Ind. J. Pharm. Edu. Res., 2026; 60(2s):s721-s727.
https://www.ijper.org

Original Article

High-Dose Ashwagandha Extract: A Toxicological Assessment in Male Wistar Rats Using a Dose-Dependent Approach

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ABSTRACT

Background/Aim: The rising utilization of Ashwagandha (*Withania somnifera*), a well-known adaptogen and herbal supplement, has prompted apprehensions over its safety at elevated dosages. This study examines the potential hazardous consequences of different high dosages of Ashwagandha, seeking to elucidate its safety profile at elevated concentrations. **Materials and Methods:** Over 30 days, four groups of laboratory animals (male rats) were given varying high dosages of Ashwagandha root extract (2000 mg to 5000 mg per day). The groups were divided as follows: (1) Control group: administered a single oral dose of saline (15 mL/kg, once daily), (2) Diseased group (2000): administered a single oral dose of Ashwagandha (2000 mg/kg, once daily), (3) Diseased group (3000): administered a single oral dose of Ashwagandha (3000 mg/kg, once daily), (4) Diseased group (5000): administered a single oral dose of Ashwagandha (5000 mg/kg, once daily). Upon completion of the trial, scarification was performed on all rats, followed by examining several toxicity biomarkers, including liver and kidney function and hematological parameters, utilizing colorimetric and ELISA kits. Furthermore, the histological evaluations of essential organs were also conducted. **Results:** Results demonstrated dose-dependent changes in hepatic enzyme activity, renal function, and cellular integrity, indicating that excessive consumption may have detrimental effects. These findings emphasize the necessity for prudent dosage recommendations and long-term clinical research to determine safe usage protocols for high-dose Ashwagandha supplementation. **Conclusion:** This study offers essential insights into the toxicological concerns linked to excessive Ashwagandha utilization. It highlights that doses up to 2000 mg/kg are deemed safe and exhibit no detrimental impacts on any of the organs assessed in the current research. Nevertheless, the elevated dosages of 3000 and 5000 mg/kg elicited hazardous effects on the evaluated organs.

Keywords: Ashwagandha, Roots, High doses, Rats, Organ Toxicity.

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Received: 28-11-2025;
Revised: 02-01-2026;
Accepted: 12-02-2026.

INTRODUCTION

Adaptogenic, anti-inflammatory, and stress-reducing capabilities are the most widely recognized medicinal herb ashwagandha (*Withania somnifera*) applications in traditional Ayurvedic medicine.[1] Theories of the natural supplement's capacity to enhance mood, cognition, immunity, and physical performance have significantly fueled Ashwagandha's rapid increase in popularity in recent years. The standard recommended daily dosage of Ashwagandha is 300-600 milligrams (mg), with studies confirming that these quantities are safe and efficacious.[2]

Nonetheless, with the burgeoning consumer interest in herbal supplements, there is a notable trend towards exceeding suggested dosages, frequently attaining levels between 2000 mg and 5000 mg daily.[2,3] This highlights the requirement to consider the potential adverse effects of extended high-dose consumption, a field still unexplored.

The pharmacological advantages conferred by Ashwagandha are principally linked to its bioactive constituents, particularly withanolides, alkaloids, and saponins, which reveal efficacy in anti-stress, anti-inflammatory, antioxidant, and immunomodulatory capabilities.[4,5] Such compounds engage with the body's hormonal, immunological, and neurological systems, facilitating the maintenance of homeostasis during stress. At therapeutic concentrations, Ashwagandha has proven the ability to lower cortisol levels, improve cognitive function, and boost vitality. Extensive animal and clinical research have established the plant's safety at appropriate dosages (300 mg to 600 mg daily), corroborating its adaptability advantages and negligible adverse

