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Safety assessment of *Withania somnifera* extract standardized for Withaferin A: Acute and sub-acute toxicity study

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ABSTRACT

Background: The use of *Withania somnifera* is increasing due to a number of its chemical constituents found useful for health.
Objective: The present study was carried out to investigate the potential adverse effects (if any) of a standardized *Withania somnifera* extract (WSE) in rats following acute and sub chronic administration.
Materials and methods: The toxicity study was performed in Wistar rats by oral administration. An acute toxicity study was done at the dose of 2000 mg/kg. In the sub-acute study, Wistar rats (10/sex/group) were administered via gavage 0 (control), 500, 1000, 2000 mg/kg body weight/day of WSE for 28 days. Among two additional satellite groups, one group did not receive any drug while the second group received 2000 mg/kg/day for 28 days. At the end of study, the animals sacrificed and their body weight, hematology, serum chemistry, and histopathology evaluation was done.
Results: 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.
Conclusion: Based on this study, the no-observed-adverse-effect-level of WSE is 2000 mg/kg body weight, the highest level tested.
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1. Introduction

Withania somnifer Dunal, commonly known as Ashwagandha, is an important medicinal plant in Ayurvedic and Unani system of medicine. Various preparations of *Withania somnifera* (WS) are available in the market. A significant anti-oxidant effect of WS has been seen in various rat brain areas, including striatum [1–4]. The use of WS is increasing due to a number of chemical constituents present in it are found useful for health [5]. Withaferin A is shown to exert potent anti-angiogenic activity *in vivo* at doses that are 500-fold lower than those previously reported to exert the anti-tumor activity *in vivo*. In conclusion, our findings identified a novel mode of action of Withaferin A, which highlights the potential use of this natural product for the cancer treatment or prevention. The major constituents present in WS root are steroidal alkaloids and steroidal lactones in a class of constituents called withanolides [6]. The current study evaluated whether Ashwagandha can be used safely in clinical trials for the treatment of Alzheimer's disease, cancer, and Parkinson's disease.

2. Materials and Methods

The present study was conducted at Sardar Patel College of Pharmacy, after the Institutional Animal Ethics Committee approval of the protocol. The protocol approval number of the study is SPCP/IAEC/RP-010/2012-13. The study protocol was prepared as per the Organization for Economic Co-operation and Development (OECD) Guidelines for testing the chemicals for repeated dose 28 day oral

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toxicity study in rodents, Section 407 (OECD Guideline, 2001) (Original 1981, revised in 1995) and Section 408 (OECD Guideline, 1995). The study was conducted in compliance with the OECD principle of good laboratory practices [22,23].

2.1. *Test substance*

The WS root extract was supplied by Pharmanza Herbal Pvt. Ltd., Gujarat, India. It was a brown powder, standardized to contain no <3% Withaferin A by high-performance liquid chromatography (HPLC) analysis (USP method). The appropriate amount of WS extract (WSE) was dissolved in distilled water to make a homogeneous solution. Solutions containing concentrations of 200, 100, 50, and 0 mg/ml were prepared daily prior to administration. The required solutions were administered to rats daily via oral gavage.

2.2. *Preparation of extract*

Authenticated WS roots were used to prepare the extract. Roots were powdered using a grinder and then extracted with methanol. Extract thus obtained was concentrated on rotator evaporator to distil methanol and to obtain a thick paste. 250 g of the thick paste of methanolic extract again extracted with 500 ml of ethyl acetate in Soxhlet extractor for 3 h at 65 °C temperature and then concentrated on rotator evaporator. Concentrated extract finally put in the drier to get dry powder.

2.3. *High-performance liquid chromatography profiling of W. somnifera extract*

The sample was prepared by dissolving 250 mg of WSE (Withania somnifera extract) in 50 ml methanol. The sample was passed through membrane filter before injecting 20 µl in column (Phenomenex, LUNA C18 (2), 5u250X4.6). The mobile phase used in HPLC was the combination of 1 mM $KH_2PO_4 + 0.05\%$ phosphoric acid and acetonitrile. Flow rate was maintained at 1.5 ml/min and ultraviolet detection performed at 227 nm [Fig. 1]. Ninety-seven percent pure Withaferin A used as a primary reference standard [Fig. 2].

2.4. *Quantification of Withaferin A*

Concentration and area values of standard Withaferin A and sample

	Standard Withaferine A	Sample
Concentration in µg/ml	42.45	5000
Percentage of purity	97.00	–
Area of Withaferin A	744,107	4,065,405

Formula to calculate percentage of Withaferin A in WSE:

$$\text{Amount of active present } (\%) = \frac{\text{Area of sample}}{\text{Area of standard}} \times \frac{\text{Concentration of standard}}{\text{Concentration of sample}} \times \text{Purity of standard}$$

Percentage of Withaferin A in WSE is 4.5.

