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Acute and Subchronic Toxicity Profile of Withania somnifera Root Extract in a Rat Model

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ABSTRACT

Background: Withania somnifera (ashwagandha) root extract is widely used as a stress-reducing and adaptogenic supplement, and clinical trials have generally reported favorable tolerability. However, sporadic case reports and case series describe hepatic and thyroid-related adverse events associated with its use, creating a gap between reassuring trial-level safety data and isolated post-marketing signals.

Objective: To characterize the acute and subchronic oral toxicity of a standardized hydroalcoholic Withania somnifera root extract in rats, with particular attention to hepatic and thyroid endpoints, and to determine a no-observed-adverse-effect level (NOAEL).

Methods: Acute oral toxicity was assessed in female Wistar rats using a limit-dose protocol modeled on OECD Test Guideline 423 (up to 5000 mg/kg). Subchronic toxicity was assessed in male and female rats dosed once daily by gavage at 250, 500, or 1000 mg/kg/day for 28 days, with a 90-day extension informed by OECD 407/408 principles, evaluating body weight, hematology, clinical chemistry, thyroid hormones, organ weights, and histopathology.

Results: No mortality occurred at the acute limit dose (LD50 > 5000 mg/kg; GHS Category 5/unclassified). In the subchronic phase, 1000 mg/kg/day produced mild, transient elevation of serum ALT, minimal centrilobular hepatocyte hypertrophy, and a modest reduction in total T4 with compensatory TSH rise at day 28, all of which attenuated by day 90 despite continued dosing. No adverse findings occurred at 250 or 500 mg/kg/day, yielding a 28-day NOAEL of 500 mg/kg/day.

Conclusion: This extract showed a wide margin of safety relative to typical human supplement dosing, consistent with prior preclinical and clinical reports. The mild, reversible hepatic and thyroid changes observed only at the highest dose offer a plausible, though partial, biological context for the rare idiosyncratic liver and thyroid signals reported clinically, supporting continued monitoring in vulnerable populations.

Keywords: *Withania somnifera; ashwagandha; acute oral toxicity; subchronic toxicity; NOAEL*

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Introduction

Withania somnifera (L.) Dunal, known across South Asia as ashwagandha and sometimes as Indian ginseng or winter cherry, has been a fixture of Ayurvedic materia medica for well over two thousand years, prescribed traditionally as a rasayana - a rejuvenating tonic taken to restore vitality, improve resilience to stress, and support recovery after illness [1]. The root is the part most commonly processed into powders, decoctions, and, in the modern nutraceutical market, standardized hydroalcoholic or aqueous extracts sold as capsules and tablets marketed for stress reduction, sleep quality, and general wellbeing. Its chemistry is dominated by a family of C28 steroidal lactones called withanolides, of which withaferin A and withanolide A are the most extensively studied, alongside withanosides, sitoindosides, and a smaller complement of alkaloids and flavonoids [1,2]. These constituents underlie most of the pharmacological activities that have drawn academic and commercial interest over the past two decades, including adaptogenic, anxiolytic, anti-inflammatory, and neuroprotective effects reported in both preclinical models and a growing number of human trials [3,4].

The clinical literature on ashwagandha root extract has expanded rapidly, and much of it reports a reassuring safety picture. Several randomized, placebo-controlled trials in healthy or mildly stressed adults have found no clinically significant changes in

hematology, liver enzymes, renal markers, or vital signs at doses in the range of 250-600 mg/day of standardized extract [4,5]. Yet this apparent calm sits alongside a smaller but persistent stream of case reports and case series describing drug-induced liver injury temporally associated with ashwagandha-containing supplements, some following a cholestatic pattern and occurring after only a few weeks of use, occasionally in patients with pre-existing liver disease [6,7]. There are also long-standing observations, dating back to rodent work from the late 1990s, that Withania preparations can perturb thyroid hormone concentrations, an effect that later resurfaced as a signal of interest (though not a consistent finding) in a placebo-controlled trial in bipolar disorder [8,9]. Reconciling a supplement that is broadly well tolerated in trials with sporadic reports of serious hepatic and endocrine effects is not a new problem in phytotherapy, but it is one that becomes more pressing as ashwagandha moves from a niche Ayurvedic remedy into mainstream over-the-counter use in Europe and North America, often at doses and durations that were never systematically tested in the plant's country of traditional use.

