



Original Research Article

In-vivo safety profile of *Withania somnifera* in Charles Foster rats

Ruchi Kumari¹, Anirban Pal¹, Madan Mohan Gupta², Dayanandan Mani¹, Debabrata Chanda^{1*}

¹In-vivo Testing Facility, Bioprospection and Product Development, Central Institute of Medicinal and Aromatic Plants (CSIR), Lucknow – 226015, India

²Analytical Chemistry Division, Central Institute of Medicinal and Aromatic Plants (CSIR), Lucknow – 226015, India

Abstract

Acute and sub-acute toxicity experiments with aqueous extracts of the leaf and root of *Withania somnifera* were carried out in male and female Charles Foster (CF) rats, including HPLC fingerprinting of the extracts. In an acute toxicity study with the extracts at 2000 mg/kg body weight as a single oral dose, no significant changes were found in the observational parameters, body weight, hematological and biochemical parameters, including gross pathological examinations with either of the extracts. Sub-acute toxicity study with the extracts at 5, 50 and 300 mg/kg body weight once orally for 28 days showed non-significant changes in observational parameters, body weights hematological and most of the biochemical parameters, except significant increase in total cholesterol and HDL cholesterol level in root extract and significant decrease in SGPT activity in leaf extract which are instead the indicator of betterment of biochemical profiles indicating that the extracts were well tolerated by the experimental animals. Additionally, HPLC analysis of the said extracts could detect the presence of withaferin A and withanolide A in leaf extract and 12-deoxywithastramonolide and withanolide A in root extracts.

Keywords: Ashwagandha, Oral toxicity, Rats, Root and leaf extracts, Safety evaluation.

Introduction

Withania somnifera Dunal (family-solanaceae) is well known for its medicinal value from time immemorial and has been an important herb in the Ayurvedic and Indian System of Medicine for over 3000 years (Ahuja et al., 2009). The plant is well known as an anti-inflammatory agent (Aphale et al., 1998), aphrodisiac, liver tonic, astringent (Ahuja et al., 2009), antistress agent (Archana et al., 1999; Bhattacharya et al., 1997; Chanda et al., 2009; Dadkar et al., 1987), antioxidant (Chanda et al., 2009) and as an immunomodulator (Devi et al., 1992). Recent studies expanded its use in many cases as an antitumor agent (Dhuley et al., 1998; Jaiswal et al., 1989), in the treatment of insomnia, senile dementia, neurological disorders (Joshua et al., 2007; Kulkarni et al., 2007) and as an adaptogen (Malhotra et al., 1965). Considering the tremendous importance of this herb, several alternative approaches have been adopted for the conservation of this herb, like the use of shoot culture as a renewable resource for the production of active molecules from *W. somnifera* (Mirjalili et al., 2009), RAPD and HPTLC analysis for bioactive compounds

from different species of Ashwagandha (Mishra et al., 2001), etc. Recently, our institute has come up with a new variety of ashwagandha, namely *Poshita*, which has higher root yield and withanoloid yield compared to the other variety available (Mishra et al., 2000). In view of the multi-dimensional use of this herb, Kulkarni and Dhir et al. (2000) reviewed literatures regarding *W. somnifera* pertaining to its different therapeutic uses and safety. However, literature on a detailed systematic toxicity study of this herb is still limited. Aphale et al. (1998) studied the sub-acute toxicity of a combination of *W. somnifera* and *Panax Ginseng* in rat but report on systematic safety evaluation of *W. somnifera* alone and the *Poshita* variety per se is still not very common. Kalaivani et al. (2023) studied Ashwagandha root extract, which failed to show any mortality in doses up to 2000 mg/kg oral dosage and did not show any mutagenic (genotoxic) effects in high concentrations. Considering the tremendous importance of *W. somnifera* in alternative medicine for multiple clinical conditions, acute and sub-acute toxicity of aqueous extracts of root and leaf were carried out using

Received: 03rd November, 2025

Accepted: 19th February, 2026

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*Corresponding author; Email: dchanda.cimap@csir.res.in

the plant materials from the *Poshita* variety of *W. somnifera* in the present experiment, including HPLC analysis of the extracts for quality control.