2.5. *Experimental animals*

Healthy young female Wistar rats were used. Though there is little difference in sensitivity to LD_{50} studies between the sexes, female rats are known to be more sensitive than males.

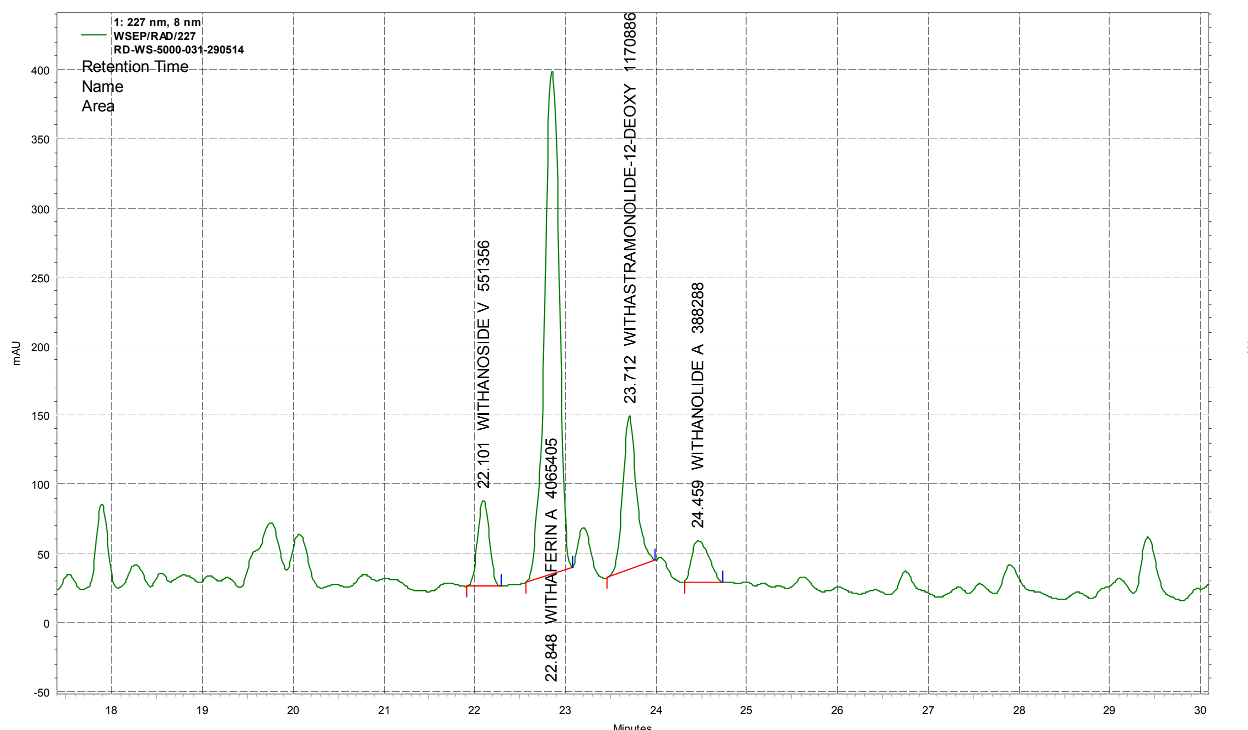

Fig. 1. Chromatogram of *Withania somnifera* extract.

