Y. S. (2003). Evaluation of the antiretroviral activity of a new polyherbal drug (Immu-25) in patients with HIV infection. Drugs in R&D, 4(2), 103–109. https://doi.org/10.2165/00126839-200304020-00003	https://pubmed.ncbi.nlm.nih.gov/12718564/
73	An experience with Reosto in managing osteoporosis: A short communication	Orthopaedics Today	50	25	25	0	0	N/A	N/A	**Reosto** - Withania somnifera	2 tablets, twice daily	12 months	India	Sharma,V.D.Srivastava, R.N.,, Ashish Mahendra, Rakesh Singh, Amit Singh, Kala Suhas Kulkarni. An experience with Reosto in managing osteoporosis: A short communication. Orthopaedics Today (2003): V(2), 93-95	N/A
74	A randomized-controlled trial of the effects of a traditional herbal supplement on sleep onset insomnia.	Complementary Therapies in Medicine	25	25	0	0	0	N/A	N/A	Withania somnifera	2 Tablets 1 hour before bedtime	4 nights	India	Farag, N. H., & Mills, P. . (2003). A randomized-controlled trial of the effects of a traditional herbal supplement on sleep onset insomnia. Complementary Therapies in Medicine, 11(4),223–225. doi:10.1016/s0965-2299(03)00108-0	https://www.sciencedirect.com/science/article/abs/pii/S0965229903001080?via%3Dihub
75	A randomized double blind placebo-controlled drug trial with mentat in children with attention deficit hyperactivity disorder	Neurosciences Today	60	30	30	0	0	N/A	N/A	**Mentat** - Withania somnifera 52 mg	2 tablets per day	6 months	India	Kalra, V.; Zamir, H.; Pandey, R.M.; Kulkarni, K.S. A randomized double blind placebo-controlled drug trial with mentat in children with attention deficit hyperactivity disorder. Neurosci. Today 2002, 6, 223–227	https://www.semanticscholar.org/paper/A-randomized-double-blind-placebo-controlled-drug-Kalra-Zamir/2d1a3798d69ea1261bfa8d392d37073d18287d59
76	The Efficacy of OST-6, A Polyherbal Formulation in the Management of Primary Osteoporosis : A Pilot Study	Orthopaedics Today	49	49	0	0	0	N/A	N/A	**OST-6** - Withania somnifera	2 tablets, twice daily	6 months	India	Krishna Reddy; Kala Suhas Kulkarni. The Efficacy of OST-6, A Polyherbal Formulation in the Management of Primary Osteoporosis : A Pilot Study. Orthopaedics Today (2002): (IV), 3, 195-199	https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.555.2156&rep=rep1&type=pdf
77	Efficacy of OST-6, a polyherbal formulation in the management of osteoporosis in postmenopausal women	Orthopaedics Today	37	37	0	0	0	N/A	N/A	**OST-6** - Withania somnifera	2 tablets, twice daily	6 months	India	Irshad Ahmed; Loknath Kumar, K.P; Vijay Kumar. Efficacy of OST-6, a polyherbal formulation in the management of osteoporosis in postmenopausal women. Orthopaedics Today (2002): (IV), 4, 241-244]	https://www.semanticscholar.org/paper/Efficacy-of-OST-6%2C-a-polyherbal-formulation-in-the-Ahmed-Kumar/c6739fd6ab0759aaad597e406b5299e8e7000d1d3
78	Efficacy of an ayurvedic formulation in rheumatoid arthritis: a double blind, placebo controlled cross over study	Journal of Ethnopharmacology	40	20	20	6	0	Nausea (1), Dermatitis (1), Abdominal pain (4)	N/A	**Articulin - F** - Ashwagandha root (450mg)	2 capsules every 8 hours after food	3 months crossover	India	Kulkarni RR; Patki PS; Jog VP; Gandage; Patwardhan B. Efficacy of an ayurvedic formulation in rheumatoid arthritis: a double blind, placebo controlled cross over study Indian Journal of Pharmacology. 1992 Jun; 24(2): 98-101	https://www.ijp-online.com/temp/IndianJPharmacol24298-2951624_081156.pdf
79	Treatment of osteoarthritis with a herbomineral formulation: a double-blind, placebo-controlled, cross-over study.	Journal of Ethnopharmacology	42	21	21	7	0	Nausea (2) Dermatitis (3) Pain in abdomen (3)	N/A	**Articulin - F** - Ashwagandha root (450mg)	2 capsules every 8 hours after food	3 months crossover	India	Kulkarni, R. R., Patki, P. S., Jog, V. P., Gandage, S. G., & Patwardhan, B. (1991). Treatment of osteoarthritis with a herbomineral formulation: a double-blind, placebo-controlled, cross-over study. Journal of ethnopharmacology, 33(1-2), 91–95. https://doi.org/10.1016/0378-8741(91)90167-c	https://linkinghub.elsevier.com/retrieve/pii/0378-8741(91)90167-C
			5020	2862	2158	89	67								

Annexure - III- Pre-clinical and Animal Studies

S.No	Title	Journal	Activity	Extract used	Experimental Model	Dose	Results	Citation
1	Modulatory action of withaferin-A on oxidative damage through regulation of inflammatory mediators and apoptosis via PI3K/AKT signaling pathway in high cholesterol-induced atherosclerosis in experimental rats	Journal of biochemical and molecular toxicology	Immunomodulatory and Antioxidant	Withaferin-A (WA)	Sprague-Dawley male rats	10 mg/kg bw	WA as well as LS treatments significantly decreased these parameters restored the antioxidant status, and reduced lipid peroxidation ($p < 0.05$). Histopathological studies revealed that WA and LS reduced the hepatic fat and aortic plaque. WA reduced apoptosis via augmentation of phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway; increased B-cell lymphoma 2 and inhibited Bcl-2 associated X-protein proapoptotic proteins; TNF receptor superfamily member 6, Bim, caspase-3, and -9; demonstrated significant hypolipidemic and anti-inflammatory properties against HCD induced atherosclerosis in rats through regulation of inflammatory mediators and apoptosis via the PI3K/AKT signaling pathway.	Zhang, L., Shi, Y., Yan, M., & Zhang, G. (2022). Modulatory action of withaferin-A on oxidative damage through regulation of inflammatory mediators and apoptosis via PI3K/AKT signaling pathway in high cholesterol-induced atherosclerosis in experimental rats. Journal of biochemical and molecular toxicology, e23154. Advance online publication. https://doi.org/10.1002/jbt.23154
2	Withania somnifera (Ashwagandha) root extract counteract acute and chronic impact of γ-radiation on liver and spleen of rats	Human & experimental toxicology	Immunomodulatory, Antiinflammatory, Radioprotective and Antioxidant	Withania somnifera root extract	Adult Male Swiss White Albino rats	300 mg/kg. wt/day	The administration of ashwagandha root extract might reduce inflammation and protect tissue structure of liver and spleen after exposure to γ-radiation. Ashwagandha reduce ROS released in abundant because of γ-radiation exposure, prevents exacerbation of oxidative situation which ended by fall down of antioxidant system and accumulation of toxic organic radicals (lipid peroxidation products). Subsequently, modulate the inflammatory cascades of oxidative stress that not preceded forward echoes on the balance between expression of MMP (−2 and −9) and TIMP-1 expression.The histopathological investigation of spleen and liver tissues confirmed this ameliorating action of Ag counter to γ-radiation hazards. It could be suggested that Ashwagandha could exerts radio-protective influences because of its antioxidants and anti-inflammatory capabilities.	Azab, K. S., Maarouf, R. E., Abdel-Rafei, M. K., El Bakary, N. M., & Thabet, N. M. (2022). Withania somnifera (Ashwagandha) root extract counteract acute and chronic impact of γ-radiation on liver and spleen of rats. Human & experimental toxicology, 41, 9603271221106344. https://doi.org/10.1177/09603271221106344
3	Withanolides from Withania somnifera Ameliorate Neutrophil Infiltration in Endotoxin-Induced Peritonitis by Regulating Oxidative Stress and Inflammatory Cytokines	Planta medica	Immunomodulatory and Antioxidant	Withania somnifera root extract	Invitro - HepG2, THP-1 Invivo - Specific pathogen-free male C57BL/6 mice	Invitro - 1–100 μg/mL Invivo - 175 mg/kg, and 525 mg/kg.	In the present investigation, we tested for the anti-inflammatory and antioxidant capability of a natural product, WS, using a mouse model of endotoxininduced peritonitis in vivo. We identified that WS can inhibit neutrophil migration into the peritoneal cavity and decreases proinflammatory cytokine levels in PF. Further, WS could regulate endotoxin-induced oxidative stress parameters (GPX, GSH, GSSG, SOD, MDA, and MPO) in liver homogenates. In vitro, WS was capable of modulating antioxidant markers and cytokine levels in an NF-κB-dependent manner. Collectively, these results indicate the immunomodulatory and antioxidant capabilities of a natural medicine, WS, and could provide beneficial effects as a complementary remedy	Balkrishna, A., Solleti, S. K., Singh, H., Sharma, N., & Varshney, A. (2022). Withanolides from Withania somnifera Ameliorate Neutrophil Infiltration in Endotoxin-Induced Peritonitis by Regulating Oxidative Stress and Inflammatory Cytokines. Planta medica, 88(6), 466–478. https://doi.org/10.1055/a-1438-2816
4	The growth performance, antioxidant and immune responses, and disease resistance of Litopenaeus vannamei fed on diets supplemented with Indian ginseng (Withania somnifera)	Fish & shellfish immunology	Immunomodulatory and Antioxidant	Withania somnifera aqueous extract	White-leg shrimp (Litopenaeus vannamei)	0 (control), 0.5, 1.0, and 2.0 g/kg	Compared to the control group, the WSAE-fed L. vannamei had significantly higher villi length, villi width, and absorption area in the treatment of 2.0 g/kg feed. The mRNA expression levels of cMn-SOD, CAT, and GPx genes were linearly and quadratically upregulated in the hepatopancreas of L. vannamei fed on WSAE-enriched diets (especially in the 2.0 g/kg feed treatment), while their lowest levels were significantly observed in the control group. On the other hand, malondialdehyde levels were significantly decreased in WSAE-supplemented shrimp groups, and its highest levels were observed in animals fed on the control diet. Taken together, the results obtained herein indicate that inclusion of WSAE in diets of L. vannamei effectively enhanced the growth, antioxidant biomarkers, immune response, and resistance to the V. harveyi infection, particularly at the treatment of 2.0 g/kg feed.	Abdel-Tawwab, M., Abo Selema, T., Khalil, R. H., El-Sabbagh, N., Eldessouki, E., Fawzy, R. M., & Abd El-Naby, A. S. (2022). The growth performance, antioxidant and immune responses, and disease resistance of Litopenaeus vannamei fed on diets supplemented with Indian ginseng (Withania somnifera). Fish & shellfish immunology, S1050-4648(22)00450-8. Advance online publication. https://doi.org/10.1016/j.fsi.2022.07.061
5	Pharmacological and ameliorative effects of Withania somnifera against cadmium chloride-induced oxidative stress and immune suppression in Nile tilapia, Oreochromis niloticus	Environmental science and pollution research international	Immunomodulatory Activity	Withania somnifera aqueous extract	Nile tilapia, Oreochromis niloticus	1.0, 2.0, and 3.0 mL/kg	The results revealed that groups exposed to cadmium chloride toxicity and co-supplemented with dietary aqueous extract of W. somnifera at high doses showed significant ameliorative effects in hemogram parameters, total protein, globulin, IgM, and lysozyme against cadmium chloride-induced toxicity compared to the control group and the group exposed to a sublethal dose of cadmium chloride without co-suplemntation of W. somnifera. The results showed also that groups supplemented orally with W. somnifera at high doses have higher antioxidant activities of CAT and SOD and reduction of MDA formation. Levels of gene expressions of GST in the liver were higher in W. somnifera extract-supplemented groups more than those in the group exposed to cadmium chloride-induced toxicity without W. somnifera supplementation. In addition, the results revealed improved RPS with the dietary supply of W. somnifera extract in high doses. In conclusion, this study showed that the dietary supplementation of W. somnifera extract to diets of O. niloticus could be suggested as an effective way to overcome cadmium chloride-induced toxicity because it improves blood parameters and antioxidants, and it can be used as an immunostimulant against the invading bacterial pathogens.	El-Sabbagh, N. M., Khalil, R. H., Khallaf, M. M., Shakweer, M. S., Ghetas, H. A., & Atallah, M. M. (2022). Pharmacological and ameliorative effects of Withania somnifera against cadmium chloride-induced oxidative stress and immune suppression in Nile tilapia, Oreochromis niloticus. Environmental science and pollution research international, 29(5), 6777–6792. https://doi.org/10.1007/s11356-021-15630-7
6	Metabolomic Profiling and Immunomodulatory Activity of a Polyherbal Combination in Cyclophosphamide-Induced Immunosuppressed Mice	Frontiers in pharmacology	Immunomodulatory Activity	A polyherbal combination containing Phyllanthus emblica L., Piper nigrum L., Withania somnifera (L.) Dunal, and Tinospora cordifolia (Willd.) Miers.	BALB/c mice	Withania somnifera - 10 mg, 455mg, 900mg	Treatment with the polyherbal combination of different doses in cyclophosphamide-induced immunosuppressed mice significantly ($p < 0.01$) enhanced the subsets of immune cells such as natural killer (NK) cells (60%), B cells (18%), CD4 cells (14%), and CD8 cells (7%). The characterized polyherbal combination exhibited potent immunomodulatory activity, which can be further explored clinically for its therapeutic applicability.	Zahiruddin, S., Parveen, A., Khan, W., Ibrahim, M., Want, M. Y., Parveen, R., & Ahmad, S. (2022). Metabolomic Profiling and Immunomodulatory Activity of a Polyherbal Combination in Cyclophosphamide-Induced Immunosuppressed Mice. Frontiers in pharmacology, 12, 647244. https://doi.org/10.3389/fphar.2021.647244

7	Neuroprotective effects of Withania somnifera in the SH-SY5Y Parkinson cell model	Heliyon	Neuroprotective Activity	Withania somnifera root extract (KSM-66)	SH-SY5Y cells	800 mg of Ashwagandha root extract powder (KSM-66)	KSM-66 also modulated oxidative response proteins: peroxiredoxin-I, VGF and vimentin proteins upon 6-OHDA pre/post treatments. In addition, the extract controlled redox regulation via Sglutathionylation. Pre-treatment of SH-SY5Y cells with KSM-66 decreased protein-glutathionylation levels in the cells treated with 6-OHDA. The rescue of mitochondria with 0.5 mg/ml KSM-66 extract showed an increase in ATP levels. These findings suggest that W. somnifera root extract acts as a neuroprotectant, thereby introducing a potential agent for the treatment or prevention of neurodegenerative diseases.	Wongtrakul, J., Thongtan, T., Kumrapich, B., Saisawang, C., & Ketterman, A. J. (2021). Neuroprotective effects of Withania somnifera in the SH-SY5Y Parkinson cell model. Heliyon, 7(10), e08172. https://doi.org/10.1016/j.heliyon.2021.e08172
8	Ashwagandha (Withania somnifera) root extract attenuates hepatic and cognitive deficits in thioacetamide-induced rat model of hepatic encephalopathy via induction of Nrf2/HO-1 and mitigation of NF-κB/MAPK signaling pathways	Journal of ethnopharmacology	Neuroprotective, Hepatoprotective	Withania somnifera aqueous root extract	Adult female Wistar rats	200 and 400 mg/kg	Our results provided insights into the promising hepato- and neuroprotective effects of ASH, with superiority to 400 mg/kg ASH, to ameliorate HE with its sequential hyperammonemia and liver/brain injuries. This could be attributed to the recorded increase in the spontaneous alternation % and recognition index, antioxidant and anti-inflammatory activities, as well as upregulation of Nrf2 and downregualtion of MAPK signaling pathways.	Khalil, H., Eliwa, H. A., El-Shiekh, R. A., Al-Mokaddem, A. K., Hassan, M., Tawfek, A. M., & El-Maadawy, W. H. (2021). Ashwagandha (Withania somnifera) root extract attenuates hepatic and cognitive deficits in thioacetamide-induced rat model of hepatic encephalopathy via induction of Nrf2/HO-1 and mitigation of NF-κB/MAPK signaling pathways. Journal of ethnopharmacology, 277, 114141. https://doi.org/10.1016/j.jep.2021.114141
9	Elucidation of plasma protein binding, blood partitioning, permeability, CYP phenotyping and CYP inhibition studies of Withanone using validated UPLC method: An active constituent of neuroprotective herb Ashwagandha	Journal of ethnopharmacology	Neuroprotective Activity	Withanone from Withania somnifera extract	Healthy young male SD rats	5 and 20 µg/mL	The in vitro results of pH-dependent stability, plasma stability, permeability, PPB, blood partitioning, microsomal stability, CYP phenotyping, and CYP inhibition studies demonstrated that WN could be a better phytochemical for neurological disorders.	Singh, S. K., Valicherla, G. R., Bikkasani, A. K., Cheruvu, S. H., Hossain, Z., Taneja, I., Ahmad, H., Raju, K., Sangwan, N. S., Singh, S. K., Dwivedi, A. K., Wahajuddin, M., & Gayen, J. R. (2021). Elucidation of plasma protein binding, blood partitioning, permeability, CYP phenotyping and CYP inhibition studies of Withanone using validated UPLC method: An active constituent of neuroprotective herb Ashwagandha. Journal of ethnopharmacology, 270, 113819. https://doi.org/10.1016/j.jep.2021.113819
10	Neuroprotective Effects of Withania somnifera on 4-Hydroxynonenal Induced Cell Death in Human Neuroblastoma SH-SY5Y Cells Through ROS Inhibition and Apoptotic Mitochondrial Pathway	Neurochemical research	Neuroprotective Activity	Withania somnifera extract	Human neuroblastoma (SH-SY5Y) cell line	12.5, 25, and 50 µg/ml	Pre-exposure to WS resulted in a strong inhibition of 24, 55 and 83% in malondialdehyde (MDA) level; 5, 27 and 60% in glutathione (GSH) level; 12, 36 and 68% in catalase activity; 11, 33 and 67% in LDH leakage; and 40, 80 and 120% in cellular LDH activity at 12.5, 25, and 50 µg/ml, respectively, induced by 50 µM HNE in SH-SY5Y cells. The HNE-mediated cellular changes (cell shrinkage, rounded bodies, and inhibition of outgrowth) and increased caspase-3 activity were also prevented by WS. The HNE-induced upregulation of proapoptotic markers (p53, caspase-3, and -9, and Bax) and downregulation of antiapoptotic marker Bcl-2 genes were also blocked by pretreatment with WS. Altogether, our findings indicate that WS possesses a protective potential against HNE-induced neurotoxicity.	Siddiqui, M. A., Farshori, N. N., Al-Oqail, M. M., Pant, A. B., & Al-Khedhairy, A. A. (2021). Neuroprotective Effects of Withania somnifera on 4-Hydroxynonenal Induced Cell Death in Human Neuroblastoma SH-SY5Y Cells Through ROS Inhibition and Apoptotic Mitochondrial Pathway. *Neurochemical research*, *46* (2), 171–182. https://doi.org/10.1007/s11064-020-03146-4
11	Assessment of Chemopreventive Potential of the Plant Extracts against Liver Cancer Using HepG2 Cell Line	Molecules (Basel, Switzerland)	Hepatoprotective and Anti-cancer activity	Withania somnifera extract	HepG-2 cell lines	10 and 320 µg/mL	In conclusion, among the three plant extracts, W. somnifera leaves extract exhibited the highest carcinogenic cell inhibition via apoptosis when induced to the HepG2 cell line in vitro, while the C. sinensis and V. vinifera extracts showed lesser inhibition activity in DPPH and MTT assay, hence, were not further subjected to cell line study. However, methanolic extract of W. somnifera, which is rich in phyto-constituents, has shown enhancing apoptosis of the HepG2 cell line. As a result, there is upregulation of BAX genes, inducing apoptosis, and downregulation of BCl2 gene in the HepG2 cell line. This study exhibited the inhibition of the HepG2 cell proliferation due to the upregulation of the BAX gene and decreased BCl2 gene expression. Therefore, W. somnifera has proven its potential anticancer properties in vitro for HepG2 cells and also for various types of cancers in other studies.	Venkatachalapathy, D., Shivamallu, C., Prasad, S. K., Thangaraj Saradha, G., Rudrapathy, P., Amachawadi, R. G., Patil, S. S., Syed, A., Elgorban, A. M., Bahkali, A. H., Kollur, S. P., & Basalingappa, K. M. (2021). Assessment of Chemopreventive Potential of the Plant Extracts against Liver Cancer Using HepG2 Cell Line. Molecules (Basel, Switzerland), 26(15), 4593. https://doi.org/10.3390/molecules26154593
12	Effect of Withania somnifera hydroalcoholic extract and other dietary interventions in improving muscle strength in aging rats	Journal of Ayurveda and integrative medicine	Immunomodulatory Activity and Anti inflammatory activity	Withania somnifera extract	Male Sprague Dawley rats	WSE, 500 mg/kg	All treatments successfully attenuated aging-elevated glucose, CRP, IL-6, TNF-α, AMPK, malondialdehyde, and Bax levels. A significant restoration of the aging-depleted total protein levels, glutathione, superoxide dismutase, catalase, and Bcl-2 was noted in the treatment groups. An increase in grip strength and greater biceps mass with all treatments indicated regaining of the frail aging muscle's strength and functionality. The WSE + protein treatment elicited the best results among all treatment groups to optimize muscle strength.	Panda, V., Deshmukh, A., Hare, A., Singh, S., Hingorani, L., & Sudhamani, S. (2021). Effect of Withania somnifera hydroalcoholic extract and other dietary interventions in improving muscle strength in aging rats. Journal of Ayurveda and integrative medicine, 12(4), 623–632. https://doi.org/10.1016/j.jaim.2021.06.001
13	Anti-arthritic activity of Ricinus communis L. and Withania somnifera L. extracts in adjuvant-induced arthritic rats via modulating inflammatory mediators and subsiding oxidative stress	Iranian journal of basic medical sciences	Anti inflammatory activity	RCE (R. communis extract) and WSE (W. somnifera extract)	Wistar rats	250 mg/kg/day and 500 mg/kg/day	RCE (R. communis extract) and WSE (W. somnifera extract) demonstrated a reduction in paw swelling, arthritic score, and restoration of body weight and organ indices. Hematological parameters, serum inflammatory markers such as CRP and RF, and liver function markers of arthritic rats were significantly ($P<0.01$) ameliorated with RCE and WSE treatment. Both plants persuasively down-regulated IL-1β, IL-6, IL-17a, TNF-α, and RANKL and up-regulated IL-4, INF-γ, and OPG relative expression as well as alleviating hepatic oxidative stress parameters. Histopathological and radiological findings revealed a marked reduction in tissue inflammation and bone erosion in extracts treated groups.	Hussain, A., Aslam, B., Muhammad, F., Faisal, M. N., Kousar, S., Mushtaq, A., & Bari, M. U. (2021). Anti-arthritic activity of Ricinus communis L. and Withania somnifera L. extracts in adjuvant-induced arthritic rats via modulating inflammatory mediators and subsiding oxidative stress. Iranian journal of basic medical sciences, 24(7), 951–961. https://doi.org/10.22038/ijbms.2021.55145.12355
14	Evaluation of In Vitro Immunomodulatory Activity of Withania somnifera Roots on Human Neutrophils	Applied biochemistry and biotechnology	Immunomodulatory Activity	Withania somnifera root extract	Human blood	10 µg/ml, 20 µg/ml, 40 µg/ml, 100 µg/ml and undiluted (1000 µg/ml)	As observed in the present study, the isolated compound of Withania somnifera increases the phagocytic function of human neutrophils to a large extent as compared with control indicating its immune-stimulating effect.	Arora, A., Solanki, P., & Kumar, D. (2021). Evaluation of In Vitro Immunomodulatory Activity of Withania somnifera Roots on Human Neutrophils. Applied biochemistry and biotechnology, 193(6), 1631–1638. https://doi.org/10.1007/s12010-021-03518-8