DOI: 10.5530/ijper.20263686

effects. The safety of Ashwagandha at low to moderate dosages is well-established; however, the effects of high-dose intake (2000 to 5000 mg) are less understood. Toxicological issues emerge from the idea that excessive intake of any supplement may surpass the body's detoxifying and metabolic capabilities. The therapeutic window for Ashwagandha remains inadequately characterized; nonetheless, research indicates that elevated dosages may lead to detrimental effects on liver and renal function and result in electrolyte imbalances, hematological irregularities, and hormonal disturbances.[6]

The main point of discussion regarding high-dose herbal supplementation is its influence on the functionality of organs. A recent study,[7] Ingesting substantial dosages of Ashwagandha, up to 2000 mg/kg for 90 days in rats, provoked a minor spike in liver enzymes, signifying potential hepatotoxicity. Likewise, research on elevated doses of many herbs has underscored the potential for nephrotoxicity, wherein the kidneys are overwhelmed by the metabolism of an excessive quantity of bioactive chemicals.[8] Despite the absence of specific data regarding the effects of high-dose Ashwagandha in certain trials, it is plausible to infer that these organs may exhibit increased vulnerability to oxidative stress and inflammation, given the established effects of other herbal supplements. Excessive use of Ashwagandha may adversely impact hormonal control and blood parameters. In substantial amounts, the withanolides in Ashwagandha may affect thyroid function, potentially leading to hypothyroidism or hyperthyroidism.[9] Furthermore, studies indicate that elevated doses of adaptogens can result in abnormal blood glucose levels, potentially endangering patients with pre-existing metabolic abnormalities.[10]

Cellular morphology modifications, particularly in the liver and kidney tissues, have been observed in histopathological investigations of animals that were administered high doses of Ashwagandha. Notwithstanding the prevalent utilization of Ashwagandha and its advantageous attributes, a significant deficiency exists in the literature about the safety of high-dose Ashwagandha, specifically within the dosage range of 2000 mg to 5000 mg daily. Current research predominantly relies on clinical trials utilizing moderate dosages and animal studies emphasizing overall safety rather than high-dose toxicity. Therefore, well-controlled toxicological studies are necessary to investigate the dose-response relationship of Ashwagandha and its effects on organ systems, blood composition, and endocrine regulation at these higher levels.

MATERIALS AND METHODS

Experimental Animals and Design

24 adults male Wistar albino rats, aged 12-16 weeks and weighing 160-180 g, were acquired from the laboratory animal section of the College of Clinical Pharmacy in Hfouf, Saudi Arabia. Throughout the 7-day acclimation period, the animals were provided with a standard control diet and water ad libitum. The Animal Ethics Committee of the College of Clinical Pharmacy, King Faisal University, Hfouf, Saudi Arabia, approved the experimental protocol. Our protocol including the protocols strictly adhered to both ARRIVE guidelines and the NIH Principles of Laboratory Animal Care (NIH Publication No. 85-23, 1996 revision). The mice were randomly allocated into four experimental groups (n = 6); the control group received a single administration of physiological saline (15 mL/kg; i.p.). Infected cohorts: (1) administered a singular oral dosage (2000 mg/kg, once a day, for 30 consecutive days) of Ashwagandha (Ashw.) suspension[11] (2) treated with a single oral administration of Ashwagandha (Ashw.) suspension at a dose of 3000 mg/kg, once daily for 30 consecutive days. (3) treated with a single oral dose of Ashwagandha (Ashw.) suspension (5000 mg/kg), once daily for 30 consecutive days.

N.B. Ashwagandha root extract prepared using aqueous maceration and then supernatant collection performed.

Samples Collection

At the end of the research experiment, after four weeks, blood samples were extracted from the retro-orbital venous sinus of each animal into unadulterated test tubes. Following a 10-min clotting period, the samples were centrifuged at 3000 rpm (4°C) to isolate the serum, which was subsequently kept at -80°C for future biochemical biomarker analysis. Subsequently, the rats were sedated and euthanized using cervical dislocation to retrieve the necessary tissues from each specimen. A minor segment of each organ (lungs, kidneys, and brain) was preserved in 10% neutral-buffered formalin for later histological evaluation.