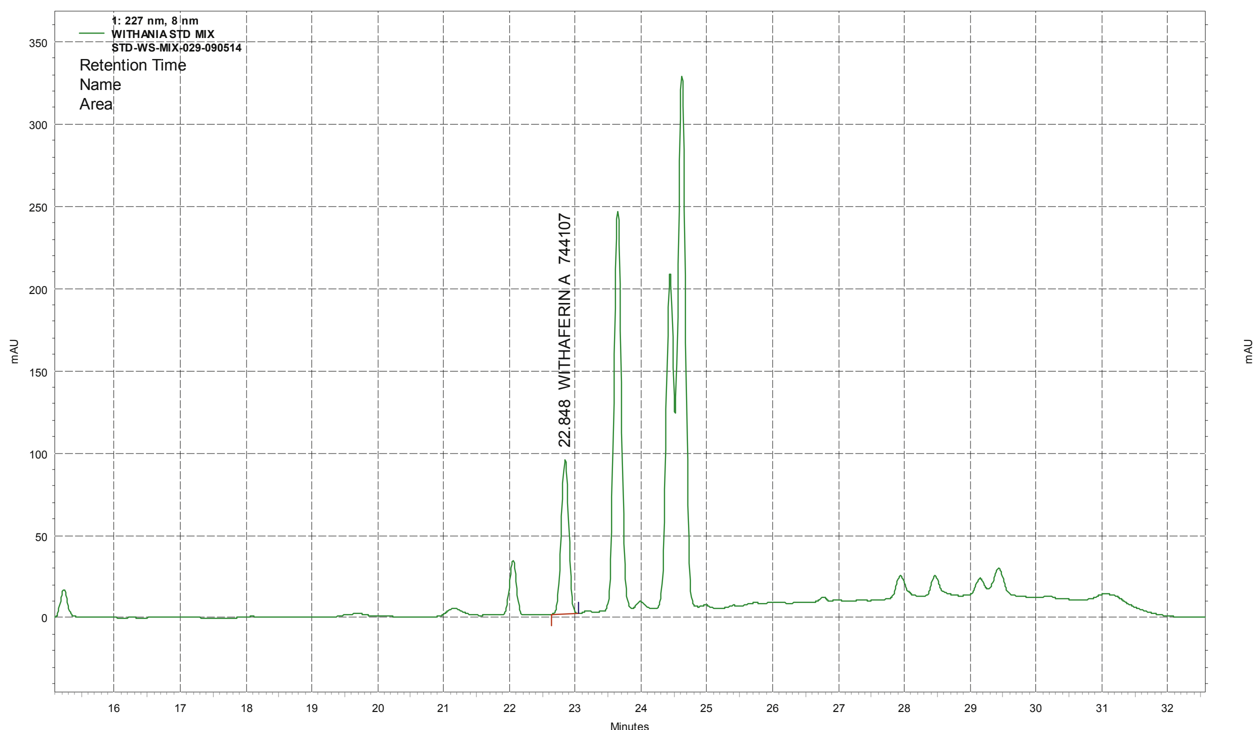

Fig. 2. Chromatogram of Withaferin A primary reference standard.

Nonpregnant rats were randomly selected, marked for identification, and housed in polypropylene cages in groups of five for 5 days prior to dosing. The ambient temperature in the experimental laboratory was maintained at 22 °C (±3 °C). Using artificial lighting, maintaining 12 h light and 12 h dark cycles were maintained. Standard pellet diet was given with water *ad libitum*.

For experiment designed to determine oral LD_{50} of WSE sighting study was done on one rat which was administered 2000 mg/kg WSE. Signs of toxicity were observed for 14 days. No toxic effects were seen in the sighting study; hence 2000 mg/kg dose was selected for the main study in five rats. Rats were administered the dose of 2000 mg/kg of WSE and signs and symptoms of toxicity were observed. On day 15, all the animals were euthanized and gross pathological examinations were done.

For sub-acute study, young, healthy rats of both sexes were used. They were divided into six groups each having five each of male and female. Rats were given oral (gavage) WSE of root in the doses of 0 (Group I control), 500 (Group II low-dose), 1000 (Group III mid dose), 2000 (Group IV high-dose) mg/kg/day for 28 days. Two additional satellite groups of animals received 0 (Group V) and 2000 (Group VI) mg/kg/day for 28 days. During the course of sub-acute study, all animals were provided an *ad libitum* feed, until the day prior to the scheduled euthanasia. The signs and symptoms of toxicity were observed for 28 days and body weight was measured on 0, 7, 14, 21, and 28 days. On day 28, animals from Groups I to IV were euthanized, blood was collected from a retro orbital vein for hematological and biochemical estimations. Then animals were sacrificed for organ weight, gross necropsy, and histopathology. Group V and VI were observed further for 14 days after 28 days dosing. On day 42, animals of both groups were euthanized, blood was collected from the animals for hematological and biochemical estimations, and animals were sacrificed for organ weight, gross necropsy, and histopathology.

2.6. *Clinical observations and body weight*

All animals were observed twice daily for morbidity and mortality. Clinical examination included any abnormal physical and behavioral changes. The observations included change in the skin, fur, eyes, mucus membranes, secretions, excretion, and autonomic activity. Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypes or bizarre behavior were recorded. Body weight of all animals was measured on day 0, 7, 14, 21, and 28.

2.7. *Hematological parameters*

The hematological examination was done at the end of the test period, hemoglobin (Hb) concentration, erythrocyte count, reticulocytes, total and differential leucocytes count, platelet count, clotting time (CT), activated partial thromboplastin time (APTT), prothrombin time, and reticulocyte counts were performed.

2.8. *Serum chemistry*

The standardized diagnostic kits (Q Dx by Parimal Healthcare manufactured under license from Diasyn Diagnostic System GmbH) were used for estimating glucose, cholesterol, triglycerides, urea, creatinine, aspertate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphate, total bilirubin, protein, albumin, globulin, sodium, phosphorus, calcium, chloride, blood urea nitrogen, and cholinesterase estimation.

2.9. *Histopathology*

All rats in the study were subjected to detailed gross necropsy which included a careful examination of external surfaces of the body, orifices and cranial, thoracic and abdominal cavity and their

contents. The liver, kidneys, adrenals, testes, epididymides, prostate, seminal vesicles, thymus, spleen, brain, and heart of all animals were trimmed of any adherent tissues. Their wet weight was recorded. For histopathology examinations, tissues were processed into paraffin blocks; ultrathin sections were dewaxed and were stained with hematoxylin and eosin.