Materials and Methods

Animals

Female Charles Foster (CF) rats of 6 to 8 weeks of age were chosen for the study. The rats were maintained at $22 \pm 5^\circ\text{C}$ with humidity control and also on an automatic dark and light cycle of 12 hours. The animals were fed with the standard rat feed and provided *ad libitum* drinking water. Experimental protocols were duly approved by the institutional animal ethics committee (AH-2012-01) and were carried out following OECD test guideline no. 420 for acute oral toxicity and OECD test guideline no. 407 for sub-acute oral toxicity in the CF rats using aqueous extract from the roots and leaves.

Materials

Roots and leaves of *W. somnifera* were collected from the medicinal plant garden of our institute and a voucher specimen was deposited in the herbarium (CIMAP 860101). To one part of the course, powdered root or leaf, three parts of distilled water were added and left overnight for soaking. The aqueous phase was then collected, filtered first through double-layered muslin cloth and then through filter paper and the process was repeated thrice. The pooled extract thus obtained was then concentrated at 65°C in a rotary vacuum evaporator and the dried extract obtained was used for the experiment. Chemicals and reagents were obtained from E-Merck and Sigma Chemicals Ltd. Diagnostic kits used were obtained from Bayer Limited, Germany.

HPLC analysis of extracts

Both root and leaf extracts were analyzed for markers like withaferin A, 12-deoxywithastramonolide and withanolide A using HPLC (Scartezzini et al., 2007). Analysis was performed using the column Waters Spherisorb^R C₁₈ (4.6*250 mm, 10 μm ODS2), mobile phase: acetonitrile 0.1%, trifluoroacetic acid in water (40:60 v/v); flow rate 1.0 mL/min; detection wavelength 220 nm on a Shimadzu LC 20AD HPLC equipped with PDA detector (SPD 20A) (Sharma et al., 1986).

Acute oral toxicity study

Ten adult rats of 6 to 8 weeks of age were taken and divided into two groups having five animals in each group. Animals of group 1 were kept as controls and treated with distilled water, while animals of group 2 were kept as experimental and were treated with aqueous root extract of *W. somnifera* at 2000 mg/kg body weight as a single oral dose and were repeated for the acute experiment with aqueous leaf extract of *W. somnifera*. Body weights were recorded individually before dosing and on the 7th day after dosing.

Sub-acute oral toxicity study

In the first phase of the sub-acute experiment, 20 rats were used and divided into four groups, namely group 1, 2, 3 and 4, comprising five animals in each group. Animals of group 1 were kept as control and were treated with distilled water orally while animals of groups 2, 3 and 4 were treated with aqueous root extract of *W. somnifera* at 5, 50 and 300 mg/kg body weight once orally for 28 days while in second phase, experiment was carried out with aqueous leaf extract of *W. somnifera* in a similar way to that of aqueous root extract. Animals in both the phases of experiments were observed for mortality, appearance and any signs of intoxication. Body weights were recorded at the 0th day, 14th day and 28th day after treatment.

In-life observation

The animals were checked daily for mortality and any signs of ill health. A daily general case side clinical examination before and after commencement of the experiment was carried out, including changes in skin, mucous membrane, eyes, occurrence of secretion and excretion and also responses like lacrimation, piloerection, respiratory patterns, etc. Also, changes in gait, posture and response to handling were also recorded (Singh et al., 1982).

Collection of samples

Blood samples were collected from all the animals of the acute experiment on the 7th day post dosing and used for analysis of different hematological and biochemical parameters. The animals of the acute experiment were then sacrificed and used for gross necropsy. Similarly, blood samples from all groups of animals of the sub-acute experiment were collected on the 0th, 14th and 28th day post-dosing and analyzed for different hematological and biochemical parameters. On completion, the animals were sacrificed and used for gross pathological findings.