Biochemical analysis

Serum liver markers

The serum concentrations of alanine Aminotransferase (ALT) and aspartate Aminotransferase (AST) were assessed calorimetrically utilizing examination kits acquired from Teco Diagnostics, located at 1268 N Lakeview Ave, Anaheim, CA 92807, USA. Serum Gamma-Glutamyl Transferase (GGT) was evaluated using a particular rat ELISA kit (My BioSource, San Diego, USA, Cat #: MBS9343646). All procedures adhered to the manufacturer's protocol.

Serum kidney markers

Renal injury was assessed by measuring specific biomarkers, including serum urea and creatinine, utilizing colorimetric analysis with Specord spectroscopy 210 and Lipocaline software.

Serum inflammatory markers

Serum protein concentrations of TNF-alpha and Interleukin-6 (IL-6) were assessed utilizing ELISA kits (My BioSource, San Diego, USA, Cat #: MBS2507393 and Cat #: MBS9343645, respectively).

Histopathological Examination

Lung, kidney, and brain tissues were preserved in a 10% formalin solution before routine histological processing. The processes entailed sequential immersion in escalating ethanol concentrations for dehydration, succeeded by embedding in paraffin wax. Thereafter, the paraffin-embedded tissue blocks were sectioned to a thickness of approximately 4 µm and stained with Hematoxylin and Eosin (H&E), following the protocols established by Bancroft and Layton.[12]

Statistical analysis

All data were collected, processed, and statistically evaluated using IIBM SPSS Statistics for Windows, version 22.0 (IBM Corporation, Armonk, New York). The results were presented as the mean and Standard Deviation (SD). The data were represented using the statistical program SigmaPlot, version 14.0 (Systat Software, Inc., Chicago, IL, USA), data were analyzed by one-way Analysis of Variance (ANOVA). Statistical comparisons between the groups were completed using Duncan's test. p-value <0.05 was considered statistically significant.

RESULTS

High doses of Ashwagandha deteriorate the liver functions in rats

Table 1 implies that the lowest dosage of Ashwagandha (2000 mg/kg) had no harmful effects on liver enzymes AST and ALT compared to normal rats. Nevertheless, the elevated dosage (3000 mg/kg) commenced to considerably augment the serum levels of AST and ALT compared to the control animals. The maximum dose (5000 mg/kg) considerably increased serum levels of AST and ALT compared to the results from normal rats.

High doses of Ashwagandha disrupted the kidney function in rats

Table 1 indicates that the lowest dose of Ashwagandha (2000 mg/kg) did not appear to adversely affect renal function, as evidenced by blood creatinine levels, compared to normal rats. Nevertheless, the elevated dosage (3000 mg/kg) increased the blood creatinine levels considerably compared to the control animals. The maximum dose (5000 mg/kg) considerably increased the serum creatinine level compared to the results of normal rats.

High doses of Ashwagandha significantly potentiate the inflammation in rats

Table 1 reveals that the lowest dose of Ashwagandha (2000 mg/kg) resulted in a slight increase in serum levels of TNF-alpha and IL-6 compared to normal rats. Nevertheless, the elevated doses (3000 and 5000 mg/kg) resulted in a significant increase in the serum levels of TNF-alpha and IL-6 compared to the results observed in the normal rats.

Effect of High doses of Ashwagandha on histopathology of brain in rats

The brain sections at the lowest dose (2000 mg/kg) (Figure 1) exhibited a seemingly normal cerebral cortex. In contrast, the higher dosage (3000 mg/kg) indicated the presence of a limited number of degenerating neurons in the rats' brain sections. The maximum dosage (5000 mg/kg) exhibited many dark degenerating neurons in the cerebral cortex.

Effect High doses of Ashwagandha on histopathology of lungs in rats

Concerning the minimal dosage (2000 mg/kg) (Figure 2), the lung sections exhibited seemingly typical bronchioles and alveoli compared to healthy rats' lungs. In the case of the elevated dosage (3000 mg/kg), the lung sections of the rats exhibited modest infiltration of mononuclear inflammatory cells in the peribronchiolar and interstitial regions. The maximum dosage (5000 mg/kg) induced peribronchiolar fibroplasia accompanied by infiltration of mononuclear inflammatory cells.