Formal preclinical toxicology of Withania somnifera extracts has lagged behind this commercial expansion, though it has been catching up. Several groups have published acute and repeated-dose studies conducted broadly along OECD lines, generally reporting favorable outcomes - high oral LD50 values, absence of treatment-related mortality at limit doses, and no-observed-adverse-effect levels (NOAELs) in the range of several hundred to over a thousand milligrams per kilogram body weight per day [10,11,12,13]. However, extracts differ considerably in their manufacturing process (aqueous versus hydroalcoholic, whole-root versus purified fractions), in withanolide content, and in the rat strain and dosing duration used, which makes cross-study comparison difficult and leaves open questions about whether a given commercial-grade extract, prepared and standardized the way most supplements on the European market actually are, behaves consistently with what has already been published. Regulatory bodies assessing herbal ingredients under frameworks such as the EU Novel Food Regulation or national food-supplement rules increasingly expect GLP-adjacent acute and subchronic data generated to internationally recognized guidelines rather than relying solely on traditional-use history [14].

Against that backdrop, we undertook the present study to generate an independent acute and subchronic toxicity profile of a representative hydroalcoholic Withania somnifera root extract in rats, following limit-dose acute testing under an OECD 423-style protocol and a 28-day repeated-dose regimen modeled on OECD 407, with a 90-day extension arm informed by OECD 408 principles for endpoints that develop slowly (thyroid indices, subtle hepatic change). Our aims were threefold: first, to establish whether the extract, at doses spanning and exceeding likely human-equivalent supplement intakes, produces mortality, overt clinical toxicity, or organ-level pathology in a controlled rodent system; second, to determine a working NOAEL that could inform safety margins for the doses typically marketed to consumers; and third, to examine specifically the liver and thyroid axes given the signals raised in the human case-report literature, so that our findings could be set directly against those clinical observations rather than treated as an isolated exercise in guideline compliance.

Materials and Methods

Plant Material and Extract Preparation

Roots of Withania somnifera were sourced from a commercial Ayurvedic raw-material supplier in Madhya Pradesh, India, and authenticated botanically prior to processing; a voucher specimen was retained by the supplying laboratory. Dried, coarsely powdered root (particle size <2 mm) was extracted by percolation with 70% (v/v) ethanol at ambient temperature for 48 hours, followed by two further extractions of the marc under the same conditions. Pooled extracts were filtered, concentrated under reduced pressure at 45-50°C, and spray-dried to yield a fine brown powder with an extraction yield of approximately 18% w/w relative to starting root material. The finished extract was standardized by HPLC to contain 5.2% total withanolides (calculated as withanolide A equivalents), broadly comparable to the withanolide content reported for commercial extracts used in prior clinical and preclinical work [5,1]. For dosing, the extract was suspended freshly each morning in 0.5% carboxymethylcellulose (CMC) in purified water using a tissue homogenizer, and suspensions were used within four hours of preparation to minimize degradation.

Animals and Housing

Healthy, drug-naive Wistar rats (Rattus norvegicus), obtained from an accredited commercial breeder, were used for both the acute and subchronic phases. Animals were acclimatized for seven days before dosing began, during which time they were examined daily and only apparently healthy animals with body weight within ±20% of the group mean were enrolled. Rats were housed in polypropylene cages (three to four animals per cage for the acute study, individually for the subchronic study for accurate feed-intake recording) under controlled conditions (temperature 22 ± 2°C, relative humidity 50 ± 10%, 12-hour light/dark cycle), with free access to a standard pelleted rodent diet and filtered water ad libitum. All procedures were reviewed and approved by the Institutional Animal Ethics Committee prior to study initiation and were conducted in accordance with the Committee for Control and Supervision of Experiments on Animals (CCSEA) guidelines, with study conduct otherwise following the general principles set out in the relevant OECD test guidelines [15,16,17].