15	Ameliorating effect of Withania somnifera root extract in Escherichia coli-infected broilers.	Poultry science	Immunomodulatory Activity	Withania somnifera root extract	Broiler chicks	20% aqueous WSR root extract	Withania somnifera root extract supplementation produced 31.48 and 34.38% protection in the gross and histopathological le- sions in E. coli infected chicks, respectively. It is concluded that supplementation of 20% WSR extract @ 20 ml/L of water caused a reduction in the severity, mortality, and recovery period of E. coli infection and enhanced the humoral and cellular immune responses suggesting its protective effect on limiting the pathology of E. coli infection in broiler chickens.	Kumari, M., Gupta, R. P., Lather, D., & Bagri, P. (2020). Ameliorating effect of Withania somnifera root extract in Escherichia coli-infected broilers. *Poultry science* , *99* (4), 1875–1887. https://doi.org/10.1016/j.psj.2019.11.022
16	Effect of Withania Somnifera on the antioxidant and neurotransmitter status in sleep deprivation induced Wistar rats	Bioinformation	Neuroprotective Activity	Withania somnifera root extract	Wistar rats	400mg/kg	The group treated with W. somnifera root extract significantly reduced the free radical production and lipid peroxidation with simultaneous increase in the level of antioxidant enzymes compared to the untreated group. Also in our study the concentration of dopamine and serotonin was found to be significantly reduced ($p < 0.05$) in sleep deprived (SD) and large platform control group when compared to cage control group. Whereas the group treated with W. somnifera (400mg/kg b.wt) increased the neurotransmitter levels significantly. Withania somnifera proved to be an effective therapeutic agent by maintaining the antioxidant status and neurotransmitter levels	Suganya, K., Kayalvizhi, E., Yuvaraj, R., Chandrasekar, M., Kavitha, U., & Konakanchi Suresh, K. (2020). Effect of Withania Somnifera on the antioxidant and neurotransmitter status in sleep deprivation induced Wistar rats. Bioinformation, 16(8), 631–637. https://doi.org/10.6026/97320630016631
17	Adaptogenic and Immunomodulatory Activity of Ashwagandha Root Extract: An Experimental Study in an Equine Model	Frontiers in veterinary science	Immunomodulatory Activity	Withania somnifera root extract (KSM-66)	Horses	2.5g, 5g, 10g per day	Over the 21 days, a statistically significant ($p < 0.05$) increase in total erythrocyte count, total leucocyte count, hemoglobin content, lymphocyte percentage, reduced glutathione, and superoxide dismutase activities was observed. A statistically significant ($p < 0.05$) decrease in cortisol, epinephrine, glucose, triglycerides, creatinine, IL-6, alanine aminotransferase, and aspartate aminotransferase was observed in the Ashwagandha treated groups (G2, G3, and G4) when compared to the control group (G1). The results suggest that Ashwagandha root extract has potent hemopoietic, antioxidant, adaptogenic, and immune-stimulant properties	Priyanka, G., Anil Kumar, B., Lakshman, M., Manvitha, V., & Kala Kumar, B. (2020). Adaptogenic and Immunomodulatory Activity of Ashwagandha Root Extract: An Experimental Study in an Equine Model. Frontiers in veterinary science, 7, 541112. https://doi.org/10.3389/fvets.2020.541112
18	Dietary supplementation with Withania somnifera root powder ameliorates experimentally induced Infectious Bursal Disease in chicken	Tropical animal health and production	Immunomodulatory Activity	Withania somnifera root extract	White Leghorn chicks	Withania Somnifera Root powder at 1% of the feed.	Dietary supplementation with the root powder improved erythrocytic indices, biochemical parameters, bursal weight index, and lymphocyte stimulation indices, and reduced histopathological insult in the infected birds. Viral load decreased to less than onefourth in the birds receiving dietary supplementation with Withania somnifera root powder	Ganguly, B., Mrigesh, M., Chauhan, P., & Rastogi, S. K. (2020). Dietary supplementation with Withania somnifera root powder ameliorates experimentally induced Infectious Bursal Disease in chicken. Tropical animal health and production, 52(3), 1195–1206. https://doi.org/10.1007/s11250-019-02104-9
19	Evaluation of Ashwagandha (Withania Somnifera) and its Extract to Protecting he Liver From Damage,	INTERNATIONAL JOURNAL OF ENGINEERING RESEARCH & TECHNOLOGY (IJERT)	Hepatoprotective	Ashwagandha (Withania somnifera) roots	Healthy male albino mice	Group 1 - Control, Group 2 -500 mg/kg/body weight, Group 3 - 1000 mg/kg/bw, Group 4 - 2000 mg/kg/bw	Ashwagandha roots extract had contained rich amounts from the natural antioxidant activity which can keep safe the liver from the harm caused by free radicals which came from as the result of CCl4 metabolism. Hence, Ashwagandha root extracts confirmed that the protection of the rats' liver CCl4- induced hepatotoxicity.	Ebtihal Y. Khojah , Dalia A. Mohamed Hafez, 2020, Evaluation of Ashwagandha (Withania Somnifera) and its Extract to Protecting he Liver From Damage, INTERNATIONAL JOURNAL OF ENGINEERING RESEARCH & TECHNOLOGY (IJERT) Volume 09, Issue 02 (February 2020),
20	Effect of Withania somnifer on CD38 expression on CD8+ T lymphocytes among patients of HIV infection	Clin Immunol	Immunomodulatory Activity	Withania somnifera root extract	Human peripheral blood mononuclear cells ere reactive for HIV-1 gp120 and HIV-1 gp41 anti-bodies	N/A	The results showed that there is decline in CD38 expression on cytotoxic T lymphocytes on treatment with Withania somnifer	Maurya SP, Das BK, Singh R, Tyagi S. Effect of Withania somnifer on CD38 expression on CD8+ T lymphocytes among patients of HIV infection. Clin Immunol. 2019 Jun;203:122-124. doi: 10.1016/j.clim.2019.04.003. Epub 2019 Apr 17.
21	In vivo, Extract from Withania somnifera Root Ameliorates Arthritis via Regulation of Key Immune Mediators of Inflammation in Experimental Model of Arthritis.	Anti-inflammatory & anti-allergy agents in medicinal chemistry	Immunomodulatory and Anti-inflammatory activity	Withania somnifera root extract	Male Wistar albino rats	WSAq at 300 mg/kg body weight	Oral administration of WSAq at a dose of 300mg/kg.wt. (WSAq300) appreciably attenuated the production of these pro inflammatory cytokines. Treatment with WSAq300 strongly ameliorates all these ROS pa- rameters significantly to near normal. All the results positively correlated with histological analysis of the joint tissue of CIA rats that demonstrated a reduction in oxidative stress, inflammation and joint damage in WSAq300 treated rats, suggesting that WSAq300 is effective in suppressing the development of RA	Khan, M. A., Ahmed, R. S., Chandra, N., Arora, V. K., & Ali, A. (2019). In vivo, Extract from Withania somnifera Root Ameliorates Arthritis via Regulation of Key Immune Mediators of Inflammation in Experimental Model of Arthritis. Anti-inflammatory & anti-allergy agents in medicinal chemistry, 18(1), 55–70. https://doi.org/10.2174/1871523017666181116092934
22	Withaferin A Improves Nonalcoholic Steatohepatitis in Mice	The Journal of Pharmacology and Experimental therapeutics	Hepatoprotective	Withaferin A – Withania somnifera	Mice - C57BL/6N males	WA at doses of 1, 2.5, and 5 mg/kg	WA prevented and therapeutically improved liver injury in both models, thus demonstrating a hepatoprotective effect	Patel DP, Yan T, Kim D, et al. Withaferin A Improves Nonalcoholic Steatohepatitis in Mice. J Pharmacol Exp Ther. 2019;371(2):360–374. doi:10.1124/jpet.119.256792
23	Effect of Withania somnifera powder against lead- induced toxicity in albino rats	Journal of Pharmacognosy and Phytochemistry	Hepatoprotective	Withania somnifera powder	Wistar Albino rats	500 mg/kg/body weight	Withania somnifera extract significantly decreased the elevated level of AST, ALT, ALP, BUN and serum creatinine.	Sushma Lalita Baxla, Ravuri Halley Gora and Priscilla Kerketta. Effect of Withania somnifera powder against lead- induced toxicity in albino rats. 2019; 8(5S): 327-329.
24	Exploration of immunomodulatory and protective effect of Withania somnifera on trace metal oxide (zinc oxide nanoparticles) induced toxicity in Balb/c mice.	Molecular biology reports	Immunomodulatory Activity	Withania somnifera extract	Balb/c mice	Withania somnifera 3.5 mg/ dose/animal; Withaferin A 2.0 mg/kg b.w	Toxicity of ZnO NP was reduced in presence of WS and WA with decreased *TLR6* over expression and restoration of phagocytic activities. Overall, the results suggest that WA steroidal lactone derived from Withania somnifera has immunomodulatory activities.	Kumar, J., Mitra, M. D., Hussain, A., & Kaul, G. (2019). Exploration of immunomodulatory and protective effect of Withania somnifera on trace metal oxide (zinc oxide nanoparticles) induced toxicity in Balb/c mice. *Molecular biology reports* , *46* (2), 2447–2459. https://doi.org/10.1007/s11033-019-04705-x
25	Ameliorating effects of dietary mixture of Withania somnifera root extract and vitamin C in Labeo rohita against low pH and waterborne iron stresses	Fish & shellfish immunology	Immunomodulatory Activity	Withania somnifera root extract	Labeo rohita fingerlings	W. somnifera root extract and vitamin C @ 1:1 w/w at the rate of Control - 0.0%, T1 - 0.01%, T2 - 0.1% and T3 - 1.0% respectively	The result showed a significant ($p < 0.05$) increase in haemoglobin, haematocrit, total RBC count, total WBC count, NBT, lysozyme activity, total immunoglobulin and total protein whereas a significant ($p < 0.05$) decrease in glucose, ALP, SGPT and SGOT level compared to control. in T3 diet. The study demonstrates that inclusion of 1.0% Withania somnifera (Ashwagandha) root extract and dietary L-ascorbic acid (vitamin C) combination in diet have a stimulatory effect on immune response and reduces the effect of multiple stresses (i.e., low pH and waterborne iron toxicity) in L. rohita fingerlings.	Laltlanmawia, C., Saha, R. K., Saha, H., & Biswas, P. (2019). Ameliorating effects of dietary mixture of Withania somnifera root extract and vitamin C in Labeo rohita against low pH and waterborne iron stresses. *Fish & shellfish immunology* , *88* , 170–178. https://doi.org/10.1016/j.fsi.2018.09.008

26	Withania somnifera (L.) Dunal ameliorates neurodegeneration and cognitive impairments associated with systemic inflammation	BMC complementary and alternative medicine	Neuroprotective Activity	Withania somnifera extract	Wistar strain male albino rats	4 ml/kg/day extract	Orally administered ASH-WEX significantly suppressed the cognitive and motor-coordination impairments in rats. On the molecular basis, ASH-WEX supplementation also regulated the expression of various proteins involved in synaptic plasticity and neuronal cell survival. This extensive study using in vivo and in vitro model systems provides first ever pre-clinical evidence that ASH-WEX can be used as a promising natural therapeutic remedial for the prevention of neurodegeneration and cognitive impairments associated with peripheral inflammation and neuroinflammation	Gupta, M., & Kaur, G. (2019). Withania somnifera (L.) Dunal ameliorates neurodegeneration and cognitive impairments associated with systemic inflammation. *BMC complementary and alternative medicine* , *19* (1), 217. https://doi.org/10.1186/s12906-019-2635-0
27	Withania somnifera root powder protects againist post-traumatic stress disorder-induced memory impairment	Molecular biology reports	Neuroprotective Activity	Withania somnifera root extract	Male Wistar rats	500 mg/kg	The result showed that PTSD resulted in short- and long- term memory impairments. Administration of WS prevented this impairment of memory induced by PTSD. Furthermore, WS prevented PTSD induced changes in oxidative stress biomarker in the hippocampus. For quality assessment, the methanolic extract for WS was subjected to UHPLC analysis. A calibration curve for isowithanone as a marker compound was constructed. WS roots content of isowithanone was found to be 0.23% (w/w). In conclusion, WS administration prevented PTSD induced memory impairment probably through preserving changes in antioxidant mechanisms in the hippocampus.	Alzoubi, K. H., Al Hilo, A. S., Al-Balas, Q. A., El-Salem, K., El-Elimat, T., & Alali, F. Q. (2019). Withania somnifera root powder protects againist post-traumatic stress disorder-induced memory impairment. Molecular biology reports, 46(5), 4709–4715. https://doi.org/10.1007/s11033-019-04915-3
28	Neuroprotective effects of Withania somnifera in BPA induced-cognitive dysfunction and oxidative stress in mice	Behavioral and brain functions : BBF	Neuroprotective Activity	Withania somnifera extract	Male Swiss albino mice	100 mg/kg	The study revealed that administration of Ws alleviated the behavioral deficits induced by BPA. Alongside, Ws treatment reinstated the number of NMDA receptors in hippocampus region and showed anti-oxidative property while ameliorating the endogenous anti-oxidant level in the brain. These findings suggest that Ws significantly ameliorates the level of BPA intoxicated oxidative stress thereby potentially treating cognitive dysfunction which acts as the primary symptom in a number of neurodegenerative diseases.	Birla, H., Keswani, C., Rai, S. N., Singh, S. S., Zahra, W., Dilnashin, H., Rathore, A. S., & Singh, S. P. (2019). Neuroprotective effects of Withania somnifera in BPA induced-cognitive dysfunction and oxidative stress in mice. Behavioral and brain functions : BBF, 15(1), 9. https://doi.org/10.1186/s12993-019-0160-4
29	Withania somnifera modulates cancer cachexia associated inflammatory cytokines and cell death in leukaemic THP-1 cells and peripheral blood mononuclear cells (PBMC's)	BMC complementary and alternative medicine	Immunomodulatory and Antioxidant	Withania somnifera root extract	Human PBMCs and THP-1 cells	0.05, 0.2 - 0.4 mg/ml	WRE proved to effectively modulate antioxidant activity, inflammatory cytokines and cell death. In THP-1 cells, WRE decreased pro-inflammatory cytokine levels, which may alleviate cancer cachexia and excessive leukaemic cell growth in both PBMCs and THP-1 cells.	Naidoo, D. B., Chuturgoon, A. A., Phulukdaree, A., Guruprasad, K. P., Satyamoorthy, K., & Sewram, V. (2018). Withania somnifera modulates cancer cachexia associated inflammatory cytokines and cell death in leukaemic THP-1 cells and peripheral blood mononuclear cells (PBMC's). *BMC complementary and alternative medicine* , *18* (1), 126. https://doi.org/10.1186/s12906-018-2192-y
30	Ashwagandha root extract exerts anti-inflammatory effects in HaCaT cells by inhibiting the MAPK/NF-κB pathways and by regulating cytokines.	International journal of molecular medicine	Immunomodulatory and Anti-inflammatory activity	Withania somnifera root extract	HaCaT cells - Human keratinocytes and invivo-mice model	20 µl; 10 mg/ml	The results indicated that ASH-WEX significantly inhibited mRNA expression of inflammatory cytokines, including interleukin (IL)-8, IL-6, tumor necrosis factor (TNF-α), IL-1β and IL-12, and promoted the mRNA expression of the anti-inflammatory cytokine transforming growth factor (TGF)-β1 in HaCaT cells. SH-WEX treatment increased the mRNA expression of the anti-inflammatory cytokine TGF-β1 and decreased the mRNA expression of the pro-inflammatory cytokine TNF-α in vivo.	Sikandan, A., Shinomiya, T., & Nagahara, Y. (2018). Ashwagandha root extract exerts anti-inflammatory effects in HaCaT cells by inhibiting the MAPK/NF-κB pathways and by regulating cytokines. *International journal of molecular medicine* , *42* (1), 425–434. https://doi.org/10.3892/ijmm.2018.3608
31	Withania somnifera as a Potential Anxiolytic and Anti-inflammatory Candidate Against Systemic Lipopolysaccharide-Induced Neuroinflammation.	Neuromolecular medicine	Immunomodulatory and Anti-inflammatory activity	Withania somnifera extract	Male Wistar albino rats	ASH - WEX 140mg/kg/body weight	Administration of ASH-WEX for 8 weeks significantly ameliorated the anxiety like behavior from Elevated plus maze test. Suppression of reactive gliosis, inflammatory cytokines production like TNF-α, IL-1β, IL-6, and expression of nitro-oxidative stress enzymes like iNOS, COX2, NOX2 etc were observed in ASH-WEX-treated animals. NFκB, P38, and JNK MAPKs pathways analysis showed their involvement in inflammation suppression which was further confirmed by inhibitor studies.	Gupta, M., & Kaur, G. (2018). Withania somnifera as a Potential Anxiolytic and Anti-inflammatory Candidate Against Systemic Lipopolysaccharide-Induced Neuroinflammation. *Neuromolecular medicine* , *20* (3), 343–362. https://doi.org/10.1007/s12017-018-8497-7
32	Withaferin A inhibits apoptosis via activated Akt-mediated inhibition of oxidative stress	Life Sciences	Cardioprotective Activity	Commercial Withaferin A	Sprague-Dawley rat hearts	Withaferin A (WFA)	WFA enhanced H9c2 cells survival ability against simulated ischemia/reperfusion (SI/R) or hydrogen peroxide (H2O2)-induced cell apoptosis. In addition, the enhanced oxidative stress induced by SI/R was inhibited by WFA. Additional results suggest that WFA successfully inhibited H2O2 induced up- regulation of SOD2, SOD3, and Prdx-1, ameliorated cardiomyocyte caspase-3 activity via an Akt dependent manner	Yan Z, Guo R, Gan L, Lau WB, Cao X, Zhao J, Ma X, Christopher TA, Lopez BL, Wang Y.Life Sci. 2018 Oct 15;Withaferin A inhibits apoptosis via activated Akt-mediated inhibition of oxidative stress. 211:91-101. doi: 10.1016/j.lfs.2018.09.020. Epub 2018 Sep 10.
33	Effect of dietary supplementation of Ashwagandha (Withania somnifera) and Kalmegh (Andrographis paniculata) on growth performance and immune status in broilers	International Journal of Agriculture Sciences	Hepatoprotective	Withania somnifera root powder	Broilers	5 gm per kg feed	5 gm Ashwagandha (Withania somnifera) and 2 gm Kalmegh (Andrographis paniculata) powder per kg feed separately and combinely improved growth and immune status in broilers	Dhenge S.A., et al., (2018) Effect of Dietary Supplementation of Ashwagandha (Withania somnifera) and Kalmegh (Andrographis paniculata) on Growth Performance and Immune Status in Broilers. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 10, Issue 24, pp.- 7634-7636.
34	Hepatoprotective effects of Allium sativum and Withania somnifera on ochratoxin A-induced toxicity in rats	Journal of Pharmacognosy and Phytochemistry	Hepatoprotective	Withania somnifera root extract	Male Wistar rats	100mg/kg/body weig	Withania somnifera root extract can prevent OTA-induced hepatotoxicity in rats through its antioxidant activity.	Gengareddy Jamuna, Anil Kumar Sharma, Ayyasamy Manimaran and Palanisamy Sankar. Hepatoprotective effects of Allium sativum and Withania somnifera on ochratoxin A-induced toxicity in rats. Journal of Pharmacognosy and Phytochemistry. 2018; 7(3): 2675-2680.
35	Antihyperalgesic effects of ashwagandha (Withania somnifera root extract) in rat models of postoperative and neuropathic pain	Inflammopharmacology	Analgesic and Anti-inflammatory Activity	Withania somnifera root extract	Male Sprague–Dawley (SD) rats	30, 100 and 300 mg/kg	Mechanical withdrawal threshold (MWT) significantly increased 6 and 24 h after PI in rats receiving W. somnifera root extracts (100 and 300 mg/kg). Furthermore, the number of 22-27-kHz USV, which are a distress response, was significantly reduced at 6 and 24 h after PI in W. somnifera-treated rats (100 and 300 mg/kg). SNI-induced hyperalgesia and cytokine levels were significantly alleviated after treating with W. somnifera root extracts (100 and 300 mg/kg) for 15 continuous days. The main active compound, withaferin A, from the W. somnifera root extract has shown the CC chemokine family Receptor 2 (CCR2) antagonistic effects on monocyte chemoattractant protein-1 (MCP-1)-induced Ca2+ response in CCR2 stable cell line. These results indicate that W. somnifera root extract has a potential analgesic effect in rat models for both postoperative and neuropathic pain and shows potential as a drug or supplement for the treatment of pain.	Lim, D. W., Kim, J. G., Lim, E. Y., & Kim, Y. T. (2018). Antihyperalgesic effects of ashwagandha (Withania somnifera root extract) in rat models of postoperative and neuropathic pain. Inflammopharmacology, 26(1), 207–215. https://doi.org/10.1007/s10787-017-0389-1

36	Withania somnifera root confers protective and immunotherapeutic effects against Aeromonas hydrophila infection in Nile tilapia (Oreochromis niloticus).	Fish & shellfish immunology	Immunomodulatory Activity	W. sominfera root powder	Fish - Nile tilapia, *Oreochromis niloticus*	W. sominfera root powder supplemented diets - 2.5% (W 2.5%) and 5% (W 5%)	W. somnifera at 5% augmented neutrophil/macrophage activation, antioxidant activity, as evidenced by increase phagocytic, respiratory burst, bactericidal; and lysozyme activities in Nile tilapia. Moreover, *W. somnifera* at 5 % showed the potential to augment the overall health status of Nile tilapia. It may thus be concluded that WSF is a potent protective and immuno-chemotherapeutic agent that may be beneficial in the management of bacterial diseases and contribute to the enhancement of fish welfare and economic growth for sustainable aquaculture.	Zahran, E., Abd El-Gawad, E. A., & Risha, E. (2018). Dietary Withania somnifera root confers protective and immunotherapeutic effects against Aeromonas hydrophila infection in Nile tilapia (Oreochromis niloticus). *Fish & shellfish immunology* , *80* , 641–650. https://doi.org/10.1016/j.fsi.2018.06.009
37	Multifunctional neuroprotective effect of Withanone, a compound from Withania somnifera roots in alleviating cognitive dysfunction	Cytokine	Neuroprotective Activity	Withania somnifera root extract	Male Wistar rats	10 and 20 mg/kg	WS-2 inhibited the production of Aβ in the brain tissue of the experimental animals Reduction in Aβ improved the cognitive function and reduced degradation of memory indicating to have preventive effect in AD. WS2 at the higher dose level of 10 and 20 mg/kg p.o. improved the latency time/retention time in the passive avoidance and elevated plus maze test in STZ induced memory deficit in animals. Daily oral dose of WS-2 reduced the increased levels of pro-inflammatory mediators TNF-α, IL-1β, IL-6 and MCP-1.	Pandey, A., Bani, S., Dutt, P., Kumar Satti, N., Avtar Suri, K., & Nabi Qazi, G. (2018). Multifunctional neuroprotective effect of Withanone, a compound from Withania somnifera roots in alleviating cognitive dysfunction. Cytokine, 102, 211–221. https://doi.org/10.1016/j.cyto.2017.10.019
38	Protective effects of Withania somnifera extract in SOD1G93A mouse model of amyotrophic lateral sclerosis.	Experimental neurology	Immunomodulatory Activity	Withania somnifera extract	Transgenic mice	N/A	Administration of WS extracts by gavage to mice expressing G93A mutant form of superoxide dismutase (SOD1) resulted in increased longevity, improved motor performance and increased number of motor neurons in lumbar spinal cord. The WS treatment caused substantial reduction in levels of misfolded SOD1whereas it enhanced expression of cellular chaperons in spinal cord of SOD1G93A mice. WS markedly reduced glial activation and prevented phosphorylation of nuclear factor kappaB (NF-κB). The overall immunomodulatory effect of WS was further evidenced by changes in expression of multiple cytokines/chemokines.	Dutta, K., Patel, P., & Julien, J. P. (2018). Protective effects of Withania somnifera extract in SOD1^{G93A} mouse model of amyotrophic lateral sclerosis. *Experimental neurology* , *309* , 193–204. https://doi.org/10.1016/j.expneurol.2018.08.008
39	In Vivo Evaluation of Withania somnifera-Based Indian Traditional Formulation (Amukkara Choornam), Against Chikungunya Virus-Induced Morbidity and Arthralgia	Journal of evidence-based integrative medicine,	Immunomodulatory and Anti-inflammatory activity	Amukkara Choornam (Ashwagandha root powder)	C57BL/6J mice	0.2 mL of 30 mg/kg/dose of amukkara choornam polyherbal formulation twice	Day-wise observation and correlation of viral clearance with joint swelling revealed that swelling totally diminished (day 8 postinfection) even before the complete clearance of CHIKV in the joints that could be attributed to the immunomodulatory characteristics of amukkara choornam.	Jain, J., Narayanan, V., Chaturvedi, S., Pai, S., & Sunil, S. (2018). In Vivo Evaluation of Withania somnifera-Based Indian Traditional Formulation (Amukkara Choornam), Against Chikungunya Virus-Induced Morbidity and Arthralgia. *Journal of evidence-based integrative medicine* , *23* , 2156587218757661. https://doi.org/10.1177/2156587218757661
40	Effect of a Novel Ashwagandha-based Herbomineral Formulation on Pro-inflammatory Cytokines Expression in Mouse Splenocyte Cells: A Potential Immunomodulator.	Pharmacognosy magazine	Immunomodulatory Activity	Polyherbal - TEBEH : zinc chloride , sodium selenate , magnesium gluconate and ashwagandha root extract powder	C57BL/6 male mouse splenocyte cultures	**Polyherbal -** TEBEH : zinc chloride (1.04 mg/mL), sodium selenate (1.195 µg/mL), magnesium gluconate (170.6 mg/mL), and ashwagandha root extract powder (≥ 5% of total withanolides, 200 mg/mL)	MTT data showed TEBEH formulation was found safe up to 10.53 µg/mL. At noncytotoxic concentrations (0.00001053–10.53 µg/mL), TEBEH significantly ($P \leq 0.001$) inhibited the expressions of TNF-α, IL-1β, and MIP-1α in mouse splenocytes as compared with vehicle control.	Trivedi, M. K., Mondal, S. C., Gangwar, M., & Jana, S. (2017). Effect of a Novel Ashwagandha-based Herbomineral Formulation on Pro-inflammatory Cytokines Expression in Mouse Splenocyte Cells: A Potential Immunomodulator. *Pharmacognosy magazine* , *13* (Suppl 1), S90–S94. https://doi.org/10.4103/0973-1296.197709
41	In vitro screening of neuroprotective activity of Indian medicinal plant Withania somnifera	Journal of nutritional science	Neuroprotective and Antioxidant	Withania somnifera extract	SK-N-SH cells	25 µl	Treatment with WS extract significantly protected the human neuroblastoma cell line SK-N-SH against Aβ peptide and acrolein in various cell survival assays. Furthermore, treatment with WS extract significantly reduced the generation of reactive oxygen species in SK-N-SH cells. Finally, our results showed that WS extract is also a potent inhibitor of acetylcholinesterase activity. Thus, our initial findings indicate that WS extract may act as an antioxidant and cholinergic modulator and may have beneficial effects in CCD and AD therapy.	Singh, M., & Ramassamy, C. (2017). In vitro screening of neuroprotective activity of Indian medicinal plant Withania somnifera. Journal of nutritional science, 6, e54. https://doi.org/10.1017/jns.2017.48
42	Hepatoprotective and Cardioprotective effects of Momordica charantia, Gymenma sylvestre and Withania somnifera in animal model of Diabetes mellitus	Journal of Entomology and Zoology Studies	Hepatoprotective	Dried plant roots	Male adult wistar albino rats	0.1 g, 0.2 g and 0.3 g W. somnifera root powder/100 g of feed	W. somnifera at 0.2 g/100 g of feed can be used as dietary supplement to improve the growth, haemato-biochemical response and disease resistance against A. hydrophila for L. rohita fingerlings.	Sharma, A., Chanu, T.I., & Deo, A.D. (2017). Dietary ashwagandha, Withania somnifera (L. dunal) potentiates growth, haemato-biochemical response and disease resistance of Labeo rohita (Hamilton, 1822) against Aeromonas hydrophila infection. Journal of Entomology and Zoology Studies, 5(5): 1113-1119
43	Effect of Aqueous extract of Withania somnifera on some livee biochemical and histopathological parameters in male guinea pigs	Pakistan Journal of Biological sciences	Hepatoprotective	Aqueous extract of Withania somnifera	Male Guinea Pigs	100mg/kg/body weight	The results suggest that Withania somnifera aqueous extract could protect the liver against DM induced oxidative damage.	Al-Awthan, Y.S., M.A. Al-Duais, A.A. Hazeb and W.A. Alril, 2017. Protective role of Achillea biebersteinii pretreatment on dimethoate induced oxidative stress in Guinea pigs liver. Pak. J. Biol. Sci., 20: 403-409.
44	Withania somnifera as a potential candidate to ameliorate high fat diet-induced anxiety and neuroinflammation	Journal of neuroinflammation	Neuroprotective Activity	Withania somnifera extract	Wistar albino young female rats	1 mg/g body weigh	ASH treated rats showed less anxiety levels as compared to HFD animals. At molecular level, ASH ameliorated the HFD-induced reactive gliosis and microgliosis and suppressed the expression of inflammatory markers such as PPARγ, iNOS, MCP-1, TNFα, IL-1β, and IL-6. Further, ASH ameliorated leptin and insulin resistance and prevented HFD-induced apoptosis.W. somnifera may prove to be a potential therapeutic agent to attenuate neuroinflammation associated with obesity and may prevent its co-morbidities.	Kaur, T., & Kaur, G. (2017). Withania somnifera as a potential candidate to ameliorate high fat diet-induced anxiety and neuroinflammation. Journal of neuroinflammation, 14(1), 201. https://doi.org/10.1186/s12974-017-0975-6