Effect High doses of Ashwagandha on histopathology of kidneys in rats

Given the lowest dosage (2000 mg/kg) (Figure 3), the kidney sections exhibited distinctly normal renal glomeruli and tubules compared to the lungs of typical rats. In the case of the elevated dosage (3000 mg/kg), the renal sections of the rats exhibited minor congestion in both the renal glomeruli and tubules. The maximum dosage (5000 mg/kg) demonstrated renal glomerular growth and significant bleeding in the interstitial tissue, displacing the renal parenchyma.

Table 1: Effect of different high doses of Ashwagandha on AST and ALT serum levels in rats.

Ashwagandha dose	ALT (UL/L)	AST (UL/L)	Creatinine (mg/dL)	TNF-ALPHA (mg/dL)	IL-6 (mg/dL)
Normal rats	40 ± 4	130± 10	0.7± 0.05	121± 8	5.4± 0.6
2000 mg/kg	45± 6	150± 12	0.9± 0.08	123± 9	7± 0.7
3000 mg/kg	89 *± 10	170 *± 14	1.5*± 0.12	139 *± 11	11.9 *± 1.2
5000 mg/kg	115 *± 13	220 *± 18	2.3*± 0.18	210 *± 20	29.6 *± 2.5

Data is expressed as Mean +/- SD (n=6) for statistical analysis using one-way ANOVA followed by Tukey's *post hoc*-test for multiple comparison; p <0.05. ALT: alanine aminotransferase, AST: aspartate aminotransferase. * Significantly different from normal rats.

DISCUSSION

Withania somnifera (L.) is an evergreen shrub grown in tropical and subtropical regions of Asia, Africa, and Europe. The plant is widely referred to as Ashwagandha, derived from the roots' scent resembling that of a wet horse ("ashwa" meaning horse and "gandha" meaning fragrance).[13] Ashwagandha has demonstrated effectiveness in addressing stress, anxiety, and sleep issues, with therapeutic doses typically ranging from 500 mg to 1500 mg.[14] This study explores the potential side effects of high doses of Ashwagandha on various organs, including the liver, lungs, kidneys, and brain.

Examining Ashwagandha's impact on liver function by exploring the effects of varying doses on ALT and AST levels. At present, observations indicate that the lower dose of 2000 mg/kg did not have a detrimental effect on liver enzymes; conversely, the higher doses of 3000 and 5000 mg/kg adversely impacted liver enzymes. Earlier clinical studies indicated that therapeutic doses of Ashwagandha significantly reduced elevated liver enzymes and mitigated liver damage over eight months.[15,16]

Regarding the serum creatinine level, it was noted that the lower dose did not significantly increase the serum creatinine level in comparison to the normal animals. Nonetheless, the elevated doses of 3000 and 5000 mg/kg resulted in a notable increase in serum creatinine levels compared to the control group. Previous studies have demonstrated the anti-inflammatory effect of Ashwagandha at low doses of 250, 300, and 500 mg/kg.[17-19] Our study found that