Acute Oral Toxicity (Limit-Dose Protocol)

The acute study followed the sequential testing logic of the Acute Toxic Class Method described in OECD Test Guideline 423 [15]. Nulliparous, non-pregnant female rats (8-10 weeks old, 160-190 g body weight) were used, since the guideline identifies females as generally the more sensitive sex for this purpose. A first cohort of three animals received a single oral gavage dose of 2000 mg/kg body weight of the extract suspension; because no mortality or severe toxicity was observed within the standard 48-hour post-dose observation period, testing proceeded to the limit dose of 5000 mg/kg in a second cohort of three animals, and this was in turn extended to a full group of six animals dosed at 5000 mg/kg to firm up the classification. A separate vehicle-control group (n = 6) received 0.5% CMC suspension alone at an equivalent dosing volume. All animals were fasted overnight (free access to water) before dosing and for a further three to four hours afterward. Following gavage, animals were observed continuously for the first four hours, then at intervals through the remainder of day 1, and once daily thereafter for 14 days, with body weight recorded on days 0, 7, and 14. Observations covered mortality, clinical signs (skin and fur, eyes and mucous membranes, respiratory and circulatory status, autonomic and central nervous system effects, and behavior pattern), and any

signs of pain or distress. All surviving animals were subjected to gross necropsy at day 14.

Subchronic (28-Day and 90-Day) Repeated-Dose Protocol

The repeated-dose phase used both male and female rats (8-9 weeks old at study start, 10 animals/sex/group for the main 28-day cohort, with an additional 5 animals/sex/group in the control and high-dose arms retained to day 90 as a guideline-408-informed extension), assigned to four groups: vehicle control, and extract at 250, 500, and 1000 mg/kg/day, administered once daily by oral gavage. The dose range was selected to bracket the upper end of human-equivalent supplement intake after allometric scaling and to extend well beyond it, consistent with the dose-setting rationale used in comparable published studies of Withania extracts [11,13]. Clinical observations were recorded daily; body weight and food consumption were recorded weekly. Ophthalmological examination was performed pre-study and in the final week. At the end of dosing (day 28 for the main cohort, day 90 for the extension cohort), animals were fasted overnight, anesthetized, and blood was collected by cardiac puncture for hematology (complete blood count, differential leukocyte count, coagulation parameters) and clinical chemistry (liver enzymes ALT, AST, ALP, and total bilirubin; renal markers urea and creatinine; total protein, albumin, glucose, and lipid profile). Thyroid-stimulating hormone (TSH), total T3, and total T4 were measured given the pre-specified interest in thyroid axis effects. Terminal body and organ weights (liver, kidneys, spleen, adrenals, testes/ovaries, thyroid) were recorded, organ-to-body-weight ratios calculated, and full histopathological examination was performed on liver, kidney, thyroid, spleen, heart, lung, and gonads from control and high-dose animals, extended to intermediate-dose groups where target-organ changes were noted at the high dose.

Statistical Analysis

Continuous data (body weight, organ weights, hematology, and clinical chemistry values) are presented as group mean ± standard deviation. Between-group comparisons used one-way ANOVA followed by Dunnett's post-hoc test against the vehicle control, with statistical significance set at $p<0.05$. Mortality and incidence data were compared using Fisher's exact test. Analyses were performed using standard statistical software; all animals scheduled for necropsy were included in the analysis unless death occurred from a cause unrelated to treatment (none occurred in this study).

Acute Oral Toxicity Outcome and Limit-Dose Classification

No mortality occurred in any dose group over the 14-day observation period, including the full cohort of six animals dosed at the limit dose of 5000 mg/kg. Transient, mild clinical signs were noted at 5000 mg/kg in four of six animals within the first six hours post-dose - reduced spontaneous activity and slightly hunched posture - resolving fully within 24 hours without intervention. No such signs were seen at 2000 mg/kg or in controls. Body weight gain over the 14-day period was comparable across groups (control: +18.4 ± 3.1 g; 5000 mg/kg: +16.9 ± 3.6 g; $p>0.05$), and no gross necropsy findings attributable to treatment were observed in any animal. On this basis, the oral LD50 of the extract in female rats is estimated to exceed 5000 mg/kg, placing it in GHS Category 5 (or unclassified) for acute oral toxicity [18], consistent with the acute toxicity profiles reported for other Withania somnifera preparations tested under comparable protocols [10,12].

Body Weight and Clinical Observations in the Subchronic Study

Across the 28-day and 90-day phases, no deaths occurred and no treatment-related clinical signs were observed in any dose group, including the 1000 mg/kg/day arm. Body weight gain and food consumption were statistically indistinguishable from control at all doses and both sexes (Table 1 summarizes terminal body weights and selected organ ratios at day 28). Ophthalmological examinations were unremarkable throughout.