3. Results

3.1. Acute toxicity study

In acute toxicity studies, oral LD50 of WSE in Wistar rats was >2000 mg/kg body weight. The day 14 observation in acute oral toxicity study and weekly body weight measurement did not show any toxic effects in rats. There was no abnormal behavior during the first 30 min (after dosing) and periodically for first 24 h and daily thereafter for 14 days. Administrations of WSE did not produce any organ atrophy, hypertrophy, and degenerative or infiltrative lesions.

3.2. Sub-acute toxicity

All animals survived until the scheduled necropsy in both 28 days and satellite group. Physical and behavioral examination did not reveal any treatment related adverse effects in any of the groups receiving 500 mg/kg/day, 1000 mg/kg/day and 2000 mg/kg/day of WSE. As compared to control group, no biological significant effects of WSE were noted on body weight gain. These results suggest that administration of WSE up to 2000/mg/kg/day to rats for 28 days has no adverse effect on the clinical observations and body weights. Similarly in satellite group as well no adverse effects were observed.

3.3. Feed and water consumption

Feed and water consumption in a different group of animals receiving WSE was similar in both satellite and control groups. These results show that administration of WSE up to 2000 mg/kg/day for 28 days to rats did not affect water and feed consumption.

3.4. Clinical pathology

3.4.1. Hematology

There was no treatment related the adverse effect of WSE on hematological parameters in females. However, some statically significant differences were noted when control and treatment group were compared. There was statically significant increase of Hb in groups treated with 500 and 2000 mg/kg/day of WSE in white blood cell (WBC) in a group treated with 2000 mg/kg/day of WSE. There were statically significant decreases in reticulocyte at 1000 mg/kg/day dose level and prothrombin time at 2000 mg/kg/day. However, values of CT and APTT of all treatment groups were in normal laboratory range, and these changes were considered as incidental not treatment related. There was a statistically nonsignificant increase in the values of red blood cells, packed cell volume (PCV), lymphocytes and platelets; however, the changes were within normal laboratory range [Table 1].

There was no treatment related adverse effect of WSE observed in hematological parameters in male rats. However, some statistically significant differences were noted when the control group and treatment group were compared. A statistically significant increase in Hb and mean corpuscular volume was observed in the group treated with 2000 mg/kg/day of WSE and also in PCV in a group treated with 500 and 2000 mg/kg/day of WSE. The change in PCV was within normal laboratory range and considering no changes in related endpoints, was not considered treatment related. There were no changes in any other hematological measures [Table 2]. In the recovery group, there were no other statistically significant differences when the respective control and treatment groups were compared. There were statistically nonsignificant increases in WBCs, mean corpuscular hemoglobin, APTT, platelets, and Hb% in both male and female treatment group. These variations were within the normal laboratory range. Increases in these values were not considered as toxicologically relevant [Table 3].

3.4.2. Serum chemistry

There was no treatment related biologically significant adverse effects of WSE on serum chemistry of female and male rats. In the recovery group, there is a significant decreased in mean values of glucose, cholesterol, triglyceride in extract treated female and male. All these variations were marginal and within the normal laboratory ranges. Decreases in these values were not considered as toxicologically relevant. There were no other statistically significant differences when the respective control and treatment groups were compared. The results of serum chemistry analysis from the treatment and recovery groups show that administration of WSE doses up to 2000 mg/kg to rats for 29 days did not cause toxicologically significant adverse effects [Table 4].

Table 1
Effect of *Ashwagandha* root extract on hematological parameters of female rats (sub-acute).

Parameters	Unit	Control	mg/kg		
			500	1000	2000
RBC	10^6/cm	7.39 ± 0.39	7.58 ± 0.29	8.21 ± 0.57	8.95 ± 0.42
WBC	10^3/cm	8.92 ± 0.33	9.78 ± 0.90	10.32 ± 0.87	10.54 ± 0.94*
Lymphocytes	%	76.0 ± 1.04	77.6 ± 2.24	81.6 ± 2.94	77.6 ± 0.92
Neutrophils	%	23 ± 1.04	21.4 ± 2.24	17.4 ± 2.94	21.4 ± 0.92
PCV	%	47.6 ± 2.58	48.4 ± 1.74	52.6 ± 1.5	53.2 ± 1.33
MCV	fL	54.8 ± 1.46	58.2 ± 0.79	50.4 ± 0.54	50.0 ± 1.80*
MCH	Pg	15.0 ± 1.13	15.6 ± 0.92	17.4 ± 0.39	17.8 ± 0.67
Platelets	10^3/cm	901.8 ± 50.98	905 ± 31.64	908.2 ± 29.35	918.6 ± 30.29
Hemoglobin	g/dl	10.25 ± 0.4	11.98 ± 0.43	13.14 ± 0.63*	15.18 ± 1.01*
CT	min	1.34 ± 0.01	1.32 ± 0.01	1.28 ± 0.05	1.27 ± 0.04
APTT	s	25.2 ± 1.31	22.8 ± 0.79	22.6 ± 0.97	21.6 ± 1.02
Prothrombin time	s	19.2 ± 1.1	15.8 ± 1.02	15.4 ± 0.66	15.2 ± 0.7*
Reticulocytes	%	0.68 ± 0.02	0.79 ± 0.04	0.48 ± 0.087*	0.61 ± 0.01