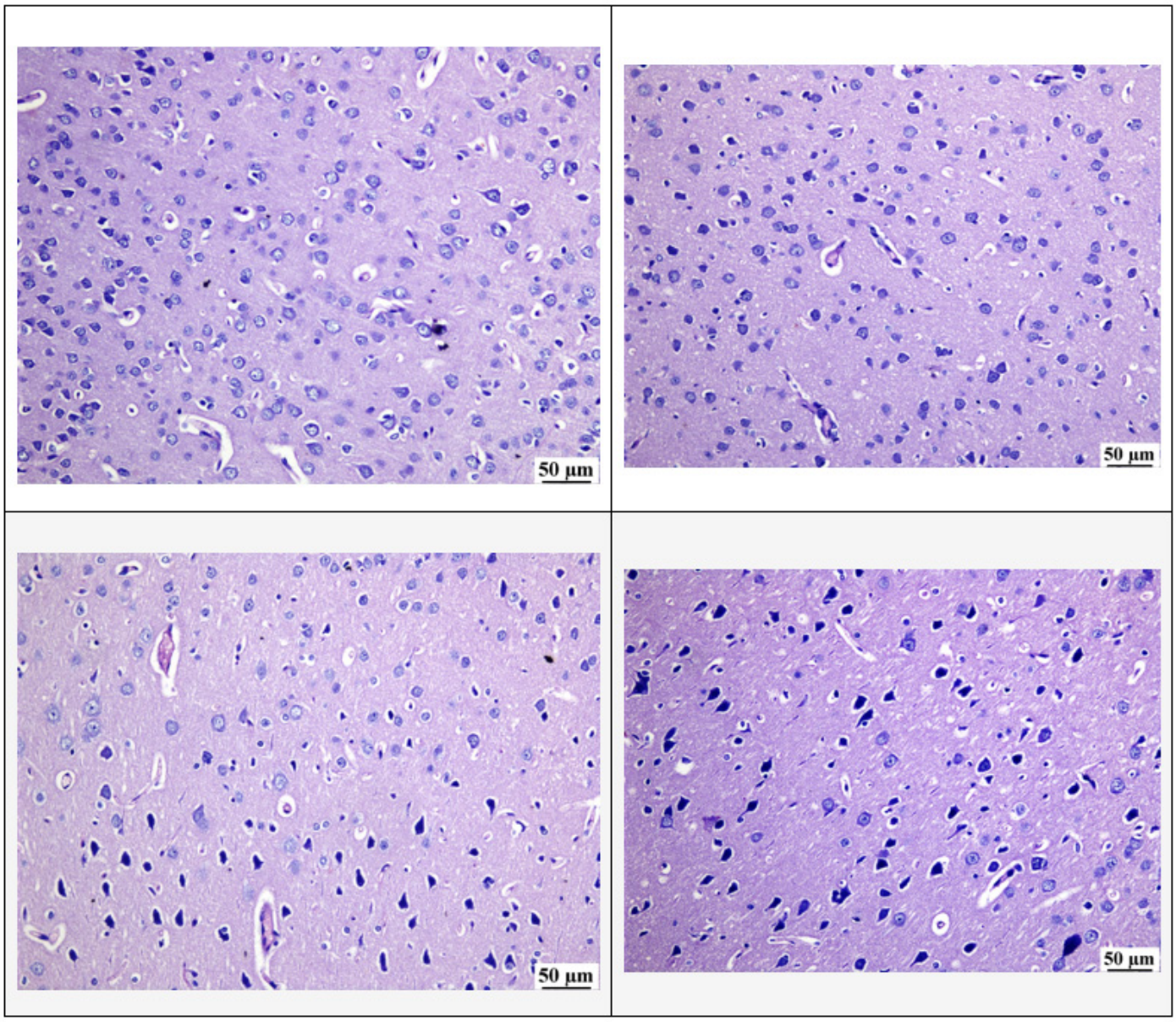

Figure 1: Photomicrograph of brain, cerebral cortex Group A (normal) showing normal cerebral cortex (H&E), cerebral cortex Group B (2000 mg/kg) showing apparently normal hippocampus (H&E), cerebral cortex Group C (3000 mg/kg) showing few dark neurons (arrow) (H&E) and cerebral cortex Group D (5000 mg/kg) showing numerous dark degenerating neurons (arrows) (H&E).