Hematological and biochemical analysis

Neubauer's chamber was used to quantify the total RBC and WBC count. Hemoglobin level was estimated by the indirect acid haematin method and expressed as g/dL (Singh et al., 1986). Serum biochemical parameters like creatinine, serum glutamate oxaloacetate transaminase (SGOT) activity, serum glutamate pyruvate transaminase (SGPT) activity, total cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol were estimated using kits from Bayer Chemicals Ltd, Germany, in a semi-auto-analyzer, RA 50 chemistry system from Bayer Ltd., Germany.

Statistical analysis

The effect of aqueous extracts of *W. somnifera* root and leaf in experimental conditions was analyzed using the INSTAT program for the results (Mean $\pm$ SE).

Results and Discussion

HPLC analysis

Aqueous extracts of *W. somnifera* leaf and root were analyzed for withaferin-A, 12-deoxywithastramonolide and withanolide-A at a wavelength of 220 nm on a Shimadzu HPLC equipped with a PDA detector. Withanolide-A was detected in both of the samples, whereas withaferin-A was detected only in the leaf extract, and also 12-deoxywithastramonolide in the root extract.

In life observations

Rats treated with aqueous extracts of *W. somnifera* root and leaf showed no observable changes either in the acute experiment at 2000 mg/kg body weight or the sub-acute experiment at 5, 50 and 300 mg/kg body weight for 28 days. No morbidity or mortality was found during the entire experimental period, both in acute and sub-acute experiments.

Body weight

Body weights of control and experimental groups of animals taken before dosing and on the 7th day after dosing in the acute experiment with *W. somnifera* leaf and root aqueous extract separately showed non-significant changes (Table 1). Similarly, in the sub-acute experiment with the same extracts, body weights taken before dosing (0 day), 14th day, and 28th day after dosing from all groups of animals also showed non-significant changes compared to the respective 0th day value (Tables 2 and 3).

Biochemical parameters

Hematological parameters like total WBC, RBC count and hemoglobin level were estimated from blood samples of both acute and sub-acute studies and on analysis showed non-significant changes in all the experimental

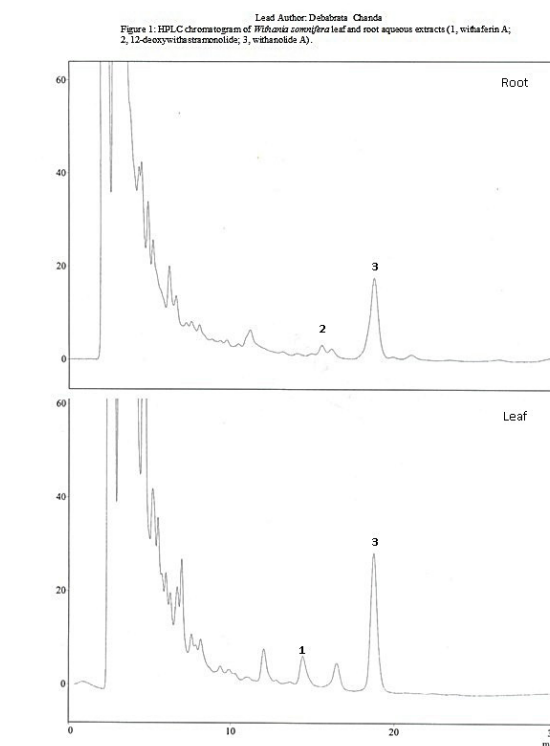


Figure 1: HPLC chromatogram of *Withania somnifera* leaf and root aqueous extracts (1, withaferin A; 2, 12-deoxywithastramonolide; 3, withanolide A)

groups compared to control in both acute and sub-acute experiments (Tables 1, 2, and 3). Serum samples from acute and sub-acute experiments were analyzed for SGOT, SGPT, creatinine and lipid indices like total cholesterol, triglycerides, HDL and LDL cholesterol. Serum biochemical analysis showed non-significant changes in any of the parameters studied in acute oral toxicity experiments, either with leaf or root aqueous

Table 1: Effect of *Withania somnifera* root and leaf extracts at 2000 mg/kg body weight once orally on body weight, haematological and serum biochemical parameters in female Charles Foster rat (n = 5; Mean ± SE).