All value are expressed as mean ± SEM ($n = 5$). The data were statistically analyzed by one-way ANOVA followed by Dunnett test. $^*P < 0.05$, statistically significant as compared to normal control. APTT: Activated partial thromboplastin time, CT: Clotting time, MCH: mean corpuscular hemoglobin, MCV: Mean corpuscular volume, PCV: Packed cell volume, RBC: Red blood cell, WBC: White blood cell, SEM: Standard error of mean.

Table 2
Effect of *Ashwagandha* root extract on hematological parameters of male rats (sub-acute).

Parameters	Unit	Control	mg/kg		
			500	1000	2000
RBC	10^6/cm	8.28 ± 0.21	8.47 ± 0.37	8.86 ± 0.52	9.04 ± 0.55
WBC	10^3/cm	10.27 ± 1.02	11.57 ± 0.71	12.05 ± 0.73	12.35 ± 0.68
Lymphocytes	%	80.2 ± 1.35	81 ± 1.13	81.4 ± 1.5	82 ± 1.94
Neutrophils	%	18.8 ± 1.35	18 ± 1.14	17.6 ± 1.5	17 ± 1.94
PCV	%	47.2 ± 1.06	50.8 ± 0.85	52 ± 1.13*	52.2 ± 1.28*
MCV	fL	50.6 ± 1.62	53.6 ± 0.39	54 ± 0.89	55.6 ± 0.92*
MCH	Pg	15 ± 0.89	16.8 ± 0.91	17.2 ± 1.1	15.6 ± 1.1
Platelets	10^3/cm	894.6 ± 31.1	906.4 ± 31.74	912.4 ± 52.77	913.6 ± 48.57
Hemoglobin	g/dl	10.13 ± 0.29	11.18 ± 0.31	11.50 ± 0.1	13.53 ± 1.05*
CT	min	1.32 ± 0.01	1.31 ± 0.03	1.13 ± 0.09	1.15 ± 0.05
APTT	s	24 ± 0.85	23.8 ± 0.15	24.2 ± 0.73	23.2 ± 0.7
Prothrombin time	s	16.8 ± 1.06	16.4 ± 1.11	16.2 ± 1.46	14 ± 0.54
Reticulocytes	%	0.74 ± 0.02	0.67 ± 0.02	0.65 ± 0.04	0.62 ± 0.06

All value are expressed as mean ± SEM ($n = 5$). The data were statistically analyzed by one-way ANOVA followed by Dunnett test. *$P < 0.05$, statistically significant as compared to normal control. APTT: Activated partial thromboplastin time, CT: Clotting time, MCH: Mean corpuscular hemoglobin, MCV: Mean corpuscular volume, PCV: Packed cell volume, RBC: Red blood cell, WBC: White blood cell, SEM: Standard error of mean.

Table 3
Effect of *Ashwagandha* root extract on hematological parameters of female and male rats (satellite groups).

Parameters	Unit	Female		Male	
		Control	2000 mg/kg	Control	2000 mg/kg
RBC	10^6/cm	7.53 ± 0.36	7.61 ± 0.29	7.87 ± 0.57	8.11 ± 0.55
WBC	10^3/cm	8.14 ± 1.00	8.66 ± 1.03	9.61 ± 1.04	10.31 ± 1.27
Lymphocytes	%	75.6 ± 1.8	78.4 ± 1.43	76.6 ± 0.80	78.0 ± 0.89
Neutrophils	%	23.4 ± 1.8	20.6 ± 1.43	22.4 ± 0.80	21.0 ± 0.89
PCV	%	41.8 ± 2.9	42.2 ± 2.58	48.2 ± 1.23	49.8 ± 1.46
MCV	fL	53.2 ± 2.12	54.4 ± 1.29	59.4 ± 0.86	60.0 ± 2.44
MCH	Pg	16.8 ± 0.7	18.2 ± 0.48	17.4 ± 0.67	19.4 ± 1.20
Platelets	10^3/cm	882.6 ± 34.7	888.6 ± 14.68	899.6 ± 35.4	903.8 ± 29.87
Hemoglobin	g/dl	10.43 ± 0.36	11.48 ± 0.45	10.68 ± 0.59	10.71 ± 0.4
CT	min	1.33 ± 0.01	1.31 ± 0.02	1.39 ± 0.04	1.35 ± 0.01
APTT	s	24.6 ± 0.79	20.6 ± 0.50*	23.8 ± 1.65	22.2 ± 1.06
Prothrombin time	s	19.4 ± 1.59	15.2 ± 1.39	19.2 ± 0.91	17.2 ± 1.23
Reticulocytes	%	0.67 ± 0.29	0.70 ± 0.03	0.80 ± 0.02	0.73 ± 0.32