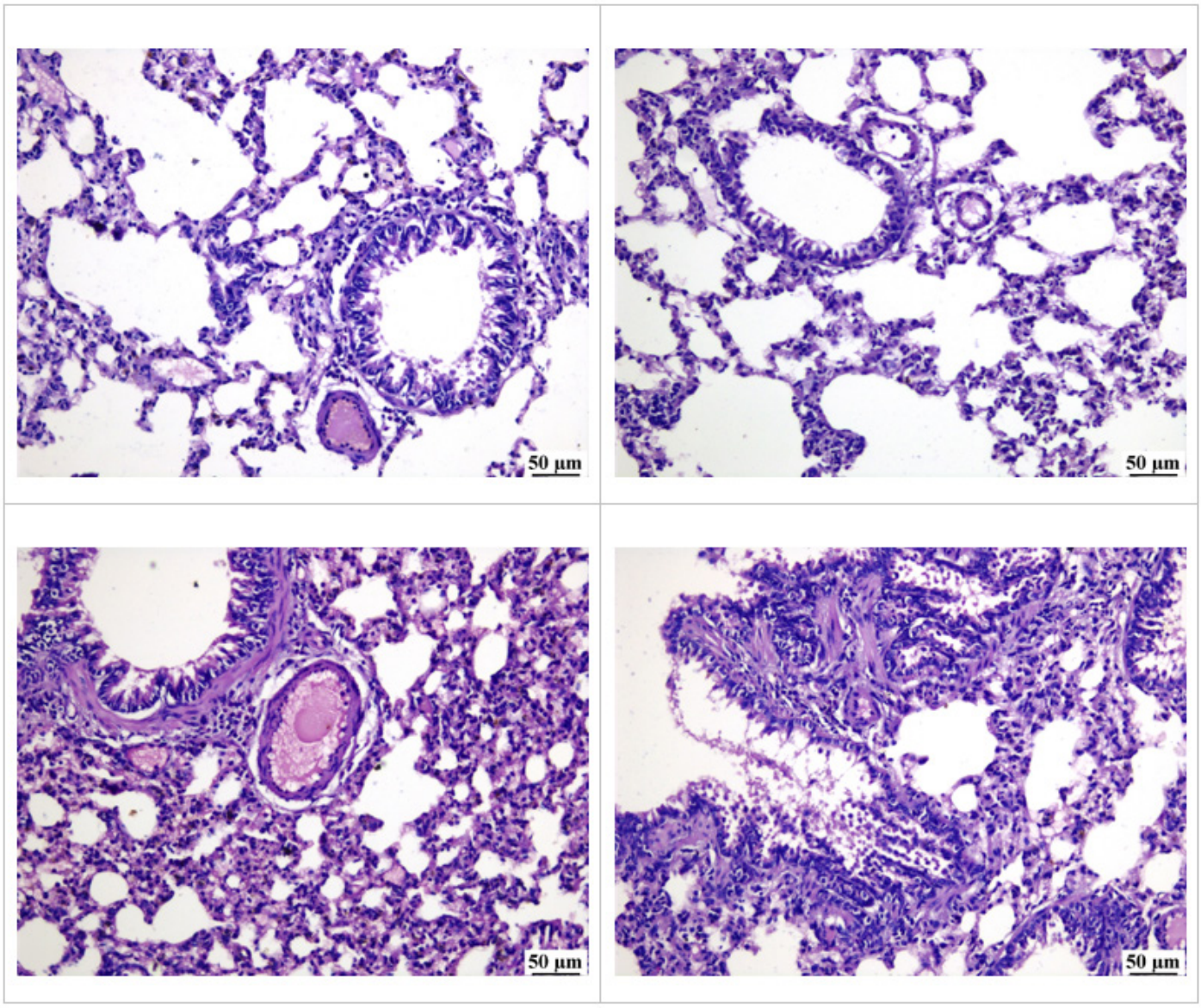

Figure 2: Photomicrograph of lungs, lungs of Group A (normal) showing normal bronchioles and alveoli (H&E), lungs of Group B (2000 mg/kg) showing apparently normal bronchioles and alveoli (H&E), lungs of Group C (3000 mg/kg) showing mild peribronchiolar and interstitial mononuclear inflammatory cells infiltration (H&E) and lungs of Group D (5000 mg/kg) peribronchiolar fibroplasia (arrow) with mononuclear inflammatory cells infiltration (H&E).

high doses of Ashwagandha harmed the inflammatory cytokines TNF-alpha and IL-6 compared to normal serum levels. The lower 2000 mg/kg dose did not adversely impact inflammatory cytokines.