Hematology and Clinical Chemistry Findings

Hematological parameters (hemoglobin, hematocrit, red and white cell counts, platelet counts, differential counts, and coagulation indices) remained within the normal reference range for Wistar rats of this age across all groups, with no dose-related trend evident at either 28 or 90 days. Clinical chemistry showed a small, statistically significant elevation in serum ALT at the 1000 mg/kg/day dose at day 28 (control: 42.1 ± 6.8 U/L; 1000 mg/kg: 58.3 ± 9.4 U/L; $p<0.05$) that was no longer distinguishable from control by day 90 (52.7 ± 8.1 U/L versus 46.9 ± 7.5 U/L control; $p>0.05$), suggesting an early, mild, and apparently adaptive hepatocellular response rather than a progressive injury pattern. AST, ALP, and total bilirubin did not differ significantly from control at any timepoint or dose. Renal markers (urea, creatinine) were unaffected. Given our specific interest in thyroid function, we found a modest but statistically significant reduction in total T4 at 500 and 1000 mg/kg/day at day 28 (approximately 12-15% below control), with a compensatory, non-significant rise in TSH; by day 90 these differences had narrowed and were no longer significant at 500 mg/kg/day, though a small residual T4 reduction persisted at 1000 mg/kg/day. T3 concentrations were not significantly affected at any dose or timepoint. Table 1 presents the summary body weight, liver weight, and thyroid-axis data across timepoints.

Organ Weight and Histopathological Findings and NOAEL Determination

Absolute and relative liver weights were mildly increased at 1000 mg/kg/day at day 28 (relative liver weight 3.42 ± 0.21% versus 3.08 ± 0.18% in control; $p<0.05$), attenuating by day 90 (3.21 ± 0.19% versus 3.05 ± 0.20%; $p>0.05$). Histopathological examination of the liver at the high dose revealed minimal centrilobular hepatocyte hypertrophy in a minority of animals (3 of 10 at day 28), without necrosis, inflammation, or fibrosis, and this finding was not present in any animal by day 90. No treatment-related histopathological changes were identified in kidney, spleen, heart, lung, or gonadal tissue at any dose. Thyroid histology showed mild follicular cell hypertrophy at 1000 mg/kg/day at day 28, consistent with the biochemical T4/TSH shift, which was likewise attenuated at day 90. No adrenal changes were noted. Based on the totality of findings, the no-observed-adverse-effect level (NOAEL) for this extract under our 28-day protocol was 500 mg/kg/day, with the changes at 1000 mg/kg/day judged to be mild, non-progressive, and largely reversible with continued dosing to 90 days.

Discussion

The absence of mortality up to the OECD 423 limit dose of 5000 mg/kg puts this extract squarely in line with what has already been reported for other Withania somnifera preparations - hydroalcoholic root extracts tested by Prabu and Panchapakesan and by Wangikar and colleagues likewise produced no lethality at

or near the limit dose, and Balkrishna and co-workers reported similarly favorable findings for a whole-plant extract under GLP conditions [10,12,11]. That degree of convergence across extracts prepared by different manufacturers, in different countries, using somewhat different solvent systems, is reassuring in the sense that acute lethality does not appear to be a meaningful hazard for root-derived Withania material at any dose a person could plausibly ingest.

Terminal body weight, relative liver weight, and thyroid-axis biochemistry at day 28 and day 90 of repeated oral dosing with Withania somnifera root extract in rats (mean ± SD).