All value are expressed as mean ± SEM ($n = 5$). The data were statistically analyzed by one-way ANOVA. *$P < 0.05$, statistically significant as compared to normal control. APTT: Activated partial thromboplastin time, CT: Clotting time, MCH: Mean corpuscular hemoglobin, MCV: Mean corpuscular volume, PCV: Packed cell volume, RBC: Red blood cell, WBC: White blood cell, SEM: Standard error of mean.

Table 4
Effect of *Withania somnifera* extract on serum chemistry parameters of female and male rats (satellite groups).

Parameters	Unit	Female		Male	
		Control	2000 mg/kg	Control	2000 mg/kg
Glucose	mg/dl	80.4 ± 2.5	73 ± 3.73*	100.2 ± 3.02	92.8 ± 3.76*
Cholesterol	mg/dl	84 ± 1.69	70.4 ± 3.04*	113 ± 3.73	92.2 ± 2.41*
Triglyceride	mg/dl	58.2 ± 3.05	53.8 ± 3.62	67.6 ± 4.02	65.0 ± 3.26
Urea	mg/dl	35.6 ± 1.04	32.4 ± 1.07	41.6 ± 0.80	40.6 ± 1.28
Creatinine	mg/dl	0.70 ± 0.03	0.67 ± 0.008	0.81 ± 0.02	0.78 ± 0.006
AST	IU/L	119 ± 2.07	120 ± 5.06	137.6 ± 4.32	139.4 ± 5.4
ALT	IU/L	48.6 ± 2.39	46.2 ± 3.26	42.2 ± 2.78	40.2 ± 3.22
ALP	IU/L	79 ± 4.21	75.6 ± 4.87	84.6 ± 4.19	82.2 ± 4.18
Total billirubin	mg/dl	0.23 ± 0.008	0.22 ± 0.008	0.45 ± 0.01	0.43 ± 0.008
Protein	g/dl	7.24 ± 0.03	7.29 ± 0.04	7.50 ± 0.12	7.74 ± 0.09
Albumin	g/dl	3.95 ± 0.07	4.08 ± 0.02	4.00 ± 0.12	4.16 ± 0.08
Globulin	g/dl	3.20 ± 0.09	3.23 ± 0.25	4.01 ± 0.34	4.26 ± 0.41
Sodium	mEq/L	134.4 ± 1.43	132.8 ± 1.98	140.4 ± 0.50	139.2 ± 1.49
Potassium	mEq/L	4.08 ± 0.23	4.18 ± 0.23	4.22 ± 0.29	4.23 ± 0.37
Phosphorus	mEq/L	4.85 ± 0.15	4.72 ± 0.09	5.11 ± 0.09	4.98 ± 0.03
Calcium	mg/dl	9.50 ± 0.37	9.00 ± 0.35	7.79 ± 0.10	7.51 ± 0.35
Chloride	mEq/L	100.8 ± 1.01	103.6 ± 1.07	106.3 ± 3.03	107.2 ± 4.24
GGT	U/L	7.382 ± 0.15	7.236 ± 0.06	7.70 ± 0.08	7.55 ± 0.15
BUN	mg/dl	17.4 ± 0.62	15.43 ± 0.75	23 ± 0.70	22.4 ± 0.80
Cholinesterase	U/ml	0.83 ± 0.02	0.722 ± 0.04	0.9 ± 0.05	0.804 ± 0.02

All value are expressed as mean ± SEM ($n = 5$). The data were statistically analyzed by one-way ANOVA. *$P < 0.05$, statistically significant as compared to normal control. ALP: Alkaline phosphate, ALT: Alanine transaminase, AST: Aspartate aminotransferase, BUN: Blood urea nitrogen, GGT: Gamma glutamyl transferase, SEM: Standard error of mean.