Histopathological examination of rats' brains indicated that the low dose of Ashwagandha at 2000 mg/kg did not impact the cerebral cortex. In contrast, the higher dose of 3000 mg/kg showed the presence of a few degenerating neurons within the cerebral cortex. The administration of the highest dose (5000 mg/kg) resulted in the observation of numerous dark degenerating neurons within the cerebral cortex. The lung results were consistent with those of normal lung tissues at the 2000 mg/kg lower dose. The elevated doses began to induce congestion in the bronchioles and alveoli, accompanied by the infiltration of inflammatory cells. The kidney sections treated with a dose of 3000 mg/kg of Ashwagandha exhibited mild congestion in the renal glomeruli and renal tubules, as indicated by the histopathological analysis. The highest dose (5000 mg/kg) demonstrated an increase in the proliferation of renal glomeruli and significant hemorrhage in the interstitial tissue, resulting in the displacement of renal parenchyma. Earlier investigations indicated that administering low doses of Ashwagandha led to significant enhancements in cognitive flexibility, visual memory, reaction time, psychomotor speed, executive functioning, and stress response over 30 days at doses of 225 or 400 mg.[20,21]

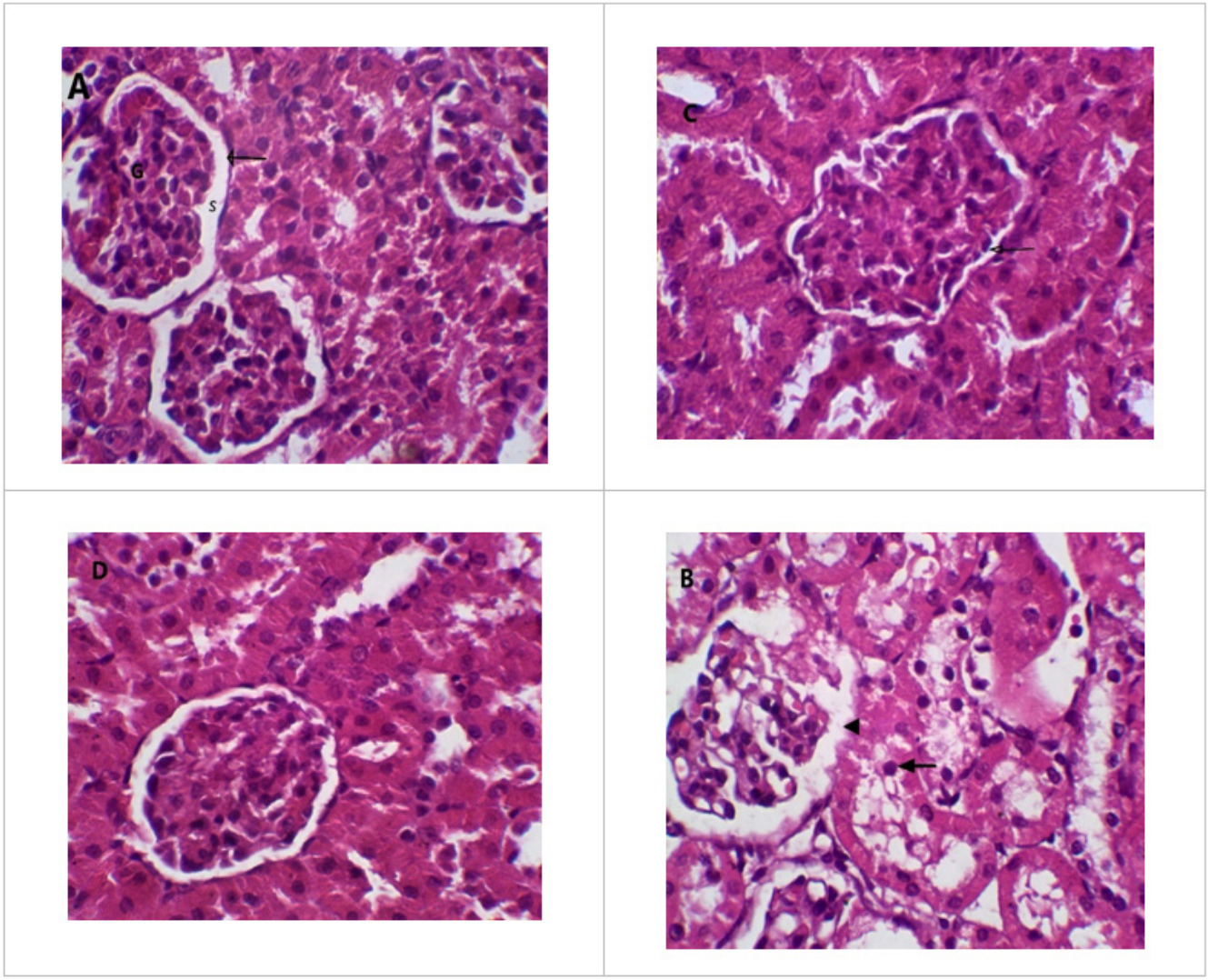

Figure 3: Photomicrograph of kidneys, kidney sections of Group A (normal) showing normal normal renal glomeruli with normal renal tubules (H&E), lungs of Group B (2000 mg/kg) showing apparently normal renal glomeruli with normal renal tubules (H&E), lungs of Group C (3000 mg/kg) showing mild congestion in renal glomeruli and renal tubules (H&E). Lungs of Group D (5000 mg/kg) showed proliferation of the renal glomeruli and marked hemorrhage in interstitial tissue displacing renal parenchyma (H&E).

CONCLUSION

In summary, the therapeutic doses of Ashwagandha demonstrated positive effects on various organs, including the liver, brain, lungs, and kidneys. Nonetheless, the current study indicates that toxicity starts to manifest when administered at elevated doses. Further investigations are necessary to explore and clarify the specific mechanisms by which Ashwagandha affects various organs when administered at high doses.