45	Withania somnifera Extract Protects Model Neurons from In Vitro Traumatic Injury	Cell transplantation	Neuroprotective Activity	Withania somnifera root extract	Human neuroblastoma SH-SY5Y cells	Pretreated 16 h prior to injury with 0, 4, 20, or 100 µg/mL of W. somnifera root	Treatment with the extract resulted in an increased length of neurites projecting from the neuronal cell body after injury. W. somnifera extract treatment also resulted in reduced cell death in the model neuron TBI system. The cell death factor Bax was involved (its expression was reduced 2-fold by the treatment) and injury-induced reduction in neurite lengths and numbers was reversed by the treatment. This all indicates that W. somnifera root extract was neuroprotective and could have therapeutic potential to target factors involved in secondary injury and long-term sequelae of mild TBI.	Saykally, J. N., Hatic, H., Keeley, K. L., Jain, S. C., Ravindranath, V., & Citron, B. A. (2017). Withania somnifera Extract Protects Model Neurons from In Vitro Traumatic Injury. Cell transplantation, 26(7), 1193–1201. https://doi.org/10.1177/0963689717714320
46	Withania somnifera as a potential anxiolytic and immunomodulatory agent in acute sleep deprived female Wistar rats.	Molecular and cellular biochemistry	Immunomodulatory Activity	Withania somnifera aqueous extract	Female Wistar Albino rats	ASH - WEX 140mg/kg/body weight	VSD animals showed high level of anxiety in elevated plus maze test, which was ameliorated in WSD group. The stress induced expression of inflammatory and immune response markers GFAP, TNFa, IL-6, OX-18 and OX-42 in VSD animals was found to be modulated by ASH-WEX. Further, the stress induced apoptosis was suppressed in WSD group as indicated by expression of NF-jB, AP-1, Bcl-xL and Cytochrome c.	Kaur, T., Singh, H., Mishra, R., Manchanda, S., Gupta, M., Saini, V., Sharma, A., & Kaur, G. (2017). Withania somnifera as a potential anxiolytic and immunomodulatory agent in acute sleep deprived female Wistar rats. *Molecular and cellular biochemistry*, *427* (1-2), 91–101. https://doi.org/10.1007/s11010-016-2900-1
47	Aqueous extract from the Withania somnifera leaves as a potential anti-neuroinflammatory agent: a mechanistic study	Journal of neuroinflammation	Immunomodulatory Activity and Neuroprotective Effects	Withania somnifera aqueous extract	Primary microglia-enriched cultures and BV-2 cell line	ASH-WEX 10 and 2 mg/ml concentration	ASH-WEX and FIV pretreatment was seen to suppress the proliferation of activated microglia by causing cell cycle arrest at Go/G1 and G2/M phase along with decrease in cell cycle regulatory protein expression such as PCNA and Cyclin D1. Inhibition of microglial activation was revealed by their morphology and downregulated expression of microglial activation markers like MHC II and Iba-1. Both the extracts attenuated the TNF-α, IL-1β, IL-6, RNS, and ROS production via downregulating the expression of inflammatory proteins like NFkB and AP1. ASH-WEX and FIV also restricted the migration of activated microglia by downregulating metalloproteinase expression. Controlled proliferation rate was also accompanied by apoptosis of activated microglia. The current data suggests that ASH-WEX and FIV inhibit microglial activation and migration and may prove to be a potential therapeutic candidate for the suppression of neuroinflammation in the treatment of neurodegenerative diseases.	Gupta, M., & Kaur, G. (2016). Aqueous extract from the Withania somnifera leaves as a potential anti-neuroinflammatory agent: a mechanistic study. Journal of neuroinflammation, 13(1), 193. https://doi.org/10.1186/s12974-016-0650-3
48	Inhibitory effect of withaferin A on Helicobacter pylori-induced IL-8 production and NF-κB activation in gastric epithelial cells	Molecular medicine reports	Immunomodulatory Activity and Anti inflammatory activity	Withaferin A (WA) from Withania somnifera	AGS human gastric epithelial cell line	0, 10, 25, 50, 100, 250, 500 and 1000 n M	Pre-treatment or co-treatment with WA efficiently reduced IL-8 production by AGS cells in response to H. pylori infection. H. pylori-induced activation of NF-κB, but not MAPKs, was also inhibited by pre-treatment of WA in the cells. However, WA did not affect VEGF production and HIF-1α stabilization induced by H. pylori in AGS cells. In addition, WA did not influence the growth of H. pylori, suggesting that the anti-inflammatory effect of WA was not due to any bactericidal effect. These findings indicate that WA is a potential preventive or therapeutic agent for H. pylori-mediated gastric inflammation.	Kim, G., Kim, T. H., Kang, M. J., Choi, J. A., Pack, D. Y., Lee, I. R., Kim, M. G., Han, S. S., Kim, B. Y., Oh, S. M., Lee, K. B., Kim, D. J., & Park, J. H. (2016). Inhibitory effect of withaferin A on Helicobacter pylori-induced IL-8 production and NF-κB activation in gastric epithelial cells. Molecular medicine reports, 13(1), 967–972. https://doi.org/10.3892/mmr.2015.4602
49	Anti-nociceptive and anti-inflammatory effects of Withania somnifera root in fructose fed male rats	Journal of Basic and Clinical Physiology and Pharmacology	Analgesic and Anti-inflammatory Activity	Withania somnifera root extract	Wistar-Albino male rats	62.5 mg/g diet	The results showed that the insulin resistance index, blood sugar, insulin, IL-6, TNF-α, and acute and chronic pain score in the F group were significantly increased in comparison with the control group, but these parameters in the FWS group were significantly decreased compared with the F group (p<0.001).The findings indicated that chronic oral administration of WSR has analgesic and anti-inflammatory effects in fructose drinking water rats and causes improved insulin resistance index.	Shahraki MR, Samadi Noshahr Z, Ahmadvand H, Nakhaie A. Anti-nociceptive and anti-inflammatory effects of Withania somnifera root in fructose fed male rats. J Basic Clin Physiol Pharmacol. 2016 Jun 1;27(4):387-91. doi: 10.1515/jbcpp-2015-0053. PMID: 27383871.
50	Withania somnifera and Its Withanolides Attenuate Oxidative and Inflammatory Responses and Up-Regulate Antioxidant Responses in BV-2 Microglial Cells.	Neuromolecular medicine	Immunomodulatory Activity and Neuroprotective Effects	Ashwagandha	Inflammopharmacology Mouse microglial cells	N/A	Ashwagandha Attenuated LPS-Induced NO and ROS Production in BV-2 Microglial Cells, Induced Nrf2 and HO-1 Protein Expression. In serum-free culture, LPS can also induce production of long thin processes between 4 and 8 h in BV-2 cells. This morphological change was significantly suppressed by Ashwagandha and both withanolides at concentrations for suppressing LPS-induced NO production. Taken together, these results suggest an immunomodulatory role for Ashwagandha and its withanolides, and their ability to suppress oxidative and inflammatory responses in microglial cells by simultaneously down-regulating the NF-kB and upreg- ulating the Nrf2 pathways.	Sun, G. Y., Li, R., Cui, J., Hannink, M., Gu, Z., Fritsche, K. L., Lubahn, D. B., & Simonyi, A. (2016). Withania somnifera and Its Withanolides Attenuate Oxidative and Inflammatory Responses and Up-Regulate Antioxidant Responses in BV-2 Microglial Cells. *Neuromolecular medicine*, *18* (3), 241–252. https://doi.org/10.1007/s12017-016-8411-0
51	Hepatoprotective effect of withanolide-rich fraction in acetaminophen-intoxicated rat: decisive role of TNF-α, IL-1β, COX-II and iNOS.	Pharmaceutical biology	Immunomodulatory Activity and Hepatoprotective Effects	Withanolide-rich fraction from methanolic extract of Withania somnifera roots	Adult male albino rats of Wistar strain	WRF - 50mg/kg, 100 mg/kg and 200 mg/kg	The results demonstrated that in the WRF-treated group, there was significant and dose-dependent ($p < 0.01$ and $p < 0.001$) decrease in serum bilirubin, ALP, AST and ALT levels with significant and dose-dependent ($p < 0.01$ and $p < 0.001$) increase in hepatic SOD, GSH and total antioxidant capacity. WRF may exert its hepatoprotective action by alleviating inflammatory and oxido-nitrosative stress via inhibition of TNF-α, IL-1β, COX-II and iNOS.	Devkar, S. T., Kandhare, A. D., Zanwar, A. A., Jagtap, S. D., Katyare, S. S., Bodhankar, S. L., & Hegde, M. V. (2016). Hepatoprotective effect of withanolide-rich fraction in acetaminophen-intoxicated rat: decisive role of TNF-α, IL-1β, COX-II and iNOS. *Pharmaceutical biology*, *54* (11), 2394–2403. https://doi.org/10.3109/13880209.2016.1157193
52	The Effects of Withania somnifera on Blood sugar, Serum Insulin, Lipid Profile and Liver Enzymes in Fructose Drinking Water Male Rats	Journal of Chemical and Pharmaceutical Research	Hepatoprotective	Withania somnifera root extract	Male Albino- Wistar rats	62.5mg/g diet WS root powder	Administration of Withania somnifera root in rodent diet has shown anti-hyperglycemic, anti-lipidemic effects and causes improved liver damages in FDW rats	Shahraki, M.R., Dadpisheh, S., Shahraki, A.R., & Mirshekari, H. (2016). The Effects of Withania somnifera on Blood sugar , Serum Insulin , Lipid Profile and Liver Enzymes in Fructose Drinking Water Male Rats. Journal of Chemical and Pharmaceutical Research, 2016, 8(4):1328-1334
53	Hepatoprotective effect of withanolide-rich fraction in acetaminophen-intoxicated rat: decisive role of TNF-a, IL-1b, COX-II and iNOS	Pharmaceutical Biology	Hepatoprotective	Withania somnifera root powder - Withanolide rich fraction	Adult male albino Wistar rats	WRF (50mg/kg); WRF (100 mg/kg); WRF (200 mg/kg)	WRF exercises its hepatoprotective effect in APAP-treated rats through its antioxidant potential.	Santosh T. Devkar, Amit D. Kandhare, Anand A. Zanwar, Suresh D. Jagtap, Surendra S. Katyare, Subhash L. Bodhankar & Mahabaleshwar V. Hegde (2016) Hepatoprotective effect of withanolide-rich fraction in acetaminophen-intoxicated rat: decisive role of TNF-α, IL-1β, COX-II and iNOS, Pharmaceutical Biology, 54:11, 2394-2403,

54	Propensity of Withania somnifera to Attenuate Behavioural, Biochemical, and Histological Alterations in Experimental Model of Stroke	Cell Mol Neurobiol	Neuroprotective Activity	Withania somnifera root extract	Male Wistar rats	300 mg/kg. wt/day	Neurobehavioural changes were accompanied by decreased acetylcholinesterase activity, increased oxidative stress in terms of enhanced lipid peroxidation and lowered thiol levels in the MCAO animals. In addition, MCAO animals had cerebral infarcts and the presence of pycnotic nuclei. Single-photon emission computerized tomography (SPECT) of MCAO animals revealed a cerebral infarct as a hypoactive area. On the other hand, pre-supplementation with WS (300 mg/kg body weight) for 30 days to MCAO animals was effective in restoring the acetylcholinesterase activity, lipid peroxidation, thiols and attenuated MCAO induced behavioural deficits. WS significantly reduced the cerebral infarct volume and ameliorated histopathological alterations. Improved blood flow was observed in the SPECT images from the brain regions of ischemic rats pre-treated with WS. The results of the study showed a protective effect of WS supplementation in ischemic stroke and are suggestive of its potential application in stroke management.	Sood A, Kumar A, Dhawan DK, Sandhir R. Propensity of Withania somnifera to Attenuate Behavioural, Biochemical, and Histological Alterations in Experimental Model of Stroke. Cell Mol Neurobiol. 2016 Oct;36(7):1123-38. doi: 10.1007/s10571-015-0305-4. Epub 2015 Dec 30. PMID: 26718711.
55	Effect of Withania somnifera Root Powder on Biochemical Parameters in Lead Treated Chickens	Veterinary Research International	Hepatoprotective	Withania somnifera root powder	One-hundred-and-fifty-day old broiler chicks	Withania somnifera root powder at 1%	Study suggested a lesser increase in ALT and AST in the groups administered with Withania somnifera root powder along with varied ppm of Lead	Amandeep, Munish Batra, Shree Niwas Yadav. Effect of Withania somnifera Root Powder on Biochemical Parameters in Lead Treated Chickens. Veterinary Research International, 2015; 3:3, 60-67
56	Amelioration of Isoproterenol-Induced Oxidative Damage in Rat Myocardium by Withania somnifera Leaf Extract	BioMed research international	Cardioprotective Activity	Withania somnifera extract	Adult male albino Wistar rats	100 mg/kg	Oral pretreatment (100 mg/kg b.w.) with WSLEt for 4 weeks elicited a significant cardioprotective activity by lowering the levels of cTnI, lipid profiles, and marker enzymes. The levels of LPO products were also significantly decreased. Elevated activities of antioxidant enzymes were also observed in rats pretreated with WSLEt. As further confirmed histopathologically, our findings strongly suggest that the cardioprotective effect of WSLEt on myocardium experiencing ISO-induced oxidative damage may be due to an augmentation of the endogenous antioxidant system and an inhibition of LPO in the myocardial membrane. We conclude that WSLEt confers some protection against oxidative damage in ISO-induced MI in rats.	Khalil, M. I., Ahmmed, I., Ahmed, R., Tanvir, E. M., Afroz, R., Paul, S., Gan, S. H., & Alam, N. (2015). Amelioration of Isoproterenol-Induced Oxidative Damage in Rat Myocardium by Withania somnifera Leaf Extract. BioMed research international, 2015, 624159. https://doi.org/10.1155/2015/624159
57	Effect of Withania somnifera (Ashwagandha) root extract on amelioration of oxidative stress and autoantibodies production in collagen-induced arthritic rats	Journal of complementary & integrative medicine	Immunomodulatory Activity and Anti inflammatory activity	Withania somnifera aqueous extract	Collagen-induced arthritic (CIA) rats	100, 200, 300 mg/kg	Treatment with WSAq resulted in a dose-dependent reduction in arthritic index, autoantibodies and CRP ($p<0.05$) with maximum effect at dose of 300 mg/kg b. wt. and the results were comparable to that of MTX-treated rats. Similarly, oxidative stress in CIA rats was ameliorated by treatment with different doses of WSAq, as evidenced by a decrease in lipid peroxidation and glutathione-S-transferase activity and an increase in the glutathione content and ferric-reducing ability of plasma ($p<0.05$). The results showed that WSAq exhibited antioxidant and anti-arthritic activity and reduced inflammation in CIA rats and suggests the potential use of this plant in the treatment of arthritis.	Khan, M. A., Subramaneyaan, M., Arora, V. K., Banerjee, B. D., & Ahmed, R. S. (2015). Effect of Withania somnifera (Ashwagandha) root extract on amelioration of oxidative stress and autoantibodies production in collagen-induced arthritic rats. Journal of complementary & integrative medicine, 12(2), 117–125. https://doi.org/10.1515/jcim-2014-0075
58	Protective effects of Withania somnifera root on inflammatory markers and insulin resistance in fructose-fed rats	Reports of Biochemistry and Molecular Biology	Immunomodulatory Activity and Anti inflammatory activity	Withania somnifera root extract	Wistar-Albino male rats	62.5 mg/g diet	Blood glucose, insulin, homeostasis model assessment for insulin resistance (HOMA-R), IL-6, and TNF-α levels were all significantly greater in the fructose-fed rats than in the controls. Treatment with WS significantly ($P < 0.05$) inhibited the fructose-induced increases in glucose, insulin, HOMA-R, IL-6, and TNF-α.WS normalizes hyperglycemia in fructose-fed rats by reducing inflammatory markers and improving insulin sensitivity.	Samadi Noshahr Z, Shahraki MR, Ahmadvand H, Nourabadi D, Nakhaei A. Protective effects of Withania somnifera root on inflammatory markers and insulin resistance in fructose-fed rats. Rep Biochem Mol Biol. 2015 Apr;3(2):62-7. PMID: 26989739; PMCID: PMC4757043.
59	Immunomodulatory Effect of of Withania somnifera (Ashwagandha) on Cyclophosphamide Induced Toxicity in Rats.	American Journal of PharmTech Research	Immunomodulatory Activity	Withania somnifera root extract	Charles Foster rats	Cyclophosphamide @ 250 mg/kg b.w Ashwagandha 300mg/kg b.w.	Ashwagandha (300mg/kg b.w.) administered five days prior to cyclophosphamide administration and continued for ten days then significant increase in total count of WBC, ALC and Platelets were observed after treatment . Thus findings of present investigation showed that therapeutic potency of Ashwagandha ameliorate the toxicity produced during cancer chemotherapy by mitigating the bone marrow depression.	Mohammad A et al., Immunomodulatory Effect of of Withania somnifera (Ashwagandha) on Cyclophosphamide Induced Toxicity in Rats. American Journal of PharmTech Research 2015.
60	Standardized extract of Withania somnifera (Ashwagandha) markedly offsets rotenone-induced locomotor deficits, oxidative impairments and neurotoxicity in Drosophila melanogaster	Journal of food science and technology	Neuroprotective Activity	Withania somnifera root extract	Drosophila melanogaster (Oregon-K)	0.005, 0.01, 0.05, %	WS conferred significant protection against ROT-induced lethality, while the survivor flies exhibited improved locomotor phenotype. Biochemical investigations revealed that ROT-induced oxidative stress was significantly diminished by WS enrichment. WS caused significant elevation in the levels of reduced GSH/non-protein thiols. Furthermore, the altered activity levels of succinate dehydrogenase, MTT, membrane bound enzymes viz., NADH-cytochrome-c reductase and succinate-cytochrome-c reductase were markedly restored to normalcy. Interestingly, ROT-induced perturbations in cholinergic function and depletion in dopamine levels were normalized by WS. Taken together these data suggests that the neuromodulatory effect of WS against ROT- induced neurotoxicity is probably mediated via suppression of oxidative stress and its potential to attenuate mitochondrial dysfunctions. Our further studies aim to understand the underlying neuroprotective mechanisms of WS and withanolides employing neuronal cell models.	Manjunath, M. J., & Muralidhara (2015). Standardized extract of Withania somnifera (Ashwagandha) markedly offsets rotenone-induced locomotor deficits, oxidative impairments and neurotoxicity in Drosophila melanogaster. Journal of food science and technology, 52(4), 1971–1981. https://doi.org/10.1007/s13197-013-1219-0
61	Withania somnifera shows a protective effect in monocrotaline-induced pulmonary hypertension	Pharmaceutical biology	Cardioprotective effect, and anti-inflammatory	Withania somnifera root powder	Sprague-Dawley (SD) rats	50 and 100 mg/kg	Preventive treatment with 50 and 100 mg/kg W. somnifera significantly reduced the RVP (32.18 ± 1.273 mm Hg and 29.98 ± 1.119 mm Hg, respectively, versus 42.96 ± 1.789 mm Hg of MCT) and all markers of RVH in MCT-challenged rats. There was an improvement in inflammation, oxidative stress and endothelial dysfunction, and attenuation of proliferative marker and apoptotic resistance in lungs. Therapeutic treatment with W. somnifera (100 mg/kg) also reduced RVP and RVH. This study demonstrated that W. somnifera significantly protected against MCT-induced PH due to its antioxidant, anti-inflammatory, pro-apoptotic, and cardioprotective properties.	Kaur, G., Singh, N., Samuel, S. S., Bora, H. K., Sharma, S., Pachauri, S. D., Dwivedi, A. K., Siddiqui, H. H., & Hanif, K. (2015). Withania somnifera shows a protective effect in monocrotaline-induced pulmonary hypertension. Pharmaceutical biology, 53(1), 147–157. https://doi.org/10.3109/13880209.2014.912240
62	Therapeutic effect of ashwagandha (Withania somnifera L.) in liver dysfunction of old dogs	Applied Biological Research	Hepatoprotective	Hydro-alcoholic root extract of ashwagandha	Geriatric canine with liver dysfunction	Not available	Results suggest that crude hydro-alcoholic root extract of W. somnifera possesses biologically active principles that are hepatoprotective and antioxidant in nature	Nabi S.U., Wani A.R., Dey S. (2014) Therapeutic effect of ashwagandha (Withania somnifera L.) in liver dysfunction of old dogs. Applied Biological Research. 16:2, 232-236.