Parameters	Aqueous root extract		Aqueous leaf extract	
	Control	Experimental	Control	Experimental
Body weight (g)	235.68 ± 3.73	230.97 ± 4.60	198.91 ± 6.98	215.78 ± 6.75
Haemoglobin (g/dL)	9.87 ± 0.61	9.49 ± 0.75	12.97 ± 0.55	12.48 ± 0.85
Total RBC (millions/mm ³)	3.85 ± 0.15	4.38 ± 0.33	4.05 ± 0.30	4.77 ± 0.21
Total WBC (thousands/mm ³)	10.67 ± 0.88	8.47 ± 0.94	10.67 ± 0.88	8.47 ± 0.94
Serum creatinine (mg/dL)	0.53 ± 0.06	0.48 ± 0.02	0.39 ± 0.11	0.33 ± 0.04
SGOT (U/L)	79.00 ± 2.72	62.20 ± 3.17	103.80 ± 9.69	136.00 ± 7.07
SGPT (U/L)	33.40 ± 3.11	42.00 ± 2.81	41.20 ± 2.22	50.20 ± 3.68
Total cholesterol (mg/dL)	96.92 ± 8.62	100.10 ± 8.20	115.34 ± 9.16	111.41 ± 8.30
HDL cholesterol (mg/dL)	75.49 ± 7.65	67.20 ± 3.02	79.07 ± 8.59	75.63 ± 7.20
LDL cholesterol (mg/dL)	18.47 ± 2.58	29.56 ± 8.63	26.31 ± 3.53	24.09 ± 3.87
Triglycerides (mg/dL)	29.67 ± 3.13	33.42 ± 3.82	49.80 ± 4.18	58.47 ± 6.82

Table 2: Effect of *Withania somnifera* root aqueous extract at 5, 50 and 300 mg/kg body weight once orally for 28 days on body weight and different biochemical and hematological parameters in Charles Foster rat (Mean $\pm$ SE). (n = 5, *p < 0.05 compared to control)