ACKNOWLEDGEMENT

This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia [Grant No. KFU253431].

ABBREVIATIONS

Ashwa: Ashwaganda; **ANOVA:** One-way analysis of variance; **ALT:** Alanine aminotransferase; **AST:** Aspartate aminotransferase; **GGT:** Gamma glutamyl transferase; **H&E:** Hematoxylin and Eosin; **IL-6:** Interleukin-6; **TNF-ALPHA:** Tumor Necrosis Factor-alpha.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

This research was funded by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia. The funding supports data collection and equipment.

ETHICAL STATEMENT

This study was conducted in accordance with the ethical standards of College of Pharmacy, King Faisal University, Saudi Arabia.

SUMMARY

In summary, *Withania Somnifera* (Ashwagandha) exhibits well-documented therapeutic benefits at low doses, especially regarding stress reduction, cognitive enhancement, and anti-inflammatory effects. This investigation emphasizes the possible toxicity linked to elevated dosages. The administration of Ashwagandha at a dosage of 2000 mg/kg showed no significant effects on organ function or histology. However, higher doses of 3000 and 5000 mg/kg were linked to increased liver enzymes, elevated serum creatinine levels, heightened expression of pro-inflammatory cytokines, and histopathological damage observed in the liver, brain, lungs, and kidneys. The results highlight the necessity of following prescribed therapeutic dosages and call for additional investigation into the mechanisms that contribute to ashwagandha-induced toxicity at elevated doses.

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Cite this article: Gad ES, Buobaid AO, ALfraih AY, Alanazi GM, Alrashid MA, Alessa MI, *et al*. High-Dose Ashwagandha Extract: A Toxicological Assessment in Male Wistar Rats Using a Dose-Dependent Approach. Indian J of Pharmaceutical Education and Research. 2026;60(2s):s721-s727.