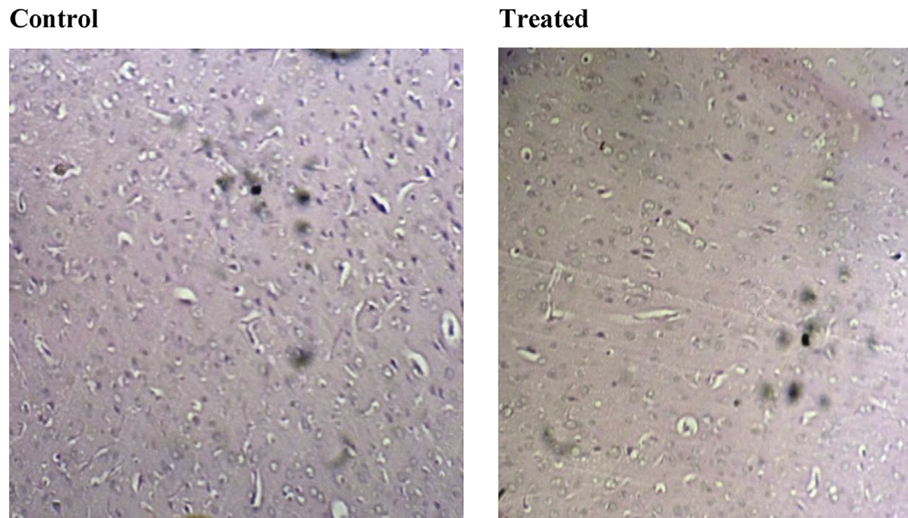

Fig. 3. Brain (H and E, ×400): Normal brain histologic structure, no signs of cell degeneration and necrosis/cell damage.

3.4.3. *Organ weight*

No treatment-related and biological significant changes in the value of organ weights were noted in female and male rats following administration of WSE.

In this study, repeated dose administration of WSE to the extent of 2000 mg/kg/day for 28 days did not produce any organ atrophy, hypertrophy, and degenerative or infiltrative lesion.

3.4.4. *Microscopic examination*

There was no treatment related histopathological findings in the acute group and satellite group. Similarly in the sub-acute group and satellite group no treatment related microscopic changes were observed. All findings observed were consistent with the normal background lesions in clinical normal rats of the age and strain used in this study. They were considered spontaneous and/or incidental in nature and unrelated to the treatment. Results from the present study suggest that administration of WSE at a dose of 2000 mg/kg/day to rats for 28 days has no adverse microscopic effects [Figs. 3–6].

4. Discussions

Root powder of WS is being used in the Ayurvedic system of medicine since ages, and it is found to be safe. Pure alcoholic or hydro alcoholic extracts of WS are not mentioned in Ayurveda, and the content of phytochemicals in such extracts is higher compared to raw powder. Animal toxicity studies with such extracts help to calculate safe starting doses in humans. A sub-acute toxicity study with hydroalcoholic extract of WS showed no evidence of toxic effect or mortality in Wistar rats [7]. Similarly, hydro alcoholic extract of WS found to be safe in sub-acute toxicity study involving rats and mice [8]. However the content of Withaferin A in these hydro alcoholic extracts are not reported. Our experience with the extraction shows that hydro alcoholic extract will have <1% of Withaferin A. Today, there is a lot of interest in the standardized extract of Ashwagandha for its use as an adjuvant therapy in cancer treatment as it is found to have radiation enhancing activity. Withaferin A and withanoloids have been tested for their anticancer activity and Withaferin A has shown promises in the treatment of cancer [9–11]. Ashwagandha extract is also found to have immunomodulatory effect in animal models [14–21]. So there was a need to conduct a study with a higher concentration of Withaferin A. We chose this extract which is easy to make as well it has a full range of withanoloids and has 4.5% of Withaferin A.

In the present study, increase in Hb and WBC count and decreases in reticulocyte and prothrombin time were observed however the values of all treatment groups were in normal laboratory range. Hence, the above changes were considered as incidental and are not treatment related. Hb is found to be increased in normal limits when the animals treated with WSE [12].

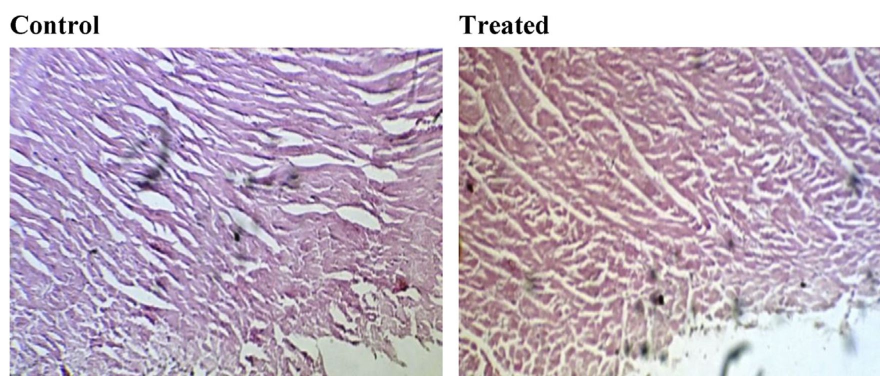

Fig. 4. Heart: Normal architecture of heart, with cardiac muscle and pericardium. No signs of degeneration and necrosis/inflammation or other changes.