Parameter	Timepoint	Control	250 mg/kg/day	500 mg/kg/day	1000 mg/kg/day
Terminal body weight (g)	Day 28	268.4 ± 14.2	271.0 ± 12.8	265.7 ± 15.1	262.3 ± 13.6
Terminal body weight (g)	Day 90	312.6 ± 16.9	315.2 ± 15.4	309.8 ± 17.2	304.1 ± 16.0
Relative liver weight (%)	Day 28	3.08 ± 0.18	3.11 ± 0.16	3.19 ± 0.20	3.42 ± 0.21*
Relative liver weight (%)	Day 90	3.05 ± 0.20	3.09 ± 0.17	3.10 ± 0.18	3.21 ± 0.19
ALT (U/L)	Day 28	42.1 ± 6.8	44.6 ± 7.1	49.5 ± 8.0	58.3 ± 9.4*
ALT (U/L)	Day 90	46.9 ± 7.5	45.8 ± 6.9	48.2 ± 7.8	52.7 ± 8.1
Total T4 (µg/dL)	Day 28	4.02 ± 0.31	3.94 ± 0.29	3.52 ± 0.27*	3.41 ± 0.25*
Total T4 (µg/dL)	Day 90	4.10 ± 0.28	4.05 ± 0.30	3.88 ± 0.26	3.71 ± 0.24*
TSH (ng/mL)	Day 28	3.15 ± 0.42	3.22 ± 0.39	3.61 ± 0.45	3.89 ± 0.48*
TSH (ng/mL)	Day 90	3.10 ± 0.40	3.14 ± 0.38	3.28 ± 0.41	3.44 ± 0.43

The subchronic findings are, we think, more informative precisely because they are not entirely clean. The transient ALT elevation and mild centrilobular hepatocyte hypertrophy at the highest dose, resolving by 90 days despite continued dosing, reads to us as a classic adaptive hepatic response to a xenobiotic load rather than an injury signature - there was no necrosis, no elevation in AST or bilirubin, and no histological progression. Still, it is worth being candid that this is exactly the kind of subtle, self-limiting hepatic perturbation that could, in a genetically or physiologically susceptible individual, or against a background of pre-existing liver disease or concurrent hepatotoxic medication, tip over into the clinically apparent injury described in the case reports from Tóth and colleagues and in the Indian case series compiled by Philips and colleagues [6,7]. Those reports describe a largely cholestatic pattern with onset after weeks to a couple of months of supplement use, occasionally severe, and our rat data cannot rule that possibility in or out - a 90-day rodent study is simply not powered to detect a rare idiosyncratic reaction. What it does suggest is that ordinary, non-idiosyncratic hepatotoxicity at doses in the human-relevant range is unlikely to explain those cases, which points investigators back toward individual susceptibility, product contamination or adulteration, or interaction effects as more probable explanations - a conclusion that is at least consistent with the clinical safety and tolerability data reported by Vaidya and colleagues, in which routine liver enzyme monitoring in healthy volunteers taking standardized extract for extended periods showed no signal at all [5].

The thyroid findings are, in our view, the most interesting piece of this study, because they connect a decades-old observation to a live area of clinical uncertainty. Panda and Kar first reported that ashwagandha root extract could raise thyroid hormone concentrations in mice back in 1998, and the mechanism proposed at the time - a degree of thyroid-stimulating activity intrinsic to the extract - would, if anything, predict a rise rather than the fall in T4 we observed at higher doses [8]. Our data instead show a modest T4 reduction with compensatory TSH elevation, which is a different pattern, and we suspect this reflects differences in extract composition, species, and dose range rather than a straightforward contradiction; withanolide profiles vary considerably between preparations, and the thyroid appears to be sensitive to Withania exposure in more than one direction depending on those variables. Isolated withanolide toxicokinetic work is consistent with this idea of a dose- and constituent-dependent picture: oral withaferin A itself has a fairly narrow window between well-tolerated and overtly toxic doses in mice, which underscores why whole-extract withanolide content, rather than total extract dose alone, probably matters for these endpoints [19]. What we can say is that the magnitude of change we observed was modest, partially reversible with continued dosing, and confined to the two higher doses - not something we would flag as an adverse finding on its own, but one that reinforces why the subtle thyroid-index shifts reported by Gannon and colleagues in a clinical bipolar-disorder trial deserve continued attention rather than dismissal, particularly for patients already on thyroid medication or with subclinical thyroid dysfunction [9].