	0 Day				14 th Day				28 th Day			
	Control	5 mg/kg	50 mg/kg	300 mg/kg	Control	5 mg/kg	50 mg/kg	300 mg/kg	Control	5 mg/kg	50 mg/kg	300 mg/kg
Body weight (g)	214.36 $\pm$ 4.69	204.05 $\pm$ 7.11	152.25 $\pm$ 6.03	223.38 $\pm$ 4.82	214.39 $\pm$ 5.65	204.24 $\pm$ 6.39	175.49 $\pm$ 6.22	226.89 $\pm$ 7.22	221.26 $\pm$ 4.52	211.94 $\pm$ 6.04	183.53 $\pm$ 5.80	233.38 $\pm$ 8.24
Hemoglobin level (g/dL)	14.23 $\pm$ 0.69	14.12 $\pm$ 0.53	14.36 $\pm$ 1.56	15.81 $\pm$ 1.68	13.23 $\pm$ 1.03	12.68 $\pm$ 0.54	11.63 $\pm$ 0.57	10.41 $\pm$ 0.52	9.93 $\pm$ 0.74	11.93 $\pm$ 1.25	13.16 $\pm$ 1.77	10.65 $\pm$ 1.14
Total WBC count (thousands/mm ³)	9.35 $\pm$ 1.01	10.01 $\pm$ 1.47	9.76 $\pm$ 0.65	9.16 $\pm$ 1.07	9.44 $\pm$ 1.16	8.44 $\pm$ 0.85	8.10 $\pm$ 0.55	9.29 $\pm$ 0.72	8.07 $\pm$ 0.47	10.67 $\pm$ 0.88	8.47 $\pm$ 0.94	8.45 $\pm$ 0.72
Total RBC count (millions/mm ³)	3.85 $\pm$ 0.13	3.42 $\pm$ 0.34	3.85 $\pm$ 0.15	4.38 $\pm$ 0.33	3.87 $\pm$ 0.24	3.95 $\pm$ 0.14	4.59 $\pm$ 0.47	3.97 $\pm$ 0.30	4.43 $\pm$ 0.28	4.83 $\pm$ 0.53	4.48 $\pm$ 0.24	4.94 $\pm$ 0.38
Serum creatinine (mg/dL)	0.54 $\pm$ 0.06	0.44 $\pm$ 0.05	0.68 $\pm$ 0.06	0.52 $\pm$ 0.03	0.61 $\pm$ 0.05	0.64 $\pm$ 0.02	0.79 $\pm$ 0.11	0.85 $\pm$ 0.08	0.70 $\pm$ 0.09	0.64 $\pm$ 0.02	0.71 $\pm$ 0.02	0.70 $\pm$ 0.03
SGOT (U/L)	116.20 $\pm$ 9.04	120.00 $\pm$ 17.24	106.60 $\pm$ 5.56	93.20 $\pm$ 9.01	131.80 $\pm$ 11.42	110.40 $\pm$ 11.30	112.80 $\pm$ 3.47	92.80 $\pm$ 6.38	117.30 $\pm$ 7.34	118.30 $\pm$ 15.54	88.90 $\pm$ 3.46	87.60 $\pm$ 5.85
SGPT (U/L)	40.00 $\pm$ 3.23	45.00 $\pm$ 6.25	46.80 $\pm$ 3.07	39.20 $\pm$ 1.53	41.00 $\pm$ 5.06	51.00 $\pm$ 4.97	41.80 $\pm$ 0.58	40.60 $\pm$ 2.11	34.40 $\pm$ 3.38	52.10 $\pm$ 10.04	33.70 $\pm$ 1.37	35.10 $\pm$ 1.68
Serum total cholesterol (mg/dL)	70.11 $\pm$ 6.89	66.02 $\pm$ 4.57	61.37 $\pm$ 4.62	64.11 $\pm$ 3.18	72.70 $\pm$ 4.81	81.59 $\pm$ 5.40	67.04 $\pm$ 4.32	83.59 $\pm$ 5.45	65.65 $\pm$ 3.60	66.37 $\pm$ 4.70	100.69 $\pm$ 13.39*	84.36 $\pm$ 5.78*
Serum triglycerides (mg/dL)	44.59 $\pm$ 5.39	46.89 $\pm$ 4.73	42.27 $\pm$ 6.59	65.04 $\pm$ 8.72	32.25 $\pm$ 4.31	28.52 $\pm$ 6.12	39.71 $\pm$ 7.50	44.68 $\pm$ 4.97	41.78 $\pm$ 1.56	38.93 $\pm$ 12.95	44.61 $\pm$ 7.09	57.25 $\pm$ 8.48
Serum HDL cholesterol (mg/dL)	46.44 $\pm$ 6.02	41.11 $\pm$ 4.02	39.83 $\pm$ 3.36	39.27 $\pm$ 5.17	48.25 $\pm$ 2.12	55.60 $\pm$ 4.73	41.92 $\pm$ 3.65	54.04 $\pm$ 3.56	40.21 $\pm$ 2.83	48.31 $\pm$ 3.65	56.01 $\pm$ 5.13*	50.46 $\pm$ 2.59*
Serum LDL cholesterol (mg/dL)	14.76 $\pm$ 1.76	15.49 $\pm$ 1.23	13.08 $\pm$ 0.94	11.85 $\pm$ 1.54	18.87 $\pm$ 4.30	21.00 $\pm$ 4.80	17.87 $\pm$ 2.05	21.11 $\pm$ 3.38	17.09 $\pm$ 4.70	10.28 $\pm$ 1.21	35.74 $\pm$ 7.56	22.46 $\pm$ 5.30