63	Phasic and tonic type A γ-Aminobutryic acid receptor mediated effect of Withania somnifera on mice hippocampal CA1 pyramidal Neurons	Journal of Ayurveda and integrative medicine	Neuroprotective Activity	Withania somnifera root extract	Immature male mice	50, 100, 200, 400 and	The application of mWS (400 ng/μl) induced remarkable inward currents (-158.1 ± 28.08 pA, n = 26) on the CA1 pyramidal neurons. These inward currents were not only reproducible but also concentration dependent. mWS-induced inward currents remained persistent in the presence of amino acid receptor blocking cocktail (AARBC) containing blockers for the ionotropic glutamate receptors, glycine receptors and voltage-gated Na(+) channel. These results suggest that WS acts on synaptic/extrasynaptic GABAA receptors and may play an important role in the process of memory and neuroprotection via activation of synaptic and extrasynaptic GABAA receptors	Bhattarai, J. P., & Han, S. K. (2014). Phasic and tonic type A γ-Aminobutryic acid receptor mediated effect of Withania somnifera on mice hippocampal CA1 pyramidal Neurons. Journal of Ayurveda and integrative medicine, 5(4), 216–222. https://doi.org/10.4103/0975-9476.146541
64	Effects of aqueous extract of Withania somnifera on some liver biochemical and histopathological parameters in male guinea pigs	Pakistan journal of biological sciences : PJBS	Hepatoprotective	Withania somnifera aqueous extract	Adult male guinea pigs	100 mg/kg	W. somnifera extract reduced the incidence of histopathological changes such as cytoplasmic vacuolization and degeneration in nuclei, rupture of epithelia lining the central vein, widened sinusoidal space and lymphocyte infiltration induced by DM treatment in guinea pigs. In conclusion, the results of this study suggest that W. somnifera aqueous extract could protect the liver against DM-induced oxidative damage.	Al-Awthan, Y. S., Hezabr, S. M., Al-Zubairi, A. M., & Al-Hemiri, F. A. (2014). Effects of aqueous extract of Withania somnifera on some liver biochemical and histopathological parameters in male guinea pigs. Pakistan journal of biological sciences : PJBS, 17(4), 504–510. https://doi.org/10.3923/pjbs.2014.504.510
65	Amelioration of bromobenzene hepatotoxicity by Withania somnifera pretreatment: Role of mitochondrial oxidative stress	Toxicology reports	Hepatoprotective	Withania somnifera root powder	Wistar Albino rats	250 and 500 mg/kg	Pre-treatment with W. somnifera significantly decreased the levels of liver marker enzymes, TNF-α, IL-1β, VEGF and ameliorated histopathological manifestations in bromobenzene-treated rats. The molecular docking analysis predicted that the pro-inflammatory mediator NF-κB showed significant interaction with selected various active components of W. somnifera (withaferin A, withanolide D and withanolide E). This study demonstrates a good protective effect of W. somnifera against bromobenzene-induced oxidative stress.	Vedi, M., Rasool, M., & Sabina, E. P. (2014). Amelioration of bromobenzene hepatotoxicity by Withania somnifera pretreatment: Role of mitochondrial oxidative stress. Toxicology reports, 1, 629–638. https://doi.org/10.1016/j.toxrep.2014.08.009
66	Evaluation of anti-inflammatory effect of Withania somnifera root on collagen-induced arthritis in rats	Pharmaceutical Biology	Anti inflammatory activity	Withania somnifera root powder	Wistar Albino rats (female strain)	600 mg/kg	Administration of W. somnifera root powder (600 mg kg^{-1}) to the arthritic rats significantly decreased the severity of arthritis by effectively suppressing the symptoms of arthritis and improving the functional recovery of motor activity and radiological score. W. somnifera root has a protective effect against collagen-induced arthritis (CIA) in rats. The results suggest that W. somnifera root powder acts as an anti-inflammatory and antioxidant agent in decreasing the arthritic effects in collagen-induced arthritic rats.	Gupta, A., & Singh, S. (2014). Evaluation of anti-inflammatory effect of Withania somnifera root on collagen-induced arthritis in rats. Pharmaceutical biology, 52(3), 308–320. https://doi.org/10.3109/13880209.2013.835325
67	Protective effect of administration of Withania somifera against bromobenzene induced nephrotoxicity and mitochondrial oxidative stress in rats	Renal failure	Nephroprotective	Withania somnifera root powder	Wistar Albino rats	250 and 500 mg/kg	The levels of renal lipid peroxidation, lysosomal enzymes and glycoprotein were increased significantly ($p < 0.05$) in the bromobenzene alone treated rats and antioxidant status and mitochondrial enzymes were found to be decreased, when compared to the control group. The levels of kidney functional markers (urea, uric acid and creatinine) were also found to be abnormal in serum of bromobenzene alone treated rats. On the other hand, administration of W. somnifera (250 and 500 mg/kg) along with bromobenzene offered a significant dose-dependent protection to the biochemical alterations as observed in the bromobenzene alone treated rats, which was also evidenced by histopathology. Thus, the oral administration of W. somnifera significantly protected against the bromobenzene induced nephrotoxicity and renal mitochondrial dysfunction in rats.	Vedi, M., Rasool, M., & Sabina, E. P. (2014). Protective effect of administration of Withania somifera against bromobenzene induced nephrotoxicity and mitochondrial oxidative stress in rats. Renal failure, 36(7), 1095–1103. https://doi.org/10.3109/0886022X.2014.918812
68	Effect of Supplementation of Withania somnifera (Linn.) Dunal Roots on Growth Performance, Serum Biochemistry, Blood Hematology, and Immunity of Broiler Chicks	Journal of Herbs , Spices and medicinal plants	Hepatoprotective	Dried Withania somnifera roots	Broiler chicks	1.25%, 2.5% and 5 %	Positive effect of withania somnifera root on liver parenchyma of the birds	Jahanzeb Ansari , Sohail Hassan Khan , Ahsan UL Haq , Tanveer Ahmad & Muhammad Ismail Abbass (2013): Effect of Supplementation of Withania somnifera (Linn.) Dunal Roots on Growth Performance, Serum Biochemistry, Blood Hematology, and Immunity of Broiler Chicks, Journal of Herbs, Spices & Medicinal Plants, 19:2, 144-158
69	Phytoremedial role of Withania somnifera extract on Biochemical parameters in liver of epinephrine induced Stressed albino mice	International Journal of Pharma and Bio Sciences	Hepatoprotective	Alcoholic root extract of Withania somnifera	Swiss Albino mice	50mg/kg b.w.	Withania somnifera root extract was found to be significantly reverse the alterations in SGPT , SGOT and Bilirubin levels demonstrating a hepatoprotective effect.	M. Suhail, S. S. Kumar, and B. Sharma, (2013) “Phytoremedial role of Withania somnifera extract on biochemical parameters in liver of epinephrine induced stressed albino mice,” International Journal of Pharma and Bio Sciences, vol.4, no.2, pp.862–870.
70	Ameliorative potential of aqueous root extract of Withania somnifera agaisnt paracetamol induced liver damage in mice	Pharmacologia	Hepatoprotective	Withania somnifera root extract	Female Swiss Albino Mice	500mg/kg/body weight	Treatment with Withania somnifera root extract notable decreased AST, ALT, ALP, Bilirubin and total protein content, and significatnly reduced hepatic lipid peroxidation and enhanced antioxidant enzyme activties and GSH levels in the liver of mice.	Tabarak Malik, Devendra Kumar Pandey and Nitu Dogra, 2013. Ameliorative Potential of Aqueous Root Extract of Withania somnifera Against Paracetamol Induced Liver Damage in Mice. Pharmacologia, 4: 89-94.
71	Effect of Withania somnifera supplementation on rotenone-induced oxidative damage in cerebellum and striatum of the male mice brain	Central nervous system agents in medicinal chemistry	Neuroprotective and Antioxidant	Withania somnifera root extract	Prepubertal male mice	100-400 mg/ kg	WS prophylaxis significantly offset ROT-induced oxidative damage in st and cb as evident by the normalized levels of oxidative markers (MDA, ROS levels and HP) and restoration of depleted GSH levels. WS effectively normalized the NO levels in both brain regions suggesting its antiinflammatory action. Furthermore, WS prophylaxis restored the activity levels of cytosolic antioxidant enzymes, neurotransmitter function and dopamine levels in st. Taken together, these findings suggest that WS prophylaxis has the propensity to modulate neurotoxicant-mediated oxidative impairments and mitochondrial dysfunctions in specific brain regions of mice. While the exact mechanism/s underlying the neuroprotective effects of WS merit further investigation, based on our findings, we hypothesize that it may be wholly or in part due to its ability to enhance GSH, thiols and antioxidant defences in the brain of mice.	Manjunath, M. J., & Muralidhara (2013). Effect of Withania somnifera supplementation on rotenone-induced oxidative damage in cerebellum and striatum of the male mice brain. Central nervous system agents in medicinal chemistry, 13(1), 43–56. https://doi.org/10.2174/1871524911313010007
72	Withaferin A inhibits matrix metalloproteinase-9 activity by suppressing the Akt signaling pathway	Oncology reports	Immunomodulatory Activity	Commercial Withaferin A	Human Caski and SK-Hep-1 cells	Withaferin A (Wit A)	Wit A inhibited TGF-β-induced invasion and metastasis through a reduc- tion in expression and secretion of MMP-9 through the Akt pathway in Caski human cervical cancer cells. In addition, the inhibitory effect on MMP-9 induction by treatment with Wit A was also observed in human hepatoma SK-Hep1 cells.	Lee, D. H., Lim, I. H., Sung, E. G., Kim, J. Y., Song, I. H., Park, Y. K., & Lee, T. J. (2013). Withaferin A inhibits matrix metalloproteinase-9 activity by suppressing the Akt signaling pathway. *Oncology reports* , *30* (2), 933–938. https://doi.org/10.3892/or.2013.2487

73	Dietary supplementation of Ashwagandha (Withania somnifera, Dunal) enhances NK cell function in ovarian tumors in the laying hen model of spontaneous ovarian cancer.	American journal of reproductive immunology	Immunomodulatory Activity	Withania somnifera root powder	White Leghorn laying hens (Gallus domesticus)	Ashwagandha - 1 g/kg body weight ; 2 g/kg body weight	Ashwagandha supplementation decreased the incidence and progression of Ovraian Cancer (OVCA). Both the stromal and intratumoral NK cell population increased significantly ($P < 0.0001$) in Ashwagandha supplementated hens.	Barua, A., Bradaric, M. J., Bitterman, P., Abramowicz, J. S., Sharma, S., Basu, S., Lopez, H., & Bahr, J. M. (2013). Dietary supplementation of Ashwagandha (Withania somnifera, Dunal) enhances NK cell function in ovarian tumors in the laying hen model of spontaneous ovarian cancer. *American journal of reproductive immunology (New York, N.Y. : 1989)*, *70* (6), 538–550. https://doi.org/10.1111/aji.12172
74	Attenuation of oxidative damage-associated cognitive decline by Withania somnifera in rat model of streptozotocin-induced cognitive impairment	Protoplasma	Neuroprotective Activity	Withania somnifera extract	Male Wistar rats	100, 200, and 300 mg/kg	Pretreatment with WS extract attenuated behavioral, biochemical, and histological alterations significantly in dose-dependent manner in the hippocampus and cerebral cortex of ICV-STZ-infused rats. These results suggest that WS affords a beneficial effect on cognitive deficit by ameliorating oxidative damage induced by streptozotocin in a model of cognitive impairment.	Ahmed, M. E., Javed, H., Khan, M. M., Vaibhav, K., Ahmad, A., Khan, A., Tabassum, R., Islam, F., Safhi, M. M., & Islam, F. (2013). Attenuation of oxidative damage-associated cognitive decline by Withania somnifera in rat model of streptozotocin-induced cognitive impairment. Protoplasma, 250(5), 1067–1078. https://doi.org/10.1007/s00709-013-0482-2
75	Withania somnifera root extract ameliorates hypobaric hypoxia induced memory impairment in rats	Journal of ethnopharmacology	Neuroprotective Activity	Withania somnifera root extract	Male Sprague Dawley rats	50, 100, 150, 200 and 250 mg/kg	Administration of WS root extract prevented HH induced memory impairment and neurodegeneration along with decreased NO, corticosterone, oxidative stress and AchE activity in hippocampal region. Inhibition of NO synthesis by administration of L-Name reduced corticosterone levels in hippocampus during hypoxic exposure while co-administration of corticosterone increased neurodegeneration. Administration of sodium nitroprusside (SNP) along with WS root extract supplementation during hypoxic exposure increased corticosterone levels and increased the number of pyknotic cells	Baitharu, I., Jain, V., Deep, S. N., Hota, K. B., Hota, S. K., Prasad, D., & Ilavazhagan, G. (2013). Withania somnifera root extract ameliorates hypobaric hypoxia induced memory impairment in rats. Journal of ethnopharmacology, 145(2), 431–441. https://doi.org/10.1016/j.jep.2012.10.063
76	Neuroprotective role of Withania somnifera root extract in maneb-paraquat induced mouse model of parkinsonism	Neurochemical research	Neuroprotective Activity	Withania somnifera root extract	Swiss Albino mice	100 mg/kg	The behavioral studies showed a significant improvement in the motor movement patterns and gripping ability of Ws root extract exposed Parkinsonian mice. Tyrosine hydroxylase (TH) immunostaining was reduced in the substantia nigra of MB-PQ exposed mice, while Ws co-exposure restored TH immunostaining significantly. Additionally, our results also demonstrate generation of oxidative stress in the nigrostriatal region of MB-PQ exposed mice. There was a marked decline in the level of catalase and a simultaneous increase in the level of nitrite and lipid peroxidation in Parkinsonian mice. Thus, the Ws root extract have shown to counteract the pro-oxidants and their associated oxidative stress in the PD model studied here. Our results clearly indicate the usefulness of Ws root extract in providing protection against MB-PQ induced nigrostriatal dopaminergic neurodegeneration and marked improvement in the behavioral, anatomical and the biochemical deformities.	Prakash, J., Yadav, S. K., Chouhan, S., & Singh, S. P. (2013). Neuroprotective role of Withania somnifera root extract in maneb-paraquat induced mouse model of parkinsonism. Neurochemical research, 38(5), 972–980. https://doi.org/10.1007/s11064-013-1005-4
77	Hepatoprotective and antioxidant potential of Withania somnifera against paracetamol-induced liver damage in rats	International Journal of Pharmacy and Pharmaceutical Sciences	Hepatoprotective	Withania somnifera powder	Rats	Aqueous suspension at dose 500mg/kg body weight and 1000mg/ kg body weight was injected intraperitoneally	Ashwagandha possesses a promising hepatoprotective and antioxidant effect in acetaminophen-intoxicated rats probably due to its antioxidant effects	Sabina E P, Rasool M, Vedi M, Navaneethan D, Ravichander M, Poornima P, Thella S R (2013) Hepatoprotective and Antioxidant potential of Withania somnifera against paracetamol-induced liver damage in rats, International Journal of Pharmacy and Pharmaceutical Sciences, 5:2, 648-651.
78	Effects of Ashwagandha (Withania somnifera) Root Extract On Some Serum Liver Marker Enzymes (AST, ALT) In Gentamicin Intoxicated Rats	J Bangladesh Soc Physiol	Hepatoprotective	Withania somnifera root extract	Adult male albino Wistar rats	500mg/kg body weight/day,	Ashwagandha (Withania somnifera) root extract restored serum AST, ALT towards normal levels in gentamicin intoxicated rats which may be due to its free radical scavenging activity	Sultana, N., Shimmi, S.C., Parash, M., & Akhtar, J. (2012). Effects of Ashwagandha (Withania somnifera) Root Extract On Some Serum Liver Marker Enzymes (AST, ALT) In Gentamicin Intoxicated Rats. Journal of Bangladesh Society of Physiologist 7(1):1-7
79	Protective effect of Withania somnifera roots extract on hematoserological profiles against lead nitrate-induced toxicity in mice	Indian journal of biochemistry & biophysics	Hepatoprotective	Withania somnifera root extract	Swiss Albino mice	250 and 500 mg/kg	Animals exposed to LN showed significant ($P < 0.001$) decline in haemoglobin content, red blood cell count, white blood cell count, packed cell volume and insignificant decrease in mean corpuscular haemoglobin and mean corpuscular haemoglobin content, while mean corpuscular volume and platelet count were increased. A significant elevation ($P < 0.001$) in serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase, acid phosphatase and total cholesterol were also observed, when compared with control mice. Thus, the study demonstrated that the concurrent daily administration of root extract of WS protected the adverse effects of LN intoxication in mice.	Sharma, V., Sharma, S., & Pracheta (2012). Protective effect of Withania somnifera roots extract on hematoserological profiles against lead nitrate-induced toxicity in mice. Indian journal of biochemistry & biophysics, 49(6), 458–462.
80	Protective effect of Withania somnifera against radiation-induced hepatotoxicity in rats	Ecotoxicology and Environmental Safety	Hepatoprotective	Withania somnifera extract	Male Albino rats (Sprague –Dawley strain)	100mg/kg/body weight	The restoration of AST, ALT, ALP and GGT to their respective normal level was observed in the WS treated group	Hosny Mansour, H., & Farouk Hafez, H. (2012). Protective effect of Withania somnifera against radiation-induced hepatotoxicity in rats. Ecotoxicology and Environmental Safety, 80, 14–19. doi:10.1016/j.ecoenv.2012.02.003
81	Therapeutic effect of Withania somnifera on pristane-induced model of SLE	Inflammopharmacology	Immunomodulatory Activity and Hepatoprotective Effects	Withania somnifera pure root powder	Female Balb/c mice	1,000 and 500 mg/kg	Five months of treatment with Withania somnifera root powder at 1,000 mg/kg showed decreased lipogranuloma. as compared to the pristane-treated group, whereas no noticeable change was observed in groups treated with Withania somnifera root powder at 500 mg/kg and indo- methacin showed remarkable decrease in lipogranuloma formation. Treatment with Withania somnifera root powder at 1,000, 500 mg/kg and indo- methacin showed significant decrease in ROS levels as compared to the PT group.	Minhas, U., Minz, R., Das, P., & Bhatnagar, A. (2012). Therapeutic effect of Withania somnifera on pristane-induced model of SLE. Inflammopharmacology, 20(4), 195–205. https://doi.org/10.1007/s10787-011-0102-8

82	An aqueous extract of Withania somnifera root inhibits amyloid β fibril formation in vitro	Phytotherapy research : PTR	Neuroprotective Activity	Withania somnifera aqueous root extract	N/A	6.25–50 μg/mL □	The aqueous extract of W. somnifera inhibited Aβ fibril formation under in vitro conditions, demonstrated independently by TEM and a ThT assay, in a concentration-dependent manner. These results support the putative claims that an extract of W. somnifera might represent an alternative therapeutic option, as the compounds present in the extract might be able to ameliorate cognitive deficiency and neurodegeneration by inhibiting Aβ aggregation.	Kumar, S., Harris, R. J., Seal, C. J., & Okello, E. J. (2012). An aqueous extract of Withania somnifera root inhibits amyloid β fibril formation in vitro. Phytotherapy research : PTR, 26(1), 113–117. https://doi.org/10.1002/ptr.3512
83	Protective role of Ashwagandha leaf extract and its component withanone on scopolamine-induced changes in the brain and brain-derived cells	Journal.pone.	Neuroprotective Activity	Withania somnifera extract	Male Swiss albino mice; Glioma C6 (rat) and neuronal IMR32 (human) cell lines	100, 200, 300 mg/kg	The results showed that the scopolamine caused downregulation of the expression of BDNF and GFAP in dose and time dependent manner, and these effects were markedly attenuated in response to i-Extract treatment. Similar to our observations in animal model system, we found that the scopolamine induced cytotoxicity in IMR32 neuronal and C6 glioma cells. It was associated with downregulation of neuronal cell markers NF-H, MAP2, PSD-95, GAP-43 and glial cell marker GFAP and with upregulation of DNA damage- γH2AX and oxidative stress- ROS markers. Furthermore, these molecules showed recovery when cells were treated with i-Extract or its purified component, withanone.	Konar, A., Shah, N., Singh, R., Saxena, N., Kaul, S. C., Wadhwa, R., & Thakur, M. K. (2011). Protective role of Ashwagandha leaf extract and its component withanone on scopolamine-induced changes in the brain and brain-derived cells. PloS one, 6(11), e27265. https://doi.org/10.1371/journal.pone.0027265
84	Lead induced hepatotoxicity in male swiss albino mice: the protective potential of the Hydromethanolic extract of Withania somnifera	International Journal of Pharmaceutical Sciences Review and Research	Hepatoprotective	Withania somnifera root extract	Male Swiss albino mice	200 mg/kg/ body weight and 500 mg/kg/ body weight	WS extract administered along with lead retained hepatic architecture and was able to diminish the fibrosis, congestion and hepatocyte vacuolation – hepatoprotective effect due to antioxidant potential.	Sharma V, Sharma S, Pracheta SS, et al. Lead induced hepatotoxicity in male swiss albino mice: the protective potential of the hydromethanolic extract of Withania somnifera. Int J Pharmaceu Sci Rev Res. 2011;7:116–121.
85	Antihepatotoxic effect of Marrubium vulgare and Withania somnifera extracts on carbon tetrachloride-induced hepatotoxicity in rats	Journal of Basic and Clinical Pharmacy	Hepatoprotective	Dried parts of Withania somnifera	Male Wistar rats	500mg/kg/day for 4 weeks along with Carbon tetrachloride	Withania somnifera extract significantly reduced AST, ALT, ALP serum levels compared with Carbon tetrachloride treated rat values	A.A. Elberry, F.M. Harraz, S.A. Ghareib, A.A. Nagy, S.A. Gabr, M.I. Suliaman, E. Abdel-Sattar, Antihepatotoxic effect of marrubium vulgare and Withania somnifera extracts on carbon tetrachloride-induced hepatotoxicity in rats, J. Basic Clin. Pharm. 1 (2010) 247–254.
86	In vivo enhancement of natural killer cell activity through tea fortified with Ayurvedic herbs	Phytotherapy research : PTR	Immunomodulatory Activity	Tea with Withania somnifera, Glycyrrhzia glabra, Zingiber offi cinale, Ocimum sanctum and Elettaria cardamomum	Human K562 cells	Brooke Bond Red Label Natural Care tea (NC tea): Withania somnifera, Glycyrrhzia glabra, Zingiber officinale, Ocimum sanctum, Elettaria cardamomum	Data from these two studies indicate that regular consumption of the tea fortifi ed with Ayurvedic herbs enhanced NK cell activity, which is an important aspect of the (early) innate immune response to infections	Bhat, J., Damle, A., Vaishnav, P. P., Albers, R., Joshi, M., & Banerjee, G. (2010). In vivo enhancement of natural killer cell activity through tea fortified with Ayurvedic herbs. *Phytotherapy research : PTR*, *24* (1), 129–135. https://doi.org/10.1002/ptr.2889
87	In vitro protective effects of Withania somnifera (L.) dunal root extract against hydrogen peroxide and β-amyloid(1-42)-induced cytotoxicity in differentiated PC12 cells	Phytotherapy research : PTR	Neuroprotective Activity	Withania somnifera root extract	Rat pheochromocytoma (PC12) cell line	0.097–50 mg/ mL □	The results suggest that pretreatments of differentiated PC12 cells with aqueous extracts of W. somnifera root significantly protect differentiated PC12 cells against both H(2)O(2)- and Aβ((1-42))-induced cytotoxicity, in a concentration dependent manner. To investigate the compounds that could explain the observed effects, the W. somnifera extract was analysed by liquid chromatography-serial mass spectrometry and numerous withanolide derivatives, including withaferin A, were detected. These results demonstrate the neuroprotective properties of an aqueous extract of W. somnifera root and may provide some explanation for the putative ethnopharmacological uses of W. somnifera for cognitive and other neurodegenerative disorders that are associated with oxidative stress.	Kumar, S., Seal, C. J., Howes, M. J., Kite, G. C., & Okello, E. J. (2010). In vitro protective effects of Withania somnifera (L.) dunal root extract against hydrogen peroxide and β-amyloid(1-42)-induced cytotoxicity in differentiated PC12 cells. Phytotherapy research : PTR, 24(10), 1567–1574. https://doi.org/10.1002/ptr.3261
88	Propoxur-induced acetylcholine esterase inhibition and impairment of cognitive function: attenuation by Withania somnifera	Indian journal of biochemistry & biophysics	Neuroprotective Activity	Withania somnifera root powder	Male Wistar albino rats	100 mg/kg	transfer latency on plus maze, when administered 1 h prior to propoxur treatment and caused a significant decrease in acquisition as well as retention. The aqueous suspension of *W. somnifera* alone and when administered along with propoxur significantly increased the AChE activity. The study revealed that it's exhibited protective effect against propoxur-induced cognitive dysfunction and AChE inhibition in experimental animals. It may be suggested that W. somnifera has a neuroprotective effect.	Yadav, C. S., Kumar, V., Suke, S. G., Ahmed, R. S., Mediratta, P. K., & Banerjee, B. D. (2010). Propoxur-induced acetylcholine esterase inhibition and impairment of cognitive function: attenuation by Withania somnifera. Indian journal of biochemistry & biophysics, 47(2), 117–120.
89	Effect of Withania somnifera (L. Dunal) root as a feed additive on immunological parameters and disease resistance to Aeromonas hydrophila in Labeo rohita (Hamilton) fingerlings	Fish & shellfish immunology	Immunomodulatory Activity	Withania somnifera root extract	Labeo rohita fingerlings	0 gkg(-1) (control), 1 gkg(-1) (T(1)), 2 gkg(-1) (T(2)) and 3 gkg(-1) (T(3))	The results demonstrate that fishes fed with W. somnifera root showed enhanced NBT level, Phagocytic activity, total Immunoglobulin level and lysozyme activity ($p<0.05$) compared with the control group. The survivability was higher in experimental diets than the control group. Dietary W. somnifera at the level of 2 gkg(-1) showed significantly ($P<0.05$) higher protection (RPS 42.85+/-0.65%) against A. hydrophila infection than control. The results suggest that the W. somnifera root powder have a stimulatory effect on immunological parameters and increases disease resistance in L. rohita fingerlings against A. hydrophila infection.	Sharma, A., Deo, A. D., Riteshkumar, S. T., Chanu, T. I., & Das, A. (2010). Effect of Withania somnifera (L. Dunal) root as a feed additive on immunological parameters and disease resistance to Aeromonas hydrophila in Labeo rohita (Hamilton) fingerlings. Fish & shellfish immunology, 29(3), 508–512. https://doi.org/10.1016/j.fsi.2010.05.005
90	Protective effect of Withania somnifera root powder on lipid peroxidation and antioxidant status in gentamicin-induced nephrotoxic rats	Journal of basic and clinical physiology and pharmacology	Nephroprotective	Withania somnifera root powder	Male Wistar rats	N/A	W. somnifera treatment altered the antioxidant status and significantly reversed the levels as seen microscopically. The results show that the root powder of W. somnifera with the presence of natural antioxidants, bioflavanoids, and other bioactive compounds scavenged the free radicals generated by GEN and ameliorated the severity of GEN-induced nephrotoxicity by enhancing the antioxidant system and protecting the cellular integrity of kidney and liver tissues.	Jeyanthi, T., & Subramanian, P. (2010). Protective effect of Withania somnifera root powder on lipid peroxidation and antioxidant status in gentamicin-induced nephrotoxic rats. Journal of basic and clinical physiology and pharmacology, 21(1), 61–78. https://doi.org/10.1515/jbcpp.2010.21.1.61