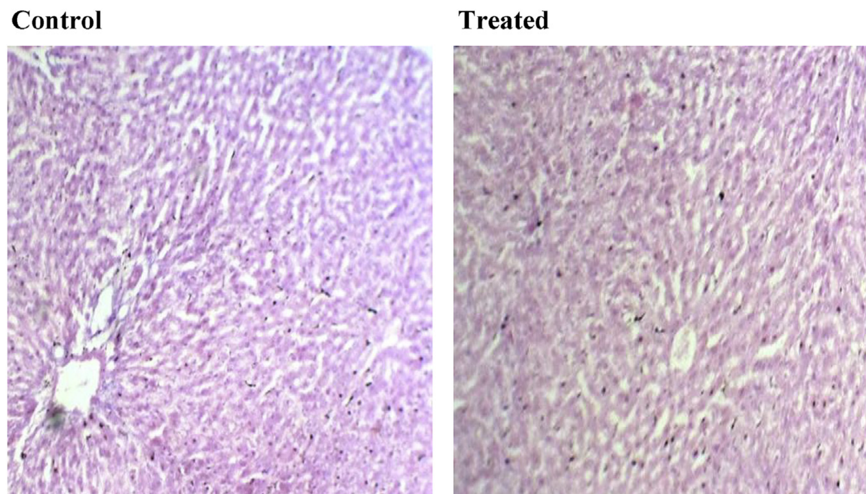

Fig. 5. Liver (H and E, ×400): Normal liver archistructure at high power, no hypostatic congestion at portal vein, no signs of tissue damage or malignancy.

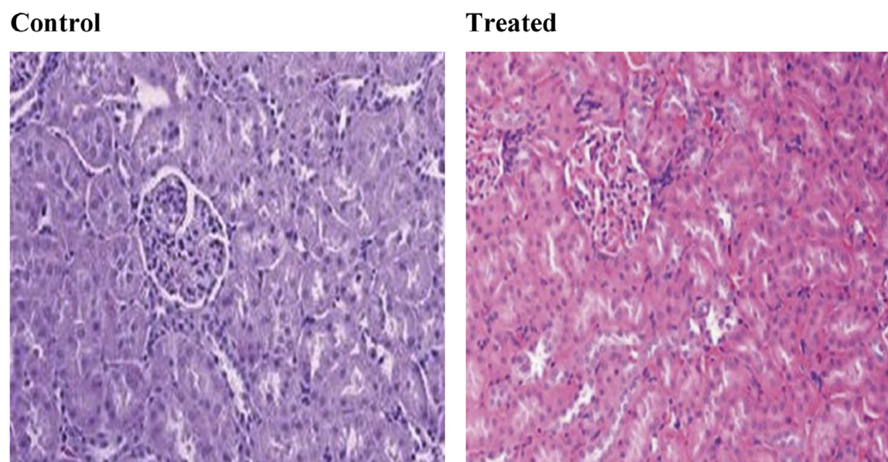

Fig. 6. Kidney: Normal tubular structure and glomerular structure in treated and control group. No signs of damage to tubules.

Administration of WSE at a dose of 1000 and 2000 mg/kg/day showed a significant increase in AST. Compared to control group, a significant increase in bilirubin in the group receiving 1000 and ALT in groups receiving 2000 mg/kg/day of WSE was observed. The increase in activities of AST and ALT represent liver damage but an increase of levels of AST; ALT and bilirubin were in normal range, so they were not considered toxicologically significant. Even these changes did not reflect histopathology changes of the liver. One sub-acute toxicity study of a combination of Ashwagandha and Ginseng reported two pathological incidents of emphysema and focal calcification in seminiferous tubules in rats. Such pathological incident may have caused due to the combination or ginseng only because no such pathological incidents seen in the present study where WSE used contains a higher percentage of Withaferin A [12].

Human clinical study with water extract of Ashwagandha and an animal toxicity study with water extract of Ashwagandha and Ginseng reported the increase in body weight and appetite. But a hydro alcoholic extract of WS and the extract used in the present study did not cause any organ or body weight gain. The appetite was also unchanged [7,12].

Though exact extrapolation of animal toxicity data to humans is difficult, some hematological findings of present study such as decrease in the levels of triglyceride, cholesterol, and urea are also get to see in an exploratory clinical study of Ashwagandha in healthy human volunteers [13]. In summary, the results of sub-acute toxicity suggest that oral administration of WSE containing 4.5% of Withaferin A at level up to 2000 mg/kg/day does not cause adverse effects in male and female rats.

5. Conclusion

Based on the study, the no-observed effect level of the extract was found to be 2000 mg/kg/day. Still we recommend 90 days sub-acute toxicity study as well as prenatal developmental toxicity study of WSE containing 4.5% of withaferin for further safety evaluation.

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Conflicts of interest

Dr. Lal L. Hingorani is an employee of Pharmanza Herbal Pvt. Ltd., Shruti B. Patel and Nirav J. Rao has declared that they have no competing interests.

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