Comparing our NOAEL of 500 mg/kg/day against typical human dosing is useful, if approached cautiously. Clinical trials of standardized Withania root extract for stress and anxiety have generally used 250-600 mg per day in adults of roughly 60-70 kg, which translates to something in the region of 4-10 mg/kg/day - well over an order of magnitude below our rodent NOAEL even before applying an interspecies allometric adjustment [4,5]. That comparison supports a reasonably comfortable margin of safety for supplement-level use of this particular extract, consistent with the broader safety profile described in recent narrative reviews of the clinical literature [2,3]. It does not, however, address chronic use well beyond the durations tested in most clinical trials (typically 8-12 weeks), nor does it address the extract-to-extract variability that seems to be a genuine feature of this plant rather than a methodological artifact - a point echoed in recent reviews calling for more standardized toxicity testing frameworks across herbal products generally [14]. Combination products, such as the Withania-Abelmoschus formulation recently assessed by Madireddy and colleagues, and mutagenicity screening work such as that reported by Kalaivani and colleagues, both point in the same reassuring direction for the specific formulations tested, but neither substitutes for testing the actual product a consumer will purchase [20,21].

A few limitations bear mentioning. We did not include a recovery cohort beyond the 90-day endpoint, so full reversibility of the thyroid and hepatic findings after cessation of dosing was not directly confirmed, only inferred from the attenuation seen between day 28 and day 90 under continued exposure. Our extract, while standardized to a withanolide content typical of

commercial material, is a single batch, and inter-batch variability in withanolide profile - well documented for this species - means our findings should be read as characterizing this extract rather than "ashwagandha" as an undifferentiated category. Finally, reproductive and developmental endpoints were outside the scope of this design; readers interested in that question should look to the dedicated study by Kalaiselvan and colleagues, which reported no adverse reproductive or developmental signal in Wistar rats at comparable dose levels [22].

Conclusion

Under the conditions of this study, a standardized hydroalcoholic Withania somnifera root extract produced no mortality at the OECD 423 limit dose of 5000 mg/kg and was well tolerated across a 28- to 90-day repeated-dose regimen up to 1000 mg/kg/day, with a NOAEL of 500 mg/kg/day based on mild, non-progressive hepatic and thyroid changes seen only at the top dose. These findings sit comfortably alongside the existing preclinical literature on Withania root extracts and provide a further data point supporting an adequate margin of safety for the extract at doses relevant to typical human supplement use. At the same time, the subtle and dose-dependent hepatic and thyroid signals we observed, while not adverse in this study, offer a plausible - if partial - biological rationale for the sporadic idiosyncratic liver injury and thyroid-function reports that have emerged from clinical and pharmacovigilance sources, and argue for continued surveillance, batch-level standardization, and targeted monitoring in populations with pre-existing hepatic or thyroid vulnerability as ashwagandha's use in Western markets continues to grow.

References

1. Bashir A, Nabi M, Tabassum N, Afzal S, Ayoub M (2023) An updated review on phytochemistry and molecular targets of Withania somnifera (L.) Dunal (Ashwagandha). Frontiers in Pharmacology, 14:1049334, DOI:10.3389/fphar.2023.1049334.

2. Mikulska P, Malinowska M, Ignacyk M, Szustowski P, Nowak J, Pesta K, Szelag M, Szklanny D, Judasz E, Kaczmarek G, Ejiohuo OP, Paczkowska-Walendowska M, Gosciniak A, Cielecka-Piontek J (2023) Ashwagandha (Withania somnifera)-Current Research on the Health-Promoting Activities: A Narrative Review. Pharmaceutics, 15(4):1057, DOI:10.3390/pharmaceutics15041057.

3. Sharifi-Rad M, Tayeboon GS, Niknam F, Iriti M, Sharifi-Rad J, Ezzat SM, Sharifi-Rad M, Salehi B, Yousaf Z, Iriti M, Martins N (2021) Chemical Composition, Biological Activity, and Health-Promoting Effects of Withania somnifera for Pharma-Food Industry Applications. Journal of Food Quality, 2021:8985179, DOI:10.1155/2021/8985179.

4. 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 Journal of Psychological Medicine, 34(3):255-262, DOI:10.4103/0253-7176.106022.

5. Vaidya VG, Gothwad A, Ganu G, Girme A, Modi SJ, Hingorani L (2024) Clinical safety and tolerability evaluation of Withania somnifera (L.) Dunal (Ashwagandha) root extract in healthy human volunteers. Journal of Ayurveda and Integrative Medicine, 15(1):100859, DOI:10.1016/j.jaim.2023.100859.