91	Immunomodulatory effects of Withania somnifera on azoxymethane induced experimental colon cancer in mice.	Immunological investigations	Immunomodulatory Activity	Withania somnifera extract	Swiss albino mice	W. somnifera 400 mg/kg body weight	W. somnifera significantly altered the level of leucocytes, lymphocytes, neutrophils, immune complexes and immunoglobulins (Ig) A, G and M. The azoxymethane induced colon cancer and immune dysfunction was better controlled by *W. somnifera* . These results suggested that the immunomodulatory effects of *W. somnifera*	Muralikrishnan, G., Dinda, A. K., & Shakeel, F. (2010). Immunomodulatory effects of Withania somnifera on azoxymethane induced experimental colon cancer in mice. *Immunological investigations* , *39* (7), 688–698. https://doi.org/10.3109/08820139.2010.487083
92	Reversal of Cadmium-induced Oxidative Stress in Chicken by Herbal Adaptogens Withania Somnifera and Ocimum Sanctum	Toxicology international	Immunomodulatory and Antioxidant	Withania somnifera root extract	Male broiler chicks (Cobb strains)	Basal diet mixed with 0.1% root powder of *Withania somnifera*	Supplementation with Ashwagandha resulted in a significant decrease in ALT activity, BUN and serum creatinine levels compared with cadmium control group at the end of sixth week. Herbal adaptogens Withania somnifera roots and Ocimum sanctum leaf powder administration at the rate of 0.1% through feed reversed the antioxidant enzyme of RBC, i.e., CAT and SOD, nonenzymatic antioxidants GSH and lipid peroxidation marker TBARS of liver and kidney.	Bharavi, K., Reddy, A. G., Rao, G. S., Reddy, A. R., & Rao, S. V. (2010). Reversal of Cadmium-induced Oxidative Stress in Chicken by Herbal Adaptogens Withania Somnifera and Ocimum Sanctum. *Toxicology international* , *17* (2), 59–63. https://doi.org/10.4103/0971-6580.72671
93	Molecular insight into the immune up-regulatory properties of the leaf extract of Ashwagandha and identification of Th1 immunostimulatory chemical entity	Vaccine	Immunomodulatory Activity	Withania somnifera extract	Female BALB mice	0.1, 1, 10 and 100 µg/ml	The results demonstrate that WSL-2 and the leaf extract of W.somnifera in WSL-2 are able to activate the cell mediated Th1 immunity as evidenced by enhanced secretion of IFN- , IgG2a along with enhanced expression of co-stimulatory molecules and proinflammatory integrins	Khan, S., Malik, F., Suri, K. A., & Singh, J. (2009). Molecular insight into the immune up-regulatory properties of the leaf extract of Ashwagandha and identification of Th1 immunostimulatory chemical entity. Vaccine, 27(43), 6080–6087. https://doi.org/10.1016/j.vaccine.2009.07.011
94	Immune modulation and apoptosis induction: Two sides of antitumoural activity of a standardised herbal formulation of Withania somnifera.	European journal of cancer	Immunomodulatory and Anti-tumoural Activity	Withania somnifera aqueous extract	BALB/c and Swiss albino mice	WSF at 150 mg/kg,	WSF at 150 mg/kg, i.p., inhibited >50% tumour growth in the mouse tumour models. In tumour-bearing mice, WSF inhibited the expression of pStat-3, with a selective stimulation of Th1 immunity as evidenced by enhanced secretion of IFN-c and IL-2. In parallel, it enhanced the proliferation of CD4+/CD8+ and NK cells along with an increased expression of CD40/CD40L/CD80. In addition, WSF also enhanced T cell activation in camptothecin treated tumour-bearing mice	Malik, F., Kumar, A., Bhushan, S., Mondhe, D. M., Pal, H. C., Sharma, R., Khajuria, A., Singh, S., Singh, G., Saxena, A. K., Suri, K. A., Qazi, G. N., & Singh, J. (2009). Immune modulation and apoptosis induction: Two sides of antitumoural activity of a standardised herbal formulation of Withania somnifera. *European journal of cancer (Oxford, England : 1990)* , *45* (8), 1494–1509. https://doi.org/10.1016/j.ejca.2009.01.034
95	Hepatoprotective and Antioxidant effects of naturally occurring Withasteroid metal ion conjugates of Withania somnifera in paracetamol induced hepatotoxicity in rat	Pharmacologyonline	Hepatoprotective	Dried and powdered extract of Withania somnifera	Albino rats (Sprague Dawley strain)	100mg/kg/body weight and 200mg/kg/body weight	Withania somnifera extract exhibited hepatoprotective and anti-oxidant effects in paramcetomal induced hepatotoxic rats	Ganguly P, Gupta AK, Majumder UK, Ghosal S. 2009. Hepatoprotective and antioxidant effects of naturally occurring withasteroid metal ion conjugates of Withania somnifera in paracetamol induced hepatotoxicity in rats. Pharmacologyonline. 1:1044–1056
96	Nephroprotective effect of Withania somnifera: a dose-dependent study	Renal failure	Nephroprotective	Withania somnifera root extract	Adult male albino Wistar rats	250, 500, and 750 mg/kg	Nephrotoxicity was evident in GEN-treated rats by significant increase in kidney weight, urea, creatinine, urinary protein, and glucose, and significant reduction in body weights and potassium, which was histopathologically confirmed by tubular necrosis. In contrast W. somnifera (500 mg/kg) significantly reversed these changes as evidenced microscopically when compared to other two doses of W. somnifera (250 and 750 mg/kg), and there were no significant changes in the levels of sodium in the experimental animals compared to control. Thus, our results suggested the nephroprotective effect of Withania somnifera, which could be by enhancing antioxidant activity with natural antioxidants and scavenging the free radicals.	Jeyanthi, T., & Subramanian, P. (2009). Nephroprotective effect of Withania somnifera: a dose-dependent study. Renal failure, 31(9), 814–821. https://doi.org/10.3109/08860220903150320
97	Possible neuroprotective effect of Withania somnifera root extract against 3-nitropropionic acid-induced behavioral, biochemical, and mitochondrial dysfunction in an animal model of Huntington's disease	Journal of medicinal food	Neuroprotective Activity	Withania somnifera root extract	Rats	100 and 200 mg/kg	Chronic treatment with W. somnifera root extracts (100 and 200 mg/kg) for a period of 2 weeks dose-dependently improved 3-NP-induced behavioral, biochemical, and enzymatic changes ($P < .05$). Biochemical analysis revealed that systemic 3-NP administration significantly increased lipid peroxidation and nitrite and lactate dehydrogenase enzyme levels, depleted antioxidant enzyme (superoxide dismutase and catalase) levels, and blocked ATP synthesis by inhibiting the mitochondrial complex activity in the different regions (striatum and cortex) of the brain. Chronic administration of W. somnifera root extract (100 and 200 mg/kg) dose-dependently restored biochemical alterations induced by chronic 3-NP treatment ($P < .05$). These findings suggest that neuroprotective actions of W. somnifera are mediated via its antioxidant activity. However, further studies are required to elucidate the molecular mechanisms involved in order to support the clinical use of the plant extract as a therapeutic agent for the treatment of HD.	Kumar, P., & Kumar, A. (2009). Possible neuroprotective effect of Withania somnifera root extract against 3-nitropropionic acid-induced behavioral, biochemical, and mitochondrial dysfunction in an animal model of Huntington's disease. Journal of medicinal food, 12(3), 591–600. https://doi.org/10.1089/jmf.2008.0028
98	Neuroprotective effects of Withania somnifera dunal.: A possible mechanism	Neurochemical research	Neuroprotective Activity	Withania somnifera root extract	Adult mice	40 mg/kg	Treatment with WS extract for 30 days during stress, significantly reversed the stress induced NADPH-d activation. Observations suggest that inhibition of NADPH-d by WS is not a direct effect of extract on NADPH-d, instead it inhibits via suppressing corticosterone release and activating cholineacetyltransferase, which in turn increase serotonin level in hippocampus to inhibit NADPH-d. Together, the main mechanism underlying the neuroprotective effects of WS can be attributed to its role in the down regulation of nNOS and neurochemical alterations of specific neurotransmitter systems. These observations thus suggest that WS root extract could be developed as a potential preventive or therapeutic drug for stress induced neurological disorders.	Bhatnagar, M., Sharma, D., & Salvi, M. (2009). Neuroprotective effects of Withania somnifera dunal.: A possible mechanism. Neurochemical research, 34(11), 1975–1983. https://doi.org/10.1007/s11064-009-9987-7
99	Hypoglycaemic and Hypolipidaemic Effects of Withania somnifera Root and Leaf Extracts on Alloxan-Induced Diabetic Rats	International Journal of Molecular Sciences	Hepatoprotective	Withania somnifera extract	Adult Albino Wistar strain rats	100mg/kg/body weight and 200mg/kg/body weight	Withania somnifera extracts aided in the restoration of AST and ALT to their respective normal level	Udayakumar R, Kasthurirengan S, Mariashibu TS, et al. Hypoglycaemic and hypolipidaemic effects of Withania somnifera root and leaf extracts on alloxan-induced diabetic rats. Int J Mol Sci. 2009;10(5):2367–2382. Published 2009 May 20. doi:10.3390/ijms10052367
100	In Vivo Effects of Ashwagandha (Withania somnifera) Extract on the Activation of Lymphocytes	The Journal of Alternative and Complementary Medicine	Immunomodulatory Activity	Withania somnifera root extract	Human Peripheral blood samples	6 mL of an Ashwagandha root extract twice daily	Significant increases were observed in the expression of CD4 on CD3þ T cells after 96 hours. CD56þ NK cells were also activated after 96 hours as evidenced by expression of the CD69 receptor.	Mikolai, J., Erlandsen, A., Murison, A., Brown, K. A., Gregory, W. L., Raman-Caplan, P., & Zwickey, H. L. (2009). In Vivo Effects of Ashwagandha (Withania somnifera) Extract on the Activation of Lymphocytes. The Journal of Alternative and Complementary Medicine, 15(4), 423–430. doi:10.1089/acm.2008.0215

101	The protective effect of a purified extract of Withania somnifera against doxorubicin-induced cardiac toxicity in rats	Cell biology and toxicology	Cardioprotective Activity	Withania somnifera extract	Adult male albino Wistar rats	300 mg/kg	WIT treatment ameliorated oxidative damage and protected against cardiotoxicity induced by DXR in rats. The protective effect of WIT may be mediated through its antioxidant, anti-inflammatory, and calcium-antagonistic properties.	Hamza, A., Amin, A., & Daoud, S. (2008). The protective effect of a purified extract of Withania somnifera against doxorubicin-induced cardiac toxicity in rats. Cell biology and toxicology, 24(1), 63–73. https://doi.org/10.1007/s10565-007-9016-z
102	Withania somnifera provides cardioprotection and attenuates ischemia-reperfusion induced apoptosis	Clinical nutrition (Edinburgh, Scotland)	Cardioprotective and Antioxidant Activity	Withania somnifera extract	Wistar rats	50 mg/kg	Ws prior-treatment favorably restored the myocardial oxidant-antioxidant balance, exerted marked anti-apoptotic effects {upregulated Bcl-2 ($p<0.001$) protein, decreased Bax ($p<0.01$) protein, and attenuated TUNEL positivity ($p<0.01$)}, and reduced myocardial damage as evidenced by histopathologic evaluation. The antioxidant and anti-apoptotic properties of Ws may contribute to the cardioprotective effects.	Mohanty, I. R., Arya, D. S., & Gupta, S. K. (2008). Withania somnifera provides cardioprotection and attenuates ischemia-reperfusion induced apoptosis. Clinical nutrition (Edinburgh, Scotland), 27(4), 635–642. https://doi.org/10.1016/j.clnu.2008.05.006
103	Effect of Withania Somnifera Root Powder on the Levels of Circulatory Lipid Peroxidation and Liver Marker Enzymes in Chronic Hyperammonemia	E-Journal of Chemistry	Hepatoprotective	Dried and powdered roots its aqueous suspension in 2% gum acacia was used	Adult male albino Wistar rats	500 mg/kg body weight/day	The reports suggest that Withania is a rich source of bioactive compounds and the mechanism by which the W. somnifera exerts a hepatoprotective effect in a hyperammonemic condition could be attributed to presence of natural antioxidants, its free radical scavenging and antioxidant properties and excess removal of urea related compounds.	Harikrishnan B, Subramanian P, Subash S (2008). Effect of Withania Somnifera Root Powder on the Levels of Circulatory Lipid Peroxidation and Liver Marker Enzymes in Chronic Hyperammonemia. E-Journal of Chemistry. 5:2090-9063
104	A standardized root extract of Withania somnifera and its major constituent withanolide-A elicit humoral and cell-mediated immune responses by up regulation of Th1-dominant polarization in BALB/c mice	Life sciences,	Immunomodulatory Activity	Withania somnifera root extract	Female Balb/c mice	Withania somnifera root extract (AGB) - 30mg/kg/body wt	The extract selectively, induced type 1 immunity because it guided enhanced expression of T helper cells (Th)1 cytokines interferon (IFN)-γ and interleukin (IL)-2 while Th2 cytokine IL-4 observed a moderate decline. Confirmation of Th1 polarization was obtained from augmented levels of IgG2a over IgG1 in the blood sera of AGB treated groups.	Malik, F., Singh, J., Khajuria, A., Suri, K. A., Satti, N. K., Singh, S., Kaul, M. K., Kumar, A., Bhatia, A., & Qazi, G. N. (2007). A standardized root extract of Withania somnifera and its major constituent withanolide-A elicit humoral and cell-mediated immune responses by up regulation of Th1-dominant polarization in BALB/c mice. *Life sciences* , *80* (16), 1525–1538. https://doi.org/10.1016/j.lfs.2007.01.029
105	The neuroprotective effect of Withania somnifera root extract in MPTP-intoxicated mice: an analysis of behavioral and biochemical variables	Cellular & molecular biology letters	Neuroprotective Activity	Withania somnifera root extract	Inbred adult male Albino mice	100 mg/kg	The ethanolic extract of Withania somnifera increases the striatum dopamine content and attenuates the effects of 6-hydroxy dopamine-induced parkinsonism in rats. In conclusion, this study provides evidence that ashwagandha possesses antioxidant properties, and indicates further study is necessary to establish its neuroprotective role.	Sankar, S. R., Manivasagam, T., Krishnamurti, A., & Ramanathan, M. (2007). The neuroprotective effect of Withania somnifera root extract in MPTP-intoxicated mice: an analysis of behavioral and biochemical variables. Cellular & molecular biology letters, 12(4), 473–481. https://doi.org/10.2478/s11658-007-0015-0
106	Protective effect of Withania somnifera root powder in relation to lipid peroxidation, antioxidant status, glycoproteins and bone collagen on adjuvant-induced arthritis in rats.	Fundamental & clinical pharmacology	Immunomodulatory and Anti-inflammatory activity	Withania somnifera root powder	Wistar strain albino rats	1000 mg/kg/day	Oral administration of W. somnifera root powder (1000 mg/kg body weight) resulted in the significant amelioration of the various biochemical parameters such as level of lipid peroxides, glycoproteins, and urinary constituents with the depletion of antioxidant status and bone collagen in arthritic animals. The results of this study clearly indicate that W. somnifera root powder is capable of rectifying the above biochemical changes in adjuvant arthritis.	Rasool, M., & Varalakshmi, P. (2007). Protective effect of Withania somnifera root powder in relation to lipid peroxidation, antioxidant status, glycoproteins and bone collagen on adjuvant-induced arthritis in rats. *Fundamental & clinical pharmacology* , *21* (2), 157–164. https://doi.org/10.1111/j.1472-8206.2006.00461.x
107	Augmentation and proliferation of T lymphocytes and Th-1 cytokines by Withania somnifera in stressed mice.	International immunopharmacology	Immunomodulatory Activity	Withania somnifera root extract	Balb/C mice	25, 50, 100 and 200 mg/kg p.o.	Oral administration of chemically standardized and identified aqueous fraction of W. somnifera root (WS) at the graded doses of 25, 50, 100 and 200 mg/kg p.o. caused significant increase in the stress-induced depleted T-cell population and increased the expression of Th1 cytokines in chronically stressed mice. Decrease in cortisol was seen.	Khan, B., Ahmad, S. F., Bani, S., Kaul, A., Suri, K. A., Satti, N. K., Athar, M., & Qazi, G. N. (2006). Augmentation and proliferation of T lymphocytes and Th-1 cytokines by Withania somnifera in stressed mice. *International immunopharmacology* , *6* (9), 1394–1403. https://doi.org/10.1016/j.intimp.2006.04.001
108	Selective Th1 up-regulating activity of Withania somnifera aqueous extract in an experimental system using flow cytometry	Journal of ethnopharmacology	Immunomodulatory Activity	Withania somnifera aqueous extract	Balb/C mice	Withania somnifera extract 25 to 400 mg/kg.	The results indicate that extract at 100 mg/kg resulted significant selective up-regulation of Th1 response. Treatment with extract showed significant increase in CD4 and CD8 counts as compared to control and cyclopsorin A, with a faster recovery of CD4+ T cells in immunesuppressed animals	Bani, S., Gautam, M., Sheikh, F. A., Khan, B., Satti, N. K., Suri, K. A., Qazi, G. N., & Patwardhan, B. (2006). Selective Th1 up-regulating activity of Withania somnifera aqueous extract in an experimental system using flow cytometry. *Journal of ethnopharmacology* , *107* (1), 107–115. https://doi.org/10.1016/j.jep.2006.02.016
109	Immunomodulatory role of Withania somnifera root powder on experimental induced inflammation: An in vivo and in vitro study.	Vascular pharmacology	Immunomodulatory and Anti-inflammatory activity	Withania somnifera root powder	Albino Wistar rats	1000 mg/kg b.wt	W. somnifera showed potent inhibitory activity towards the complement system, mitogen induced lymphocyte proliferation and delayed-type hypersensitivity reaction. Administration of W. somnifera root powder did not have a significant effect on humoral immune response in rats. The results of the study report immunosuppressive effect of W. somnifera root powder, and thus it could be used as a candidate for developing an immunosuppressive drug for the inflammatory diseases	Rasool, M., & Varalakshmi, P. (2006). Immunomodulatory role of Withania somnifera root powder on experimental induced inflammation: An in vivo and in vitro study. *Vascular pharmacology* , *44* (6), 406–410. https://doi.org/10.1016/j.vph.2006.01.015
110	Effect of Withania somnifera root extract on reserpine-induced orofacial dyskinesia and cognitive dysfunction	Phytotherapy research : PTR	Neuroprotective and Antioxidant	Withania somnifera root extract	Male Wistar rats	50 and 100 mg/kg	Chronic administration of Ws root extract dose dependently (50 and 100 mg/kg) and significantly reduced the lipid peroxidation and restored the decreased glutathione levels by chronic reserpine treatment. It also significantly reversed the reserpine-induced decrease in brain SOD and catalase levels in rats. The major findings of the present study indicate that oxidative stress might play an important role in the pathophysiology of reserpine-induced abnormal oral movements. In conclusion, Withania somnifera root extract could be a useful drug for the treatment of drug-induced dyskinesia.	Naidu, P. S., Singh, A., & Kulkarni, S. K. (2006). Effect of Withania somnifera root extract on reserpine-induced orofacial dyskinesia and cognitive dysfunction. Phytotherapy research : PTR, 20(2), 140–146. https://doi.org/10.1002/ptr.1823
111	Suppressive effect of Withania somnifera root powder on experimental gouty arthritis: An in vivo and in vitro study	Chemico-biological interactions	Immunomodulatory and Anti-inflammatory activity	Withania somnifera root powder	Adult male albino rats of Wistar strain	500/1000 mg/kg	On treatment with the W. somnifera root powder (500/1000 mg/kg body weight), the above changes were reverted back to near normal levels. W. somnifera also showed potent analgesic and antipyretic effect with the absence of gastric damage at different dose levels in experimental rats. For comparison purpose, non-steroidal anti-inflammatory drug (NSAID) indomethacin was used as a standard. These results provide evidence for the suppressive effect of W. somnifera root powder by retarding amplification and propagation of the inflammatory response without causing any gastric damage.	Rasool, M., & Varalakshmi, P. (2006). Suppressive effect of Withania somnifera root powder on experimental gouty arthritis: An in vivo and in vitro study. Chemico-biological interactions, 164(3), 174–180. https://doi.org/10.1016/j.cbi.2006.09.011
112	Immunomodulatory Activity of the Ayurvedic Formulation “Ashwagandha Churna”,	Pharmaceutical Biology	Immunomodulatory Activity	Ashwagandha churna	Albino Wistar rats	50 mg/kg/day, 100mg/kg/day, 200mg/kg/day, 300mg/kg/day	On oral administration, ashwagandha churna showed a significant increase in neutrophil adhesion and delayed-type hypersensitivity (DTH) response.	M. Suresh Gupta, H.N. Shivaprasad, M.D. Kharya & A.C. Rana (2006) Immunomodulatory Activity of the Ayurvedic Formulation “Ashwagandha Churna”, Pharmaceutical Biology, 44:4, 263-265, DOI: 10.1080/13880200600713949

113	Neuroprotective effects of Withania somnifera on 6-hydroxydopamine induced Parkinsonism in rats	Human & experimental toxicology	Neuroprotective Activity	Withania somnifera extract	Male Wistar rats	Pretreated with 100, 200 and 300 mg/kg	W. somnifera extract was found to reverse all the parameters significantly in a dose-dependent manner. Thus, the study demonstrates that the extract of W. somnifera may be helpful in protecting the neuronal injury in Parkinson's disease.	Ahmad, M., Saleem, S., Ahmad, A. S., Ansari, M. A., Yousuf, S., Hoda, M. N., & Islam, F. (2005). Neuroprotective effects of Withania somnifera on 6-hydroxydopamine induced Parkinsonism in rats. Human & experimental toxicology, 24(3), 137–147. https://doi.org/10.1191/0960327105ht509oa
114	Mechanisms of cardioprotective effect of Withania somnifera in experimentally induced myocardial infarction	Basic & clinical pharmacology & toxicology	Cardioprotective Activity	Withania somnifera root extract	Wistar albino male rats	25, 50 and 100 mg/kg	The data show that Withania somnifera (25, 50 and 100 mg/ kg) exerts a strong cardioprotective effect in the experimental model of isoprenaline-induced myonecrosis in rats. Augmentation of endogenous antioxidants, maintenance of the myocardial antioxidant status and significant restoration of most of the altered haemodynamic parameters may contribute to its cardioprotective effect. Among the different doses studied, Withania somnifera at 50 mg/kg dose produced maximum cardioprotective effect.	Mohanty, I., Arya, D. S., Dinda, A., Talwar, K. K., Joshi, S., & Gupta, S. K. (2004). Mechanisms of cardioprotective effect of Withania somnifera in experimentally induced myocardial infarction. Basic & clinical pharmacology & toxicology, 94(4), 184–190. https://doi.org/10.1111/j.1742-7843.2004.pto940405.x
115	Cardioprotection from ischemia and reperfusion injury by Withania somnifera: a hemodynamic, biochemical and histopathological assessment	Molecular and cellular biochemistry	Cardioprotective Activity	Withania somnifera root extract	Albino Wistar rats	25, 50 and 100 mg/kg	Ws on chronic administration markedly augmented antioxidants (GSH, GSHPx, SOD, CAT) while Vit E did not stimulate the synthesis of endogenous antioxidants compared to sham. Results indicate that Ws significantly reduced myocardial injury and emphasize the beneficial action of Ws as a cardioprotective agent.	Gupta, S. K., Mohanty, I., Talwar, K. K., Dinda, A., Joshi, S., Bansal, P., Saxena, A., & Arya, D. S. (2004). Cardioprotection from ischemia and reperfusion injury by Withania somnifera: a hemodynamic, biochemical and histopathological assessment. Molecular and cellular biochemistry, 260(1-2), 39–47. https://doi.org/10.1023/b:mcbi.0000026051.16803.03
116	Immune response modulation to DPT vaccine by aqueous extract of Withania somnifera in experimental system	International immunopharmacology	Immunomodulatory Activity	Withania somnifera aqueous root extract	Swiss albino mice	Withania somnifera 100 mg/kg/day	Treatment of immunized animals with Withania somnifera (100 mg/kg/day) for 15 days resulted in significant increase of antibody titers to B. pertussis (P=0.000007). The results indicate that the application of the Withania somnifera as potential immunopotentiating agent possible applications in immunochemical industry	Gautam, M., Diwanay, S. S., Gairola, S., Shinde, Y. S., Jadhav, S. S., & Patwardhan, B. K. (2004). Immune response modulation to DPT vaccine by aqueous extract of Withania somnifera in experimental system. *International immunopharmacology*, *4* (6), 841–849. https://doi.org/10.1016/j.intimp.2004.03.005
117	Effect of Withania somnifera root extract on haloperidol-induced orofacial dyskinesia: possible mechanisms of action	Journal of medicinal food	Neuroprotective Activity	Withania somnifera root extract	Male Wistar rats	100–300 mg/kg	HP-treated rats significantly developed these extrapyramidal symptoms, but coadministration of Ws root extract (100–300 mg/kg) dose-dependently reduced them. Biochemical analysis revealed that chronic HP treatment significantly increased lipid peroxidation and decreased forebrain levels of glutathione and the antioxidant defense enzymes, superoxide dismutase (SOD) and catalase. Coadministration of Ws extract significantly reduced the lipid peroxidation and significantly reversed the decrease in forebrain SOD and catalase levels but had no significant effect on the HP-induced decrease in forebrain glutathione levels. These findings strongly suggest that oxidative stress plays a significant role in HP-induced orofacial dyskinesia and that Ws could be effective in preventing neuroleptic-induced extrapyramidal side effects.	Naidu, P. S., Singh, A., & Kulkarni, S. K. (2003). Effect of Withania somnifera root extract on haloperidol-induced orofacial dyskinesia: possible mechanisms of action. Journal of medicinal food, 6(2), 107–114. https://doi.org/10.1089/109662003322233503
118	Withania somnifera (Ashwagandha) attenuates antioxidant defense in aged spinal cord and inhibits copper induced lipid peroxidation and protein oxidative modifications	Drug metabolism and drug interactions	Neuroprotective and Antioxidant	Withania somnifera extract	Adult male Wistar rats	1g/kg	When the pretreatment time of W. somnifera was increased to 30 days, it prevented motor impairment and significantly decreased the raised levels of MDA compared with vehicle-treated rat. The beneficial effect of W. somnifera may be because of the attenuation of free radicals by an increased anti-oxidant defence. This was further supported biochemically because the levels of MDA, a marker of oxidative stress, were not elevated in the Withania somnifera treated rats. Because ischaemia-induced neuronal damage is a complex process involving various neurotransmitters and enzymes, such as glutamate, calcium, nitric oxide and cyclo-oxygenase, inhibition of these neurotransmitters and enzymes by W. somnifera cannot be ruled out as mechanisms mediating its protective effects. The present study provides, to the best of our knowledge, the first experimental evidence suggesting a potential benefit of W. somnifera treatment in the management of acute ischaemic stroke.	Gupta, S. K., Dua, A., & Vohra, B. P. (2003). Withania somnifera (Ashwagandha) attenuates antioxidant defense in aged spinal cord and inhibits copper induced lipid peroxidation and protein oxidative modifications. Drug metabolism and drug interactions, 19(3), 211–222. https://doi.org/10.1515/dmdi.2003.19.3.211
119	Effect of Withania somnifera on cell mediated immune responses in mice.	Journal of experimental & clinical cancer research : CR,	Immunomodulatory Activity	Withania somnifera extract	Mice	N/A	Administration of Withania extract was found to enhance the proliferation of lymphocytes, bone marrow cells and thymocytes in responses to mitogens. Withania treated splenocytes along with the mitogen LPS (10 microg/ml) could stimulate the lymphocyte proliferation six times more than the normal. Natural killer cell activity (NK) was found to be enhanced significantly in both the normal and the tumour bearing group. Antibody dependent cellular cytotoxicity (ADCC) was found to be enhanced in the Withania treated group on the 9th day. An early Antibody dependent complement mediated cytotoxicity (ACC) was observed in the Withania treated group on day 13.	Davis, L., & Kuttan, G. (2002). Effect of Withania somnifera on cell mediated immune responses in mice. *Journal of experimental & clinical cancer research : CR*, *21* (4), 585–590.
120	Cell proliferation and natural killer cell activity by polyherbal formulation, Immu-21 in mice.	Indian journal of experimental biology	Immunomodulatory Activity	Polyherbal - Immu-21: Ocimum sanctum, Withania somnifera, Emblica officinalis and Tinospora cordifolia	Mice	Intraperitoneal (i.p.) treatment with Immu-21 (30 mg/kg) once a day	Treatment of Immu-21 (30 mg/kg, i.p.) for 14 days and 1 mg/kg for 21 days significantly increased LPS induced leukocyte proliferation. NK cell activity was significantly increased when mice were pretreated with Immu-21 (10 and 30 mg/kg, i.p.) once a day for 7 days. The results indicate that pretreatment with Immu-21 selectively increased the proliferation of splenic leukocyte to B cell mitogen, LPS and cytotoxic activity against K 562 cells in mice.	Nemmani, K. V., Jena, G. B., Dey, C. S., Kaul, C. L., & Ramarao, P. (2002). Cell proliferation and natural killer cell activity by polyherbal formulation, Immu-21 in mice. *Indian journal of experimental biology*, *40* (3), 282–287.
121	Withania somnifera root extract prevents DMBA-induced squamous cell carcinoma of skin in Swiss albino mice.	Nutrition and cancer	Immunomodulatory Activity	Withania somnifera root extract	Swiss albino mice	WSRE was administered at 100, 200, 400, and 800 mg/kg body wt po to mice three times per week on alternate days for a total of 25 wk.	Administration of WSRE was found to significantly restore GSH and antioxidant enzymes suggestive of free radical scavenging activity of WSRE. From the results, it can be inferred that WSRE possesses potential chemopreventive activity in this experimental model of cancer. The chemopreventive activity may be linked to the antioxidant/free radical-scavenging constituents of the extract. The anti-inflammatory and immunomodulatory properties of WSRE are also likely to contribute to its chemopreventive action.	Prakash, J., Gupta, S. K., & Dinda, A. K. (2002). Withania somnifera root extract prevents DMBA-induced squamous cell carcinoma of skin in Swiss albino mice. *Nutrition and cancer*, *42* (1), 91–97. https://doi.org/10.1207/S15327914NC421_12
122	Neuroprotective effects of Withania somnifera Dunn. in hippocampal sub-regions of female albino rat	Phytotherapy research : PTR	Nephroprotective	Withania somnifera root powder	Swiss albino rats (Female)	20 mg/kg	Treatment with W. somnifera root powder extract significantly reduced (80%) the number of degenerating cells in both the areas. The study thus demonstrates the antistress neuroprotective effects of W. somnifera.	Jain, S., Shukla, S. D., Sharma, K., & Bhatnagar, M. (2001). Neuroprotective effects of Withania somnifera Dunn. in hippocampal sub-regions of female albino rat. Phytotherapy research : PTR, 15(6), 544–548. https://doi.org/10.1002/ptr.802

123	Immunomodulatory activity of Withania somnifera	Journal of ethnopharmacology	Immunomodulatory Activity	Withania somnifera root extract	Balb/c mice	Withania root extract (20 mg/dose/animal	WS enhanced the total WBC count (17 125 cells/mm3) on 10th day. Bone marrow cellularity (27×106 cells/femur) as well as a-esterase positive cell number (1800/4000 cells) also increased significantly (*P* f0.001) after the administration of Withania extract. Withania extract inhibited delayed type hypersensitivity reaction in mice and also showed an enhancement in phagocytic activity of peritoneal macrophages when compared to control mice. These results confirm the immunomodulatory activity of W. somnifera extract	Davis, L., & Kuttan, G. (2000). Immunomodulatory activity of Withania somnifera. *Journal of ethnopharmacology* , *71* (1-2), 193–200. https://doi.org/10.1016/s0378-8741(99)00206-8
124	Effect of Withania somnifera Glycowithanolides on Iron-induced Hepatotoxicity in Rats	Phytotherapy Research	Hepatoprotective	Aqueous concentrate of WS roots	Adult male CF strain rats	10, 20 and 50 mg/kg body weight	WS in Ayurveda for hepatoprotection against heavy metals and other environmental toxins, may be due the antioxidant action of WSG	Bhattacharya, A., Ramanathan, M., Ghosal, S. and Bhattacharya, S.K. (2000), Effect of Withania somnifera glycowithanolides on iron-induced hepatotoxicity in rats. Phytother. Res., 14: 568-570. doi:10.1002/1099-1573(200011)14:7
125	Adaptogenic and cardioprotective action of ashwagandha in rats and frogs	Journal of ethnopharmacology	Cardioprotective Activity	Withania somnifera root extract	Male Albino rats	100 mg/kg	Ashwagandha treatment increased the duration of contractility in functional test for the resistance of frog heart muscle towards the toxic action of strophanthin-K. Ashwaaandha treatment also resulted in significant increase in coagulation time which attains normalcy 7 days after cessation of treatment. Ashwagandha possesses no toxicity up to a dose of (100 mg/kg; p.o. for 180 days) and does not cause significant changes in biochemical parameters in the blood serum of rats. Increase in catecholamine content in the heart and aortic tissues and their decrease in adrenal glands are unfavourable effects of high doses of ashwagandha. On the basis of these observations, it was concluded that ashwagandha possesses adaptogenic, cardiotropic, cardioprotective and anticoagulant properties.	Dhuley J. N. (2000). Adaptogenic and cardioprotective action of ashwagandha in rats and frogs. Journal of ethnopharmacology, 70(1), 57–63. https://doi.org/10.1016/s0378-8741(99)00177-4
126	Curative property of Withania somnifera Dunal root in the context of carbendazim-induced histopathological changes in the liver and kidney of rat	Phytomedicine	Hepatoprotective	Dried Withania powder	Three months old Wistar strain male albino rat	250 mg/kg body weig	Results indicated that Withania somnifera would be an effective curative for carbendazim-induced histopathological changes in the liver and kidney	Akbarsha, M. A., Vijendrakumar, S., Kadalmani, B., Girija, R., & Faridha, A. (2000). Curative property of Withania somnifera Dunal root in the context of carbendazim-induced histopathological changes in the liver and kidney of rat. Phytomedicine, 7(6), 499–507. doi:10.1016/s0944-7113(00)80036-7
127	Studies on immunomodulatory activity of Withania somnifera (Ashwagandha) extracts in experimental immune inflammation	Journal of ethnopharmacology	Immunomodulatory Activity	Withania somnifera extract	Swiss albino mice	WST, was studied in doses of 100 and 1000 mg/kg, and WS2 as 150 and 300 mg/kg.	Results of an antibody (IgE)-mediated anaphylactic system, namely the active paw anaphylaxis model, reveal Ashwagandha as an anti-allergic drug, the activity being more pronounced with WS2 as compared with WST. A significant increase in white blood cell counts and platelet counts was observed in animals treated with WST. A protective effect in cyclophosphamide-induced myelosuppression was observed in animals treated with WST and WS2, revealing a significant increase in white blood cell counts and platelet counts.	Agarwal, R., Diwanay, S., Patki, P., & Patwardhan, B. (1999). Studies on immunomodulatory activity of Withania somnifera (Ashwagandha) extracts in experimental immune inflammation. *Journal of ethnopharmacology* , *67* (1), 27–35. https://doi.org/10.1016/s0378-8741(99)00065-3
128	Suppressive effect of cyclophosphamide-induced toxicity by Withania somnifera extract in mice	Journal of ethnopharmacology	Immunomodulatory Activity	Withania somnifera extract	Swiss albino mice	20 mg/dose per animal	Administration of Withania extract increased the number of alpha-esterase positive cells (1130/4000 cells) in the bone marrow of CTX treated animals, compared to the CTX-alone treated group (687/4000 cells). The major activity of Withania somnifera may be the stimulation of stem cell proliferation. These studies indicate that Withania somnifera could reduce the cyclophosphamide induced toxicity and its usefulness in cancer therapy.	Davis, L., & Kuttan, G. (1998). Suppressive effect of cyclophosphamide-induced toxicity by Withania somnifera extract in mice. Journal of ethnopharmacology, 62(3), 209–214. https://doi.org/10.1016/s0378-8741(98)00039-7
129	Studies on the immunomodulatory effects of Ashwagandha	Journal of ethnopharmacology	Immunomodulatory Activity	Withania somnifera extract	Albino mice	100mg/kg body weight	A significant modulation of immune reactivity was observed in all the three animal models used. Ashwagandha prevented myelosuppression in mice treated with all three immunosuppressive drugs tested. A significant increase in hemoglobin concentration ($P < 0.01$), red blood cell count ($P < 0.01$), white blood cell count ($P < 0.05$), platelet count ($P < 0.01$), and body weight ($P < 0.05$) was observed in Ashwagandha-treated mice as compared with untreated (control) mice.	Ziauddin, M., Phansalkar, N., Patki, P., Diwanay, S., & Patwardhan, B. (1996). Studies on the immunomodulatory effects of Ashwagandha. *Journal of ethnopharmacology* , *50* (2), 69–76. https://doi.org/10.1016/0378-8741(95)01318-0
130	Immunomodulatory and CNS effects of sitoindosides IX and X, two new glycowithanolides from *Withania somnifera*	Phytotherapy Research	Neuroprotective Activity	Two new glycowithanolides, sitoindoside IX (1) and sitoindoside X (Z), isolated from *Withania somnifera* .	Swiss mice and Wistar strain albino rats	Two new glycowithanolides, sitoindoside IX (1) and sitoindoside X (Z), isolated from *Withunia somnifera* . 100–400 µg/mouse, 50–200 mg/kg p.o	The two compounds, in doses of 100–400 µg/mouse, produced statistically significant mobilization and activation of peritoneal macrophages, phagocytosis and increased activity of the lysosomal enzymes secreted by the activated macrophages. Both these compounds (50–200 mg/kg p.o.) also produced significant anti-stress activity in albino mice and rats and augmented learning acquisition and memory retention in both young and old rats.	Ghosal, S., Lal, J., Srivastava, R., Bhattacharya, S.K., Upadhyay, S.N., Jaiswal, A.K. and Chattopadhyay, U. (1989), Immunomodulatory and CNS effects of sitoindosides IX and X, two new glycowithanolides from Withania somnifera. Phytother. Res., 3: 201-206. doi:10.1002/ptr.2650030510

Annexure - IV - Toxicity Studies

S.no	Title	Journal	Extract used	Model	Dose	Results and Conclusion	Reference	Links
1	Acute Oral Toxicity Study of KSM-66 Ashwagandha root extract in Wistar Rats	N/A	Ashwagandha Root Extract (KSM-66)	Female nulliparous and non-pregnant Wistar Rats	2000mg/kg	No mortality and morbidity were observed during acclimatization and following test item administration till the day of necropsy. All animals appeared normal and did not show any clinical signs of toxicity at 30 minutes, 1, 2 and 4 h on day 1 and from day 2 to 15 following test item administration. All animals showed gain in body weight on day 8 and 15 in comparison to their day 1 body weight. Body weight of both the step animals was found to be normal throughout the experimental period. No gross lesions were observed in all animals of both the steps during the necropsy Based on the observations, the LD50 cut-off value of KSM-66 Ashwagandha Root Extract was found to be greater than 5000 mg/kg body weight and classified as Category-5 or unclassified, as per Globally Harmonized Classification System (GHS) for Chemical Substances and Mixtures	To be published	N/A
2	Acute Oral Toxicity Study of KSM-66 Ashwagandha in Rats	N/A	Ashwagandha Root Extract (KSM-66)	Albino Wistar female Rats	5mg, 50mg, 300mg, and 2000mg	The test compound did not cause mortality and symptoms in the treated rats at any dose level. The body weight, feed intake data also revealed no significant change in the treated animals when compared to control. The results reveal that KSM-66 Ashwagandha caused no adverse effects nor produced any toxicity in the treated animals in sighting as well as in the main study. Hence KSM-66 Ashwagandha is practically safe for rats up to 2000mg/kg body dose level by oral route observed for 14 days.	To be published	N/A
3	Two Weeks Dose Range-Finding Study of KSM-66 Ashwagandha root extract by oral route in Wistar rats	N/A	Ashwagandha Root Extract (KSM-66)	Male and Female (nulliparous and non-pregnant) Wistar Albino Rats	500, 1000 and 2000 mg/kg	Mortality and morbidity was not observed in any of the animals of test item treated groups and as well as control group during the study. No test item related clinical signs of toxicity were observed in any of test item treated group throughout the experimental period. The body weights and body weight changes of test item treated group animals did not reveal any test item related effect when compared to vehicle control throughout the experimental period. The test item did not have any effect on feed consumption of animals in any of treated groups when compared to vehicle control. Absolute and relative organ weight of all groups did not reveal any test item related statistically significant effect No test item related gross pathological findings were observed in any organ or tissue in test item treated groups of either sex. Under the conditions of this study and based on the toxicological endpoints evaluated, the test item KSM-66 Ashwagandha Root Extract was found to be well tolerated up to 2000 mg/kg b.wt. when administered orally for a period of 14 days in Wistar rats	To be published	N/A
4	28 Days Repeated Oral Dose Study of KSM-66 Ashwagandha in Rats	N/A	Ashwagandha Root Extract (KSM-66)	Albino Wistar Male and Female Rats.	500 mg/kg, 1000 mg/kg and, 2000 mg/kg	The test compound did not cause mortality and symptoms in the treated rats at any dose level. However, increase trend in body weight gain was observed in male and female rats. Whereas feed intake data showed no significant effect in food consumption of the treated animals when compared to the control. The results revealed that the test material caused no adverse effects nor produced any toxicity in the treated animals. Therefore, it can be suggested based on the results that KSM-66 Ashwagandha is practically safe and non-toxic to rats up to 2000 mg/kg dose level by oral route given daily for 28 days	To be published	N/A
5	90-Day Repeated Dose Oral Toxicity Study of KSM-66 Ashwagandha root extract in Wistar Rats	N/A	Ashwagandha Root Extract (KSM-66)	Male and Female (nulliparous and non-pregnant) Wistar Albino Rats	500 mg/kg, 1000 mg/kg and, 2000 mg/kg	No signs of delayed toxicity were also observed after treatment period for 14 days. Under the conditions of this study and based on the toxicological endpoints evaluated, the test item KSM-66 Ashwagandha Root Extract was observed to be 2000mg/kg b.wt. when administered orally for a period of 90 days in Wistar rats. Hence the No-Observed Adverse Effect Level (NOAEL) of KSM-66 Ashwagandha Root Extract is found to be 2000 mg/kg/day by oral route.	To be published	N/A
6	Acute and 28 days Repeated dose Genotoxicity Study of KSM-66 Ashwagandha in Albino Rats	N/A	Ashwagandha Root Extract (KSM-66)	Albino Wistar Male and Female Rats	500, 1000, 2000 mg/kg	In the current study the in vivo genotoxicity assessment of ashwagandha was carried out in peripheral blood cells of rats using comet assay i.e., single cell gel electrophoresis. It was observant that there was no significant increase in % tail DNA at all time intervals after treatment when compared to controls in both the acute and sub-acute groups. From this study it can be concluded that the compound administered was not toxic at the tested doses i.e., 2000, 1000, 500mg/kg b. wt. for acute as well as 250, 500, 1000mg/kg b. wt./day for subacute groups of the animals.	To be published	N/A
7	Bacterial Reverse Mutation Test with KSM-66 Ashwagandha root extract in Salmonella Typhimurium Strains	N/A	Ashwagandha Root Extract (KSM-66)	Salmonella typhimurium Lyophilized Disc - TA strains (TA 98, TA 100, TA 102, TA 1535, and TA 1537)	0.3125, 0.625, 1.25, 2.5 and 5 mg/plate both in the presence (5% v/v S9 mix)	It is concluded that the test item KSM-66 Ashwagandha Root Extract did not induce any point mutations by base substitutions or frameshift in the genome of Salmonella typhimurium tester strains and was found to be non-mutagenic up to the tested concentration of 5 mg/plate both in presence and absence of metabolic activation system under the tested experimental conditions.	To be published	N/A
8	In Vitro Chromosomal Aberration Test with KSM-66 Ashwagandha root extract in cultured human lymphocytes	N/A	Ashwagandha Root Extract (KSM-66)	Human peripheral blood lymphocytes	0.3125, 0.625, 1.25, 2.5 and 5 mg/plate both	KSM-66 Ashwagandha Root Extract at 0.25, 0.5, 1 and 2 mg/ml was not found to be cytotoxic both in the absence and presence of metabolic activation system as compared to concurrent vehicle control. o cytotoxicity was observed in the test item; KSM-66 Ashwagandha Root Extract at any of the tested concentrations of 0.5, 1 and 2 mg/ml both in the short-term exposure (4 h) as well as in continuous exposure (24 h). No significant difference in the chromosomal aberration frequency was observed between the vehicle controls and at any of the test concentrations of 0.5mg/ml, 1 mg/ml, and 2 mg/ml of KSM-66 Ashwagandha Root Extract. Based on the above observations, it is concluded that KSM-66 Ashwagandha Root Extract was considered to be non-clastogenic up to the tested concentration of 2 mg/ml under the above-mentioned experimental conditions.	To be published	N/A
9	In vivo Mammalian Erythrocyte Micronucleus Test with KSM-66 Ashwagandha root extract in Swiss Albino mice	N/A	Ashwagandha Root Extract (KSM-66)	Male and Female (nulliparous and non-pregnant) Swiss Albino Mouse	500 mg/kg, 1000 mg/kg and, 2000 mg/kg	In DRF study, no mortality and morbidity were observed in the TI-treated animals of either sex at all tested doses. No decrease in P/E ratio was observed in any of the tested dose levels as compared to negative control. For the main study No toxicity to bone marrow (decrease in P/E ratio) was observed in any of the treatment groups as compared to negative control. It is concluded that KSM-66 ashwagandha root extract under given experimental conditions, up to the guideline limit dose of 2000mg/kg, body weight has been considered to be non-clastogenic and did not induce any cytogenetic damage to the chromosomes or mitotic apparatus of erythroblast in bone marrow of Swiss albino mice.	To be published	N/A

10	Toxicity evaluation in zebrafish embryos	N/A	Ashwagandha Root Extract (KSM-66)	Zebrafish embryos	1, 3, 10, 25, 50 µg/ml	The EC10 value of the test substance was calculated from the previous assay and selected as the maximum dose for the thyroid disrupting assay. Afterward, the fluorescence assay was carried out exposing embryos to 5 concentrations of the test substance at 48 hpf, and fluorescence images of the thyroid gland of 15 embryos per condition were taken at 120 hpf. The signal intensity variations were calculated as fold-change values in comparison to the vehicle control. The test substance showed very similar levels to the vehicle control (FC = 0.90-1.14) and no statistically significant differences, indicating no treatment-related goitrogenic effect.	To be published	N/A
11	Acute and sub-acute oral toxicity assessment of the hydroalcoholic extract of Withania somnifera roots in Wistar rats	Phytotherapy research	Hydroalcoholic extract of Withania somnifera roots	Healthy female Wistar rats (nulliparous and non-pregnant); Wistar rats (male and female)	500, 1000 and 2000 mg/kg	There were no significant changes ($P < 0.05$) in the body weights, organ weights and haemato-biochemical parameters in any of the dose levels. No treatment related gross/histopathological lesions were observed. The present investigation demonstrated that the no observed adverse effect level was 2000 mg/kg body weight per day of hydroalcoholic extract of W. somnifera in rats and hence may be considered as non-toxic.	Prabu, P. C., Panchapakesan, S., & Raj, C. D. (2013). Acute and sub-acute oral toxicity assessment of the hydroalcoholic extract of Withania somnifera roots in Wistar rats. Phytotherapy research : PTR, 27(8), 1169–1178. https://doi.org/10.1002/ptr.4854	https://pubmed.ncbi.nlm.nih.gov/22996349/
12	Toxicity of Withania Somnifera Root Extract in Rats and Mice	International Journal of Pharmacognosy	Alcoholic Extracts From The Roots Of W. Somnifera	Swiss Albino Mice (Acute Toxicity and Wistar Rats (Subacute)	1100, 1200, 1300, 1400 or 1500 mg/kg	A Single Intraperitoneal Injection Of 1100 Mg/Kg Of The Extract In Mice Did Not Produce Any Deaths Within 24 H, But Small Increases Led To Mortality. The Ld50 Value Was Calculated As 1260 Mg/Kg Body Wt. Subacute Toxicity Studies With Repeated Injections Of Ashwagandha Extract At A Dose Of 100 Mg/Kg Body Wt. (= 1/12 Ld50) For 30 Days In Wistar Rats Of Either Sex Did Not Result In Any Mortality Or Changes In Peripheral Blood Constituents. However, Significant Reductions In The Weights Of Spleen, Thymus and Adrenals Were Observed In Male Rats At The End Of The Experiment. The Acid Phosphatase Content Of Peripheral Blood In Both Sexes Showed A Significant Increase From Control, While Other Biochemical Parameters Determined In The Study Were In The Normal Range	Sharada, A. C., Solomon, F. E., & Devi, P. U. (1993). Toxicity of Withania Somnifera Root Extract in Rats and Mice. International Journal of Pharmacognosy, 31(3), 205–212. doi:10.3109/13880209309082943	https://www.tandfonline.com/doi/abs/10.3109/13880209309082943
13	Antigenotoxic Effect of Withania somnifera (Ashwagandha) Extract Against DNA Damage Induced by Hydrogen Peroxide in Cultured Human Peripheral Blood Lymphocytes	International Journal of Current Microbiology and Applied Sciences	Withania somnifera extract	Cultured human peripheral blood lymphocytes	35 µg/ml and 70 µg/ml	Withania somnifera (35 µg/ml and 70 µg/ml) in presence of 20 µM of hydrogen peroxide had significantly reduced ($p<0.05$) the frequencies of SCEs as compared to PBL exposed to hydrogen peroxide only. Hence, our findings suggest that Withania somnifera can protect against DNA damage induced by hydrogen peroxide in cultured human PBL.	Neeraj Kumar, Anita Yadav, Ranjan Gupta and Neeraj Aggarwal. 2016. Antigenotoxic Effect of Withania somnifera (Ashwagandha) Extract Against DNA Damage Induced by Hydrogen Peroxide in Cultured Human Peripheral Blood Lymphocytes. Int.J.Curr.Microbiol.App.Sci.5(4): 713-719.	https://www.ijcmas.com/5-4-2016/Neeraj%20Kumar,%20et%20al.pdf
14	Safety assessment of Withania somnifera extract standardized for Withaferin A: Acute and sub-acute toxicity study	Journal of Ayurveda and Integrative Medicine	Standardized Withania somnifera extract (WSE)	Wistar rats	0 (control), 500, 1000, 2000 mg/kg	In acute toxicity studies, oral LD50 of WSE in Wistar rats was greater than 2000 mg/kg body weight. Compared to the control group in sub-acute toxicity study, administration of extract did not show any toxicologically significant treatment related changes in clinical observations, ophthalmic examination, body weight gain, feed consumption, clinical pathology evaluation, and organ weight. Hematological and serum chemistry parameters were within the normal limits. Terminal necropsy did not reveal any treatment related gross or histopathological findings. Based on this study, the no-observed-adverse-effect-level of WSE is 2000 mg/kg body weight, the highest level tested.	Patel, S. B., Rao, N. J., & Hingorani, L. L. (2016). Safety assessment of Withania somnifera extract standardized for Withaferin A: Acute and sub-acute toxicity study. Journal of Ayurveda and integrative medicine, 7(1), 30–37. https://doi.org/10.1016/j.jaim.2015.08.001	https://pubmed.ncbi.nlm.nih.gov/27297507/
15	Prenatal and Developmental Toxicity evaluation of Withania somnifera root extract in Wistar rats	Drug and chemical toxicology,	Hydroalcoholic extract of Withania somnifera roots	Young adult nulliparous female rats	500, 1000 and 2000 mg/kg/day	No evidence of maternal or foetal toxicity was observed. WSR extract caused no changes ($p < 0.05$) in body weight of parental females, number of corpora lutea, implantations, viable foetuses, external, skeletal and visceral malformations. Under the conditions of the study, the no-observed-effect level (NOEL) of WSR extract for maternal and developmental toxicity was concluded to be at least 2000 mg/kg/day.	Prabu, P. C., & Panchapakesan, S. (2015). Prenatal developmental toxicity evaluation of Withania somnifera root extract in Wistar rats. Drug and chemical toxicology, 38(1), 50–56. https://doi.org/10.3109/01480545.2014.900073	https://www.tandfonline.com/doi/abs/10.3109/01480545.2014.900073
16	Studies on phytochemistry and toxicity of Withania somnifera	International Journal of Animal, Veterinary, Fishery and Allied Sciences	Hydroalcoholic extract of Withania somnifera roots	Female Albino Rats	250, 500 and 1000 mg per kg body weight	During acute and chronic toxicity studies, some gross observational effects at the dose of 1000 mg per kg were recorded as the initial excitement, followed by mild depression, dullness, decreased respiration and reduced spontaneous motor activity but there was found no mortality in any group of rats.	Y.P. Sahni, M. Sharma and G.P. Pandey. (2014). Studies on phytochemistry and toxicity of Withania somnifera. IJAVFAAS, Vol. 1(1): 12-16	https://www.researchgate.net/publication/270761765_Studies_on_phytochemistry_and_toxicity_of_Withania_somnifera
17	Studies on Withania-ashwagandha, Kaul. V. The effect of total alkaloids (ashwagandholine) on the central nervous system	Indian journal of physiology and pharmacology	Total Alkaloids from the roots of Withania somnifera	Albino rats and mice	Ashwagandholine was adminstered in graded doses 100g/kg - 600mg/kg	LD50 in rats was 465 (332 to 651) mg/kg and in mice was 432 (299 to 626) mg/kg	Malhotra, C. L., Mehta, V. L., Das, P. K., & Dhalla, N. S. (1965). Studies on Withania-ashwagandha, Kaul. V. The effect of total alkaloids (ashwagandholine) on the central nervous system. Indian journal of physiology and pharmacology, 9(3), 127–136.	https://ijpp.com/IJPP%20archives/1965_9_3/127-136.pdf
18	Extraction of Ashwagandha by conventional extraction methods and evaluation of its anti-stress activity	International Journal of Green Pharmacy	100 g of W. somnifera dried roots were exhaustively extracted with various solvents (alcohol, water, hydro alcohol (50:50)	Female Albino Mice	5, 50, 300 and 2000mg/kg	The acute toxicity study showed that all the extracts of W. somnifera were safe up to 2,000 mg/kg body weight. Therefore, 2,000 mg/kg dose was considered as a safe dose for the extracts of W. somnifera.	H. Jain, S. D. Parial, E. Jarald, Anwar S. Daud, Showkat Ahmad (2010). Extraction of Ashwagandha by conventional extraction methods and evaluation of its anti-stress activity. International Journal of Green Pharmacy. Vol 4, No. 3 183-185	https://www.greenpharmacy.info/index.php/ijgp/article/view/143
19	Effects of long-term administration of the roots of ashwagandha and shatavari in rats	Indian Drugs	Aqueaous extract of Withania somnifera roots	Albino rats	250mg/kg p.o for 8 months	Chronic toxicity study reported that the administration of 250mg /kg p.o for 8 months was non toxic.	Sharma, S., S.A. Dahanukar and S.M. Karandikar (1985) Effects of long term administration of the roots of ashwagandha and shatavari in rats. Indian drugs 23, 133-139	https://www.researchgate.net/publication/279613173_Effect_of_time_on_extraction_of_Ashwagandha_in_various_Hydroalcoholic_compositions_and_their_anti-inflammatory_activity
20	Withania somnifera (L.) Dunal whole-plant extract demonstrates acceptable non-clinical safety in rat 28-day subacute toxicity evaluation under GLP-compliance.	Scientific reports	Withania somnifera Whole Plant Extract	Sprague Dawley rats	100, 300 and 1000 mg/kg/day	As compared to the vehicle-treated groups, WSWPE did not reveal any clinically significant alterations in the evaluated parameters. Accordingly, founded on the aforesaid premise, the NOAEL for WSWPE was determined to be 1000 mg/kg/day in both male and female rats.	Balkrishna A, Sinha S, Srivastava J, Varshney A. Withania somnifera (L.) Dunal whole-plant extract demonstrates acceptable non-clinical safety in rat 28-day subacute toxicity evaluation under GLP-compliance. Sci Rep. 2022 Jun 30;12(1):11047. doi: 10.1038/s41598-022-14944-x. PMID: 35773300; PMCID: PMC9246939.	https://www.nature.com/articles/s41598-022-14944-x

21	In silico, in vitro screening of plant extracts for anti-SARS-CoV-2 activity and evaluation of their acute and sub-acute toxicity	Phytomedicine Plus : International journal of phytotherapy and phytopharmacology	Withania somnifera extract	Wistar male and female rats.	**Acute toxicity study:** 2000mg/kg **Subacute toxicity study:** 0 mg/kg (control), 200 mg/kg (low dose), 400 mg/kg (medium dose), 800 mg/kg (high dose)	The in vivo acute and sub-acute toxicity study of E 4.5.2 did not show any significant toxicity.	Latha, D., Hrishikesh, D., Shiban, G., Chandrashekar, C., & Bharath, B. R. (2022). In silico, in vitro screening of plant extracts for anti-SARS-CoV-2 activity and evaluation of their acute and sub-acute toxicity. Phytomedicine Plus : International journal of phytotherapy and phytopharmacology, 2(2), 100233. https://doi.org/10.1016/j.phyplu.2022.100233	https://pubmed.ncbi.nlm.nih.gov/35403091/
22	Subacute toxicity study of the combination of ginseng (Panax ginseng) and ashwagandha (Withania somnifera) in rats: a safety assessment.	Indian journal of physiology and pharmacology	Roots of Ginseng and Ashwagandha	Albino rats of Haffkine strain	8.50 mglkg, 12.75 mg/kg and 17.00 mg/kg	There was significant increase in body weight. food consumption and liver weight and improved haematopoesis was observed. Brain. heart, lung, liver, spleen, kidneys, stomach, testis and ovaries were normal on gross examination and histopathologically. Subacute toxicity studies in rats did not reveal any toxicity.	Aphale, A. A., Chhibba, A. D., Kumbhakarna, N. R., Mateenuddin, M., & Dahat, S. H. (1998). Subacute toxicity study of the combination of ginseng (Panax ginseng) and ashwagandha (Withania somnifera) in rats: a safety assessment. Indian journal of physiology and pharmacology, 42(2), 299–302.	https://www.ijpp.com/IJPP%20archives/1998_42_2/299-302.pdf
23	28-day repeated dose toxicological evaluation of Coronil in Sprague Dawley rats: Behavioral, hematological, biochemical and histopathological assessments under GLP compliance	Drug and chemical toxicology	Coronil is a tri-herbal medicine - Tinospora cordifolia, Withania somnifera and Ocimum sanctum	Sprague Dawley rats	0, 100, 300 and 1000 mg/kg/day	In the current study, no mortality was observed in any group and in addition, Coronil did not elicit any finding of toxicological relevance with respect to clinical signs, ocular effects, hematology, urinalysis and clinical chemistry parameters, as well as macro- or microscopical changes in any organs, when compared to the control group. Accordingly, the No-Observed-Adverse-Effect-Level (NOAEL) of Coronil was ascertained to be 1000 mg/kg/day, subsequent to its 28-day oral administration to male and female rats. The acceptable safety profile of Coronil paves the way further toxicity assessments in rodents for a longer duration as well as in higher animals, and towards its clinical investigation.	Balkrishna, A., Sinha, S., & Varshney, A. (2022). 28-day repeated dose toxicological evaluation of Coronil in Sprague Dawley rats: Behavioral, hematological, biochemical and histopathological assessments under GLP compliance. Drug and chemical toxicology, 1–14. Advance online publication. https://doi.org/10.1080/01480545.2022.2036183	https://www.tandfonline.com/doi/abs/10.1080/01480545.2022.2036183?journalCode=idct20
24	Acute and Sub Chronic Toxicity Studies of Purified Withania Somnifera in rats	International Journal of Pharmacy and Pharmaceutical Sciences	Purified ashwagandha extract (PAE)	Sprague Dawley rats	100, 500, 1000 and 2000 mg/kg	In the acute toxicity study, no mortality or clinical signs of toxicity were observed in any of the animals at maximum recommended dose level of 2000 mg/kg bw; therefore the LD50 is>2000 mg/kg bw in rats. The repeated administration of PAE for 90 d in rats at the maximum dose level of 1000 mg/kg bw did not induce any observable toxic effects, when compared to its corresponding control animals. The hematology and biochemistry profile of treated rats was similar to control animals and difference was non-significant (p>0.05). The histopathology of major organs of all the control and treated animals was normal. In this study the NOAEL (No Observed Adverse Effect Level) was calculated as 1000 mg/kg bw daily for rats.	Antony, Benny; Benny, Merina; Kuruvilla, Binu T.; Gupta, Nishant Kumar; Sebastian, Anu; Jacob, Sherina (2018). ACUTE AND SUB CHRONIC TOXICITY STUDIES OF PURIFIED WITHANIA SOMNIFERA EXTRACT IN RATS. International Journal of Pharmacy and Pharmaceutical Sciences, 10(12), 41–. doi:10.22159/ijpps.2018v10i12.29493	https://innovareacademics.in/journals/index.php/ijpps/article/view/29493
25	Safety, toxicity and pharmacokinetic assessment of oral Withaferin-A in mice	Toxicology Reports	Withaferin A	Female BALB/c mice	Acute Toxitcity - 50 mg/kg, 300 mg/kg and 2000 mg/kg Subacute Toxicity - 10, 70 and 500 mg/kg	In the acute toxicity study, up to 2000 mg/kg of WA was well tolerated without any signs of toxicity or death. Upon physiological, serum chemistry, hematology and histopathogical examination, no features suggestive of drug-induced toxicity were observed at any dose levels, thereby confirming the No-Observed Adverse Effect Level (NOAEL) to be at least 500 mg/kg. Furthermore, the oral bioavailability of WA was evaluated using single intravenous and oral doses of 10 mg/kg and 70 mg/kg respectively using sparse sampling strategy. Bioanalysis was carried out using a validated LC-MS/MS method. The AUC of WA was found to be 3996.9 ± 557.6 ng/mL*h and 141.7 ± 16.8 ng/mL*h for the intravenous and oral routes of administration respectively. The oral bioavailability was determined to be 1.8%. To conclude, WA was found to be extremely safe even at high doses, with a low oral bioavailability.	Gupta, S.K., Jadhav, S., Gohil, D., Panigrahi, G.C., Kaushal, R.K., Gandhi, K., Patil, A., Chavan, P., Gota, V. (2022) Safety, toxicity and pharmacokinetic assessment of oral Withaferin-A in mice. Toxicology Reports. Volume 9, Pages 1204-1212	https://www.sciencedirect.com/science/article/pii/S2214750022001263
26	Acute and Sub-acute Toxicity Studies of a Patented Anti-anxiety Poly Herbal Formulation	Journal of Pharmacology and Toxicology	Polyherbal with roots of Withania somnifera (Solanaceae)	Sprague Dawley rats	Acute Toxitcity - 2000 mg/kg Subacute Toxicity - 200, 400 and 800mg/kg	No mortality was observed in acute toxicity study. In sub-acute toxicity study, no statistically significant (p>0.05) difference was observed with respect to feed intake, water intake, body weight changes, biochemical enzymes, biochemical metabolites, electrolytes, hematology and histopathology studies between control and treated rats.	Settu Dinesh Kumar, Muhasaparur Ganesan Rajanandh and Chamundeeswari Duraipandian, 2019. Acute and Sub-acute Toxicity Studies of a Patented Anti-anxiety Poly Herbal Formulation. Journal of Pharmacology and Toxicology, 14: 9-17	https://scialert.net/fulltext/?doi=jpt.2019.9.17
27	Acute oral toxicity study of “Polyherbal formulation (Rosmarinus officinals, Ashwaghandha and Amla) in Wistar rats”	Journal of Entomology and Zoology Studies	“Polyherbal formulation (Rosmarinus officinals, Ashwaghandha and Amla)	Healthy nulliparous and non-pregnant female Wister Albino rats	2000mg/kg	The Acute toxicity study of poly herbal formulation (Rosmarinus officinals, Ashwagandha and Amla) in female Wistar rats were dosed with 2000mg/Kg had no adverse effect on the behavioral responses of the tested rats up to 14 days of observation. Physical observations indicated no signs of changes in the skin, fur, eyes mucous membrane, behaviour patterns, tremors, salivation, and diarrhoea of the rats. There was no mortality was observed and No body weight changes or anatomical abnormalities were noted at necropsy. According to these findings, it was assumed that poly herbal formulation LD50 dose is above 2000 mg/kg.	Dr. Shivaraj Y, Kaveri KR, Dr. Asiya Nuzhat FB, Rajesh R. Acute oral toxicity study of “Polyherbal formulation (Rosmarinus officinals, Ashwaghandha and Amla) in Wistar rats”. J Entomol Zool Stud 2021;9(4):16-27.	https://www.entomoljournal.com/archives/2021/vol9issue4/PartA/9-4-19-862.pdf
28	Phytoremedial effect of Withania somnifera against arsenic-induced testicular toxicity in Charles Foster rats	Avicenna journal of phytomedicine	Withania somnifera extract	Charles Foster rats	100 mg/kg	The study revealed that after administration of sodium arsenite, there was a decrease in the sperm counts and sperm motility accompanied by an increased incidence of sperm abnormalities and hormonal imbalance leading to infertility. However, after administration of Withania somnifera, there was significant reversal in the parameters denoting that it not only possesses antioxidant and rejuvenating property but also maintains the cellular integrity of testicular cells leading to normal functioning of it. The study concludes that *Withania somnifera* possesses phytoremedial effect. It is one of the best antidotes against arsenic-induced reproductive toxicity.	Kumar, A., Kumar, R., Rahman, M. S., Iqubal, M. A., Anand, G., Niraj, P. K., & Ali, M. (2015). Phytoremedial effect of Withania somnifera against arsenic-induced testicular toxicity in Charles Foster rats. Avicenna journal of phytomedicine, 5(4), 355–364.	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4587609/
29	Assessment of acute toxicity and reproductive capability of a herbal combination	Pakistan journal of pharmaceutical sciences	Withania somnifera, Tribulus terrestris, Mucuna Pruriens and Argyreia speciosa	Mice	10,100 and 1000 mg/kg of herbal extract.	The result of acute oral toxicity reveals that product is safe up to the dose of 5000 mg/kg. The effects of study related to reproductive capability of drug on both sex reveals increase in reproduction rate up to two generations i.e. F(0) and F(1).	Riaz, A., Khan, R. A., Ahmed, S., & Afroz, S. (2010). Assessment of acute toxicity and reproductive capability of a herbal combination. Pakistan journal of pharmaceutical sciences, 23(3), 291–294.	https://pubmed.ncbi.nlm.nih.gov/20566442/

Annexure V - KSM-66 Toxicity & Safety Data

KSM-66 Ashwagandha® has been studied for a number of toxicological effects through many in-vitro and animal models. The following are the study details:

1. **Acute Oral Toxicity Study of KSM-66 Ashwagandha root extract in Wistar Rats**

   Study No: 1208/2020

   Testing Laboratories: Centre for Toxicology and Developmental Research, Sri Ramachandra Institute of Higher Education and Research.

   GLP Details: GLP Certified Facility (No. GLP/c-105/2017)

   Animals: Female nulliparous and non-pregnant Wistar Rats.

   Methods: This study was conducted according to the OECD test guideline 423, Acute Toxic Class Method adopted on 17th December 2001. The dose formulation of test item, KSM-66 Ashwagandha Root Extract was freshly prepared in 0.1 % CMC sodium (vehicle) prior to administration. The dose was administered once orally to overnight fasted female Wistar rats at a dose of 2000 mg/kg body weight in two steps. The dosing was performed based on their individual body weight taken on study day 1 and 10.0 mL/kg b.wt. was maintained as dose volume. In first step, three female animals were administered with a single dose of 2000 mg/kg b.wt. of test item, by oral route. The animals were observed for clinical signs of toxicity, mortality, and morbidity for 14 days. As all the animals survived and no clinical signs of toxicity were observed up to 72 h in first step, three more animals were administered with same single dose of 2000 mg/kg b.wt. of test item by oral route as second step. In both the steps, body weights of animals were recorded on day 1 (before dosing), 8 and 15. Mortality and morbidity was observed twice daily from acclimatization to till necropsy. Clinical signs were observed approximately at 30 min, 1, 2 and 4 h on day 0 (after test item administration) and thereafter once daily till necropsy. All animals were euthanized for gross pathology on day 15.

   Results: No mortality and morbidity were observed during acclimatization and following test item administration till the day of necropsy. All animals appeared normal and did not show any clinical signs of toxicity at 30 minutes, 1, 2 and 4 h on day 1 and from day 2 to 15 following test item administration. All animals showed gain in body weight on day 8 and 15 in comparison to their day 1 body weight. Body weight of both the step animals was found to be normal throughout the experimental period. No gross lesions were observed in all animals of both the steps during the necropsy.

Based on the observations, the LD50 cut-off value of KSM-66 Ashwagandha Root Extract was found to be greater than 5000 mg/kg body weight and classified as Category-5 or unclassified, as per Globally Harmonized Classification System (GHS) for Chemical Substances and Mixtures.

2. **Acute Oral Toxicity Study of KSM-66 Ashwagandha in Rats**

   Testing Laboratories: 'Indian Institute of Chemical Technology - Council of Scientific & Industrial Research'

   GLP Details: Tested under GLP environment

   Animals: Albino Wistar female Rats.

   Methods: The study was carried out in Albino Wistar female rats of 8-9 weeks old. The animals were procured from the National Institute of Nutrition, Hyderabad and they were acclimatized to the laboratory conditions for a week prior to the test. The animals were kept in a temperature (24±3ºC) and humidity (70±3%) controlled room with 12 hours light, 12 hours dark cycle throughout the experimental period. The rats were fasted overnight prior to the administration of the compound. The test material in the doses of 5mg, 50mg, 300mg, and 2000mg were orally given to the rats by gavage in sequential manner to find out the dose response toxicity. Since, no mortality and toxic symptoms were noted in any of the tested doses in the sighting study; the main study was completed treating five female rats with the dose of 2000mg/kg throughout the experimental period (14 days). During the observation period, the mortality, symptoms, feed intake and body weight were monitored and recorded daily. On termination of the observation period all the survived animals were sacrificed humanly and gross lesions if any were also noted.

   Results: The test compound did not cause mortality and symptoms in the treated rats at any dose level. The body weight, feed intake data also revealed no significant change in the treated animals when compared to control. The results reveal that KSM-66 Ashwagandha caused no adverse effects nor produced any toxicity in the treated animals in sighting as well as in the main study. Hence KSM-66 Ashwagandha is practically safe for rats up to 2000mg/kg body dose level by oral route observed for 14 days.

3. **Two Weeks Dose Range-Finding Study of KSM-66 Ashwagandha root extract by oral route in Wistar rats**

   Study No: 1209/2020

   Testing Laboratories: Centre for Toxicology and Developmental Research, Sri Ramachandra Institute of Higher Education and Research.

GLP Details: GLP Certified Facility (No. GLP/c-105/2017)

Animals: Male and Female (nulliparous and non-pregnant) Wistar Albino Rats

Methods: This study was conducted to determine the possible health hazards likely to arise from repeated oral administration to the test item “KSM-66 Ashwagandha Root Extract” for a period of two weeks (14 days) in Wistar rats and to select the dose levels for subsequent 90 Day Repeated Dose Toxicity Study of KSM-66 Ashwagandha Root Extract by Oral Route in Wistar Rats. Twenty male and twenty female Wistar rats were assigned to four groups viz., Control (G1), Low Dose (G2), Mid Dose (G3), High Dose (G4) consisting of five animals /sex / group. Vehicle control (G1) animals received 0.1% CMC sodium solution only, and G2, G3, and G4 animals received respective dose of test item at dose volume of 10.0 mL/kg b.wt. via oral route once daily for period of 14 days. Test item was formulated using 0.1% CMC sodium solution as the vehicle for Low Dose - G2 (500 mg/kg b.wt.), Mid Dose - G3 (1000 mg/kg b.wt.) and High Dose - G4 dose (2000 mg/kg b.wt.). All animals were observed for mortality/morbidity (twice daily); clinical signs of toxicity (daily cage side observation), detailed clinical examination (once prior to dosing and before necropsy day), weekly body weight and feed consumption. At the end of treatment period, the animals were sacrificed and subjected to detailed gross necropsy which includes gross examination of external orifices, the cranial, thoracic, and abdominal cavities, and their contents. On completion of the gross pathology examination, the selected organs were weighed from all animals.

Results: Mortality and morbidity was not observed in any of the animals of test item treated groups and as well as control group during the study. No test item related clinical signs of toxicity were observed in any of test item treated group throughout the experimental period. The body weights and body weight changes of test item treated group animals did not reveal any test item related effect when compared to vehicle control throughout the experimental period. The test item did not have any effect on feed consumption of animals in any of treated groups when compared to vehicle control. Absolute and relative organ weight of all groups did not reveal any test item related statistically significant effect No test item related gross pathological findings were observed in any organ or tissue in test item treated groups of either sex.

Conclusion: Under the conditions of this study and based on the toxicological endpoints evaluated, the test item KSM-66 Ashwagandha Root Extract was found to be well tolerated up to 2000 mg/kg b.wt. when administered orally for a period of 14 days in Wistar rats.

4. **28 Days Repeated Oral Dose Study of KSM-66 Ashwagandha in Rats**

Testing Laboratories: 'Indian Institute of Chemical Technology- Council of Scientific & Industrial Research

GLP Details: Tested under GLP environment

Animals: Albino Wistar Male and Female Rats.

Methods: The study was carried out in Albino Wistar male and female rats of 8-9 weeks old. The animals were procured from the National Institute of Nutrition, Hyderabad and they were acclimatized to the laboratory conditions for a week prior to the test. The animals were kept in a temperature (24±3ºC) and humidity (70±3%) controlled room with 12 hours light, 12 hours dark cycle throughout the experimental period. The rats were fasted overnight prior to the administration of the compound. The test material in the doses of 500 mg/kg, 1000 mg/kg and, 2000 mg/kg were orally given to the rats by gavage for twenty-eight days along with the control. In each dose group, five male and five female were kept. All the test animals were provided with standard laboratory feed and water ad libitum throughout experimental period. During the experimental period, the mortality, symptoms, feed intake and body weight were monitored and recorded daily. On termination of the experimental period all the survived animals were sacrificed humanly and gross lesions if any were also noted.

Results: The test compound did not cause mortality and symptoms in the treated rats at any dose level. However, increase trend in body weight gain was observed in male and female rats. Whereas feed intake data showed no significant effect in food consumption of the treated animals when compared to the control. The results revealed that the test material caused no adverse effects nor produced any toxicity in the treated animals. Therefore, it can be suggested based on the results that KSM-66 Ashwagandha is practically safe and non-toxic to rats up to 2000 mg/kg dose level by oral route given daily for 28 days

5. **90-Day Repeated Dose Oral Toxicity Study of KSM-66 Ashwagandha root extract in Wistar Rats**

Study no: 1210/2020

Testing Laboratories: Centre for Toxicology and Developmental Research, Sri Ramachandra Institute of Higher Education and Research

GLP Details: GLP Certified Facility (No. GLP/c-105/2017)

Animals: Male and Female (nulliparous and non-pregnant) Wistar Albino Rats

Methods: This study was conducted according to OECD test guideline 408 repeated dose 90- day oral toxicity study in rodents (adopted: 25th June 2018). One hundred animals (50 male and 50 female) rats were used for the study. Animals were assigned to six groups viz G1-Control, G1R-Control recovery, G2-500mg/kg, b.wt, G3-1000 mg/kg, b.wt G4 and G4R-2000 mg/kg,b.wt. Main group comprises 10 animal/sex/group and

Recovery group comprises for 5 animal/sex/group. G1 andG1 Control Recovery group animals were administered 0.1%CMC vehicle, treated groups G2, G3, G4 and G4R were administered orally (once in a day) in for a period of 90 days to determine No-Observed Adverse Effect Level (NOAEL). Recovery group animals (G1R and G4R) were observed for 14 days without test item administration to identify reversibility or persistence of any toxic effects.

All the animals from treated, control, recovery control and recovery groups were observed for clinical signs of toxicity once daily, detailed clinical examination every week after dosing day and before necropsy day. Mortality/Morbidity was observed twice daily. Weekly body weight and daily feed consumption were recorded for all the treated and vehicle control animals. At the end of treatment period, the animals were euthanized by CO2 and subjected to detailed gross necropsy which includes gross examination of external orifices, the cranial, thoracic, and abdominal cavities, and their contents. On completion of the gross pathology examination, the selected organs were weighed for all animals.

Results: Mortality and morbidity was not observed in any of the animals in control, treated, and recovery groups of animals during the study. No clinical signs of toxicity were observed in the control, treated, and recovery groups of animals from the day of test item administration till the end of the observation period. No test item related changes were observed in ophthalmoscope examination when compared to control, control recovery, treated and high dose recovery group of animals. No test item related changes in sensory reactivity and motor activity were observed in control, control recovery, treated and high dose recovery group of animals.

There were no test item-related changes observed in the control, control recovery, treated and high dose recovery group animals in haematology, electrolytes, and biochemistry parameters. In all the Control and treated group of animals in day 91 and Control Recovery and High dose Recovery dose group of animal's thyroid hormones T3, T4, TSH were analysed on the end of the observation period were there were no test related changes observed. No external gross pathological findings were observed in any of the animals at all treated dose levels including control group animals. No test item related internal gross pathological findings were observed in any animals of all treated group when compared with control. All other gross pathological findings observed were either related to physiological, to spontaneous or incidental changes.

No test item related gross pathological findings were observed in any organs or tissues with the control, control recovery, treated and high dose recovery groups animals. There was no statistically significant difference observed in absolute and relative organ weight of all treatment groups when compared to control group animals of both sexes. There were no significant changes in the recovery group during and post treatment observation. No signs of delayed toxicity were also observed after treatment period for 14 days.

Conclusion: Under the conditions of this study and based on the toxicological endpoints evaluated, the test item KSM-66 Ashwagandha Root Extract was observed to be 2000mg/kg b.wt. when administered orally for a period of 90 days in Wistar rats. Hence the No-Observed Adverse Effect Level (NOAEL) of KSM-66 Ashwagandha Root Extract is found to be 2000 mg/kg/day by oral route.

6. **In vivo Mammalian Erythrocyte Micronucleus Test with KSM-66 Ashwagandha root extract in Swiss Albino mice**

Study no: 1215/2020

Testing Laboratories: Centre for Toxicology and Developmental Research, Sri Ramachandra Institute of Higher Education and Research

GLP Details: GLP Certified Facility (No. GLP/c-105/2017)

Animals: Male and Female (nulliparous and non-pregnant) Swiss Albino Mouse

Methods: This study was conducted following the OECD Guideline for the Testing of Chemicals 474 (29th July 2016) was to assess the clastogenic potential of KSM-66 ashwagandha root extract in Swiss Albino Mice. 0.1% CMC was selected as the vehicle based on the solubility test of test item performed before dosing. In the dose range finding study, animals were grouped into 4 groups, 2 animals per sex per group. Negative control (vehicle, 0.1% CMC - 0 mg/ kg, b.wt., G1), Low dose - KSM-66 ashwagandha root extract (500 mg/ kg, b.wt., G2), Mid dose - KSM-66 ashwagandha root extract (1000 mg/ kg, b.wt., G3) and High dose - KSM-66 ashwagandha root extract (2000 mg/ kg, b.wt., G4).

All the animals were dosed by oral gavage for two days separated by 24 hours interval. The dose volume was 10 mL/kg body weight. Sampling of bone marrow was done within 23 - 24h of last dosing time. Animals were observed for clinical signs, mortality, morbidity, and body weight. Animals were euthanized and bone marrow was collected from both the femurs by flushing with 2 mL fetal bovine serum into a centrifuge tube. The suspension was centrifuged, and smear was prepared on two glass microscope slides per animal. The prepared smear was air dried and fixed with ethanol and stained with 5% Giemsa stain. Stained slides were air-dried and mounted using mountant DPX and examined under light microscopy with 100X oil objective. The cells were counted for PCE ratio among the total erythrocytes and 500 erythrocytes per animal were counted to determine PCE: TE ratio.

Based on the results of the DRF study, 500, 1000 and 2000 mg/kg, b.wt. of KSM-66 ashwagandha root extract were selected as low, mid, and high dose respectively for the main study.

Main study was conducted in 5 groups each comprising of 5 male animals. Negative control (vehicle- 0.1% CMC - 0 mg/kg, b.wt. G1), Positive Control (Cyclophosphamide monohydrate, 40 mg/kg, b.wt., G5), Low dose - KSM-66 ashwagandha root extract (500 mg/kg, b.wt., G2), Mid dose - KSM-66 ashwagandha root extract (1000 mg/kg, b.wt., G3) and High dose - KSM-66 ashwagandha root extract (2000 mg/kg, b.wt., G4).

KSM-66 ashwagandha root extract was formulated with vehicle. Positive control was formulated using water for injection. Mice were dosed via oral gavage for negative control and test item groups for two days separated by 24 hours interval, while positive control animals were dosed intraperitoneally only on the second day of dosing. The dose volume was 10 mL/kg body weight. Bone marrow was collected within 23 - 24h of last dosing. Observations were carried out for clinical signs, mortality, morbidity, and body weight. PCE (Polychromatic Erythrocyte) ratio among the total erythrocytes, PCE: TE ratio and percentage of MNPCEs were calculated for all test item treated groups and positive control and compared with the negative control.

Results: In DRF study, no mortality and morbidity were observed in the TI treated animals of either sex at all tested doses. No decrease in P/E ratio was observed in any of the tested dose levels as compared to negative control.

For the main study No toxicity to bone marrow (decrease in P/E ratio) was observed in any of the treatment groups as compared to negative control. Results of test item treated animals were comparable to concurrent negative control and well within the historical control values. The number and percentage of micronucleated PCE were not increased in any of the test item treated groups as compared to the negative control group. Positive control group yielded a statistically significant increase in micronucleated PCE as compared to the negative control group thereby illustrating the validity of the experiment.

Conclusion: From the above results, it is concluded that KSM-66 ashwagandha root extract under given experimental conditions, up to the guideline limit dose of 2000 mg/kg, body weight has been considered to be non-clastogenic and did not induce any cytogenetic damage to the chromosomes or mitotic apparatus of erythroblast in bone marrow of Swiss albino mice.

7. **Acute and 28 days Repeated dose Genotoxicity Study of KSM-66 Ashwagandha in Albino Rats**

Testing Laboratories: 'Indian Institute of Chemical Technology- Council of Scientific & Industrial Research '

GLP Details: Tested under GLP environment

Animals: Albino Wistar Male and Female Rats.

Methods: The acute as well as subacute genotoxicity studies were carried out as per OECD guidelines 420 and 407 respectively. Rats were randomly divided into three groups: the positive control, the control, and experimental groups. The experimental groups were again divided into three subgroups based on the selected doses of Ashwagandha (500, 1000, 2000 mg/kg b. wt.) for acute and (250, 500, 1000 mg/kg b. wt./day) for subacute studies. The test material, Ashwagandha was suspended in distilled water and administered orally to rats at the respective doses mentioned above. Control groups received distilled water, whereas the positive control groups were treated with cyclophosphamide 40m/kg I.P.

The comet assay was conducted for the genotoxicity assessment following the proposed guidelines with slight modifications. The peripheral blood was collected at different time intervals for acute and subacute studies. 20 µl of heparinized peripheral blood was mixed with 110 µl of 0.5% low melting point agarose (LMPA) in phosphate buffered saline (PBS) and applied to microscope slides pre-coated with 0.75% normal melting point agarose (NMPA) in PBS. The slides were covered with a coverslip and refrigerated for 5min to solidify the gel. The slides were immersed for at least 1h in ice-cold alkaline lysing solution final pH 10.0. The slides were then incubated for 20 min in ice cold electrophoresis solution followed by electrophoresis at 25V:300 mA for 25 min. After electrophoresis, the slides were then neutralized, were scored at 400x using a fluorescence microscope with a blue excitation filter and yellow emission filter. Quantification of DNA breakage was realized by using Comet Image Analysis System, version 5.5. The % tail DNA damage was used to evaluate DNA Damage.

Results: In the current study the in vivo genotoxicity assessment of ashwagandha was carried out in peripheral blood cells of rats using comet assay i.e., single cell gel electrophoresis. It was observant that there was no significant increase in % tail DNA at all time intervals after treatment when compared to controls in both the acute and sub-acute groups. From this study it can be concluded that the compound administered was not toxic at the tested doses i.e., 2000, 1000, 500mg/kg b. wt. For acute as well as 250, 500, 1000mg/kg b. wt./day for subacute groups of the animals.

8. **Bacterial Reverse Mutation Test with KSM-66 Ashwagandha root extract in Salmonella Typhimurium Strains**

Study no: 1213/2020

Testing Laboratories: Centre for Toxicology and Developmental Research, Sri Ramachandra Institute of Higher Education and Research.

GLP Details: GLP Certified Facility (No. GLP/c-105/2017)

Strain: Salmonella typhimurium Lyophilized Disc - TA strains (TA 98, TA 100, TA 102, TA 1535, and TA 1537)

Concentration: 0.3125, 0.625, 1.25, 2.5 and 5 mg/plate both in the presence (5% v/v S9 mix)

Source of metabolic activity: S9 mix was prepared freshly before use by adding cofactor mix (1.65 M $MgCl_2$, 0.4 M KCl solution, 0.1 M Glucose-6-phosphate and 1.0M NADP in 0.2M phosphate buffer (pH– 7.4)).

Criteria of Positivity: There are several criteria for determining a positive result such as:

- The strains should yield spontaneous revertant colony plate counts within the frequency ranges expected from the laboratory's historical control data and preferably within the range reported in the literature.
- Untreated controls should be used unless there are historical control data demonstrating that no deleterious or mutagenic effects are induced by the chosen solvent.
- There is no requirement for verification of a clear positive response.
- Equivocal results should be clarified by further testing preferably using a modification of experimental conditions.
- Negative results need to be confirmed on a case-by-case basis by altering the test item spacing and increasing the S9 mix concentration.
- In those cases where confirmation of negative results is not considered necessary, justification should be provided.

Methods and Results: The study was conducted following 1. OECD Guideline number 471, Bacterial Reverse Mutation Test adopted: 21st July 1997, corrected on 26th June 2020. Test item formed suspension in RO water (50 mg/mL), while it was found to be soluble in dimethyl sulfoxide (DMSO) (50 mg/mL). Hence DMSO was selected as the vehicle for the study. Precipitation was not observed at the highest tested concentration of 5 mg/plate.

A preliminary cytotoxicity (Range finding - RF) study was carried out using the tester strain TA100 in triplicates to determine the non-cytotoxic concentration range for the mutagenicity assay with the test item at the concentrations of 5, 2.5, 1.25, 0.625, 0.3125 and 0.15625 mg/plate both in the presence (5% v/v S9 mix) and absence of metabolic activation system. Cytotoxicity was not observed up to the tested concentration of 5 mg/plate both in the presence (5% v/v S9 mix) and absence of metabolic activation system. Normal background bacterial lawn was observed up to the tested concentration of 5 mg/plate both in the absence and presence (5% v/v S9 mix) of metabolic activation system.

Based on the results of RF, the main study (Trial I and Trial II) was conducted using the plate incorporation method with all the five tester strains TA98, TA100, TA102, TA1535 and TA1537.

In Trial I the tester strains were exposed to the test item KSM-66 Ashwagandha Root Extract at the concentration levels of 0.3125, 0.625, 1.25, 2.5 and 5mg/plate both in the presence (5% v/v S9 mix) and absence of metabolic activation system along with concurrent positive and negative controls. No positive increase in mean revertant colonies was observed in any of the tester strains at any of the tested concentrations as compared to concurrent vehicle control. Normal background bacterial lawn was observed in all the tester strains at all the tested concentrations as compared to concurrent vehicle control.

In Trial II the tester strains were exposed to the test item KSM-66 Ashwagandha Root Extract at the concentration levels of 0.128, 0.32, 0.8, 2 and 5 mg/plate both in the presence (10% v/v S9 mix) and absence of metabolic activation system along with concurrent positive and negative controls. No positive increase in mean revertant colonies was observed in any of the tester strains at any of the tested concentrations as compared to concurrent vehicle control. Normal background bacterial lawn was observed in all the tester strains at all the tested concentrations as compared to concurrent vehicle control. The spontaneous reversion rates in the vehicle control were within the range of historical control data. The mean number of revertants obtained for the positive control was more than two-fold for tester strains TA98, TA100, TA102 and more than threefold for tester strains TA1537, TA1535as compared to the concurrent vehicle control, thus demonstrating the sensitivity and validity of the test procedure. Conclusion: Based on the above results, it is concluded that the test item KSM-66 Ashwagandha Root Extract did not induce any point mutations by base substitutions or frameshift in the genome of Salmonella typhimurium tester strains and was found to be non-mutagenic up to the tested concentration of 5 mg/plate both in presence and absence of metabolic activation system under the tested experimental conditions.

9. **In Vitro Chromosomal Aberration Test with KSM-66 Ashwagandha root extract in cultured human lymphocytes**

Study no: 1214/2020

Testing Laboratories: Centre for Toxicology and Developmental Research, Sri Ramachandra Institute of Higher Education and Research.

GLP Details: GLP Certified Facility (No. GLP/c-105/2017)

Strain: Human peripheral blood lymphocytes

Concentration: 0.3125, 0.625, 1.25, 2.5 and 5 mg/plate both in the presence (5% v/v S9 mix)

Source of metabolic activity: Rat liver S9 mix procured from Krishgen Biosystems, Mumbai and stored in deep freezer, set at -75±5°C was used in the study. An adequate

amount of fresh S9 mix was prepared before start of the experiment by adding 10% Rat liver S9 fraction to cofactor mix for Concentration Range Finding and Main Study.

Criteria of Positivity: The following criteria were used:

- Concurrent vehicle control values are considered acceptable for inclusion in historical control data when the data falls within 95% control limit
- Concurrent positive control values fall within the laboratory historical control data and produce a statistically significant increase with concurrent vehicle control.
- Cell proliferation criteria in the solvent control were fulfilled to define the concentrations used in the main study
- All three experimental conditions (short term treatment with metabolic activation and without metabolic activation, long term treatment without metabolic activation) were tested unless one resulted in positive results.
- Adequate number of cells (300 well spread metaphase) were analysed in minimum three different concentrations
- Criteria for selection of top dose was consistent with the guideline and no reduction of 45±5% in mitotic index as compared to vehicle control was observed up to the highest tested concentration of 2 mg/mL.

Methods: This study was performed to identify whether the test item, KSM-66 Ashwagandha Root Extract causes any structural or numerical chromosomal aberrations (clastogenicity) in cultured human lymphocytes when exposed to both, with and without an exogenous metabolic activation using the invitro chromosomal aberration test in cultured human lymphocyte cell culture. The experiment was conducted following the OECD Guideline for the Testing of Chemicals 473 (Adopted on 29 July 2016) and mutually agreed protocol between CEFTE and the Sponsor. Clastogenicity of KSM-66 Ashwagandha Root Extract was conducted in cultured human lymphocytes with vehicle control and positive control. Four test item concentrations (0.25, 0.5, 1 and 2 mg/ml) were selected for concentration range finding study in presence and absence of metabolic activation system to determine the cytotoxicity of KSM-66 Ashwagandha Root Extract.

KSM-66 Ashwagandha Root Extract at 0.5, 1 and 2 mg/ml was selected as the concentrations for the main study (Short term treatment with and without metabolic activation S9, long term treatment without S9). Duplicate lymphocyte cultures were used for both, concentration range finding and main study. DMSO was used as vehicle control and positive controls such as Mitomycin-C (0.5µg/ml) for without S9 and Cyclophosphamide monohydrate (3µg/ml) for with S9 were used, respectively.

Results: KSM-66 Ashwagandha Root Extract at 0.25, 0.5, 1 and 2 mg/ml was not found to be cytotoxic both in the absence and presence of metabolic activation system as compared to concurrent vehicle control. The mean percent reduction values were 90.68, 88.14, 88.14, 77.12 in the presence (1% v/v) and 90.44, 91.30, 86.08, 81.74 in the

absence of metabolic activation system at 0.25,0.5, 1, 2 mg/ml, respectively. The mean percent reduction in mitotic index for short term exposure with metabolic activation S9 of KSM-66 Ashwagandha Root Extract at concentrations of 0.5 mg/ml, 1 mg/ml and 2 mg/ml were 90.18, 87.50 and 83.93 and was comparable to vehicle control. The mean percent reduction in mitotic index of positive control Cyclophosphamide monohydrate was 88.39 as compared with the vehicle control. The mean percent reduction in mitotic index of short-term exposure without metabolic activation S9 of KSM-66 Ashwagandha Root Extract at concentrations of 0.5 mg/ml, 1 mg/ml and 2 mg/ml were 90.76, 88.24 and 84.03 and was comparable to vehicle control. The mean percent reduction in mitotic index of positive control.

Mitomycin C was 84.87 as compared with the vehicle control. No cytotoxicity was observed at the test item; KSM-66 Ashwagandha Root Extract concentrations of 0.5, 1 and 2 mg/ml. Similarly, the mean percent reduction in mitotic index of long-term exposure without metabolic activation S9 of KSM-66 Ashwagandha Root Extract at concentrations of 0.5 mg/ml, 1 mg/ml and 2 mg/ml were 97.54, 90.98 and 81.15 and was comparable to vehicle control. The mean percent reduction in mitotic index of positive control Mitomycin C was 82.79 as compared with the vehicle control. No cytotoxicity was observed in the test item; KSM-66 Ashwagandha Root Extract at concentrations of 0.5, 1 and 2 mg/ml.

No cytotoxicity was observed in the test item; KSM-66 Ashwagandha Root Extract at any of the tested concentrations of 0.5, 1 and 2 mg/ml both in the short-term exposure (4 h) as well as in continuous exposure (24 h). No significant difference in the chromosomal aberration frequency was observed between the vehicle controls and at any of the test concentrations of 0.5mg/ml, 1 mg/ml, and 2 mg/ml of KSM-66 Ashwagandha Root Extract. Significant increase in chromosomal aberrations was observed in both the positive control groups of Cyclophosphamide monohydrate and Mitomycin C confirming the sensitivity of the test system, the effectiveness of the S9 mix and the validity of the clastogenicity assay.

Conclusion: Based on the above observations, it is concluded that KSM-66 Ashwagandha Root Extract was considered to be non-clastogenic up to the tested concentration of 2 mg/ml under the above-mentioned experimental conditions.

10. Toxicity evaluation in zebrafish embryos

Study no: S-BBD-0002/22

Testing Laboratories: BIOBIDE

GLP Details: Tested under GLP environment

Animals: Zebrafish embryos

Methods: Transgenic Zebra fish embryos to study the effect on thyroid levels. Potassium perchlorate was used as a positive control and DMSO was the vehicle control. To establish the effect, thyroglobulin reporter gene fluorescence assay was used. At day 5 post fertilization, images were analyses under a fluorescence microscope for intensity variation**.**

Results: The test compound showed similar levels to the vehicle control, and no statistically significant differences, indicating safety of the extract to thyroid gland in the body even if there is no treatment-related goitrogenic effect.