extracts of *W. somnifera* (Table 1). Similarly, serum samples from sub-acute experiments either with leaf or root extracts of *W. somnifera*, on analysis, showed non-significant changes in most of the parameters studied compared to control (Tables 2 and 3) except total cholesterol and HDL cholesterol in root extracts (Table 2) and SGPT activity (Table 3) in leaf extract. Total cholesterol level showed a significant increase in groups of animals treated with the aqueous root extract at 50 and 300 mg/kg body weight on the 28th day after treatment compared to the respective control (Table 2). On further compartmental analysis, it was observed that only the HDL component (Table 2) of the total cholesterol showed a significant increase in serum level in the said group (i.e., 50 and 300 mg/kg body weight) compared to the control. Serum triglycerides and LDL cholesterol levels remained unaffected. SGPT activity in the sub-acute experiment with *W. somnifera* leaf aqueous extract showed a significant decrease in activity in groups of animals treated with the extract at 50 and 300 mg/kg body weight on 14th and 28 day after treatment compared to respective control (Table 3). Animals of both acute and sub-acute experiments on gross pathological study after

the completion of the experiment showed no observable changes.

Summarizing the complete study, it was observed that aqueous root and leaf extracts of *W. somnifera* (Poshita variety) when administered orally to adult female CF rats at acute (2000 mg/kg body weight as a single dose) and sub-acute levels (at 5, 50 and 300 mg/kg body weight once daily for 28 days) in different phases, no morbidity, mortality or gross pathological changes could be observed. Non-significant changes were observed in absolute body weight and hematological parameters, including most of the serum biochemical parameters studied in both acute and sub-acute experiments, except for an increase in total and HDL cholesterol levels in sub-acute experiments with root extracts and a decrease in SGPT activity in sub-acute experiments with leaf extracts. Our observation is in corroboration with an earlier report on sub-acute toxicity study of a combination of *W. somnifera* and ginseng for a period of 90 days, showing no observable changes on various safety parameters like body weight, hematological, biochemical, histopathological studies, etc. Similarly, some earlier studies with alkaloid fraction of *W. somnifera* (Srivastava et al., 2008), boiled water

Table 3: Effect of *Withania somnifera* leaf aqueous extract at 5, 50 and 300 mg/kg body weight once orally for 28 days on body weight and different biochemical and hematological parameters in Charles Foster rat (Mean $\pm$ SE). (n = 5, ** p<0.01 compared to control)

	0 Day				14 th Day				28 th Day			
	Control	5 mg/kg	50 mg/kg	300 mg/kg	Control	5 mg/kg	50 mg/kg	300 mg/kg	Control	5 mg/kg	50 mg/kg	300 mg/kg
Body weight (g)	166.53 $\pm$ 7.57	174.01 $\pm$ 12.81	171.36 $\pm$ 9.66	187.31 $\pm$ 8.68	192.63 $\pm$ 9.98	201.61 $\pm$ 14.71	194.50 $\pm$ 11.40	210.42 $\pm$ 6.68	200.99 $\pm$ 9.56	205.79 $\pm$ 12.68	200.53 $\pm$ 9.10	211.57 $\pm$ 4.11
Hemoglobin level (g/dL)	13.86 $\pm$ 0.60	12.69 $\pm$ 0.86	12.79 $\pm$ 0.55	13.28 $\pm$ 0.65	12.68 $\pm$ 0.54	11.63 $\pm$ 0.57	10.41 $\pm$ 0.52	9.87 $\pm$ 0.61	11.93 $\pm$ 1.25	13.16 $\pm$ 1.77	10.65 $\pm$ 1.14	13.75 $\pm$ 1.64
Total WBC count (thousands/mm ³)	9.16 $\pm$ 1.07	9.44 $\pm$ 1.16	7.39 $\pm$ 0.82	8.10 $\pm$ 0.55	9.29 $\pm$ 0.72	8.46 $\pm$ 0.64	10.27 $\pm$ 1.01	8.47 $\pm$ 0.94	9.13 $\pm$ 0.47	8.92 $\pm$ 1.08	9.03 $\pm$ 