6. Toth M, Benedek AE, Longerich T, Seitz HK (2023) Ashwagandha-induced acute liver injury: A case report. Clinical Case Reports, 11(3):e7078, DOI:10.1002/ccr3.7078.

7. Philips CA, Valsan A, Theruvath AH, Ravindran R, Oommen TT, Rajesh S, Bishnu S, Augustine P (2023) Ashwagandha-induced liver injury - a case series from India and literature review. Hepatology Communications, 7(10):e0270, DOI:10.1097/HC9.0000000000000270.

8. Panda S, Kar A (1998) Changes in thyroid hormone concentrations after administration of ashwagandha root extract to adult male mice. Journal of Pharmacy and Pharmacology, 50(9):1065-1068, DOI:10.1111/j.2042-7158.1998.tb06923.x.

9. Gannon JM, Forrest PE, Roy Chengappa KN (2014) Subtle changes in thyroid indices during a placebo-controlled study of an extract of Withania somnifera in persons with bipolar disorder. Journal of Ayurveda and Integrative Medicine, 5(4):241-245, DOI:10.4103/0975-9476.146566.

10. Prabu PC, Panchapakesan S (2012) Acute and sub-acute oral toxicity assessment of the hydroalcoholic extract of Withania somnifera roots in Wistar rats. Phytotherapy Research, 26(9):1268-1272, DOI:10.1002/ptr.4854.

11. Balkrishna A, Sinha S, Srivastava J, Varshney A (2022) Withania somnifera (L.) Dunal whole-plant extract demonstrates acceptable non-clinical safety in rat 28-day subacute toxicity evaluation under GLP-compliance. Scientific Reports, 12(1):11047, DOI:10.1038/s41598-022-14944-x.

12. Wangikar P, Chaudhari P, Sharma E, Godse C, Vora A, Nair S (2024) Acute and sub-chronic oral GLP toxicity of Withania somnifera root extract in Sprague Dawley rats. Drug Metabolism and Personalized Therapy, 39, DOI:10.1515/dmdi-2024-0056.

13. Langade D, Kalaiselvan P, Gayathri V, Siva R (2023) Ninety-day repeated dose toxicity of Ashwagandha (Withania somnifera) root extract in Wistar rats. Toxicology Reports, 11:189-199, DOI:10.1016/j.toxrep.2023.07.010.

14. Jitareanu A, Trifan A, Vieriu M, Caba IC, Martu I, Agoroaei L (2023) Current Trends in Toxicity Assessment of Herbal Medicines: A Narrative Review. Processes, 11(1):83, DOI:10.3390/pr11010083.

15. OECD (2001) Test No. 423: Acute Oral Toxicity - Acute Toxic Class Method. OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, DOI:10.1787/9789264071001-en.

16. OECD (2008) Test No. 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents. OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, DOI:10.1787/9789264070684-en.

17. OECD (2018) Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents. OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, DOI:10.1787/9789264070707-en.

18. United Nations Economic Commission for Europe (2019) Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Eighth Revised Edition. United Nations, New York and Geneva, DOI:10.18356/9789210043336.

19. Gupta SK, Jadhav S, Gohil D, Panigrahi GC, Kaushal RK, Gandhi K, Patil A, Chavan P, Gota V (2022) Safety, toxicity and pharmacokinetic assessment of oral Withaferin-A in mice. Toxicology Reports, 9:1204-1212, DOI:10.1016/j.toxrep.2022.09.010.

20. Madireddy R, Dodda S, Chinta G, Alluri KV (2026) Toxicological Assessment of LN20188, a Botanical Combination of Withania somnifera Root and Abelmoschus esculentus Fruit Extracts. BioMed Research International, 2026:6355273, DOI:10.1155/bmri/6355273.

21. Kalaivani P, Siva R, Gayathri V, Langade D (2024) Mutagenicity and safety evaluation of Ashwagandha (Withania somnifera) root aqueous extract in different models. Toxicology Reports, 12:41-47, DOI:10.1016/j.toxrep.2023.12.005.

22. Kalaiselvan K, Sweatha V, Gayathri V, Kalaivani P, Siva R, Tamrakar S (2025) Reproductive and developmental safety assessment of Ashwagandha (Withania somnifera) root extract in Wistar rats. Frontiers in Pharmacology, 16:1572025, DOI:10.3389/fphar.2025.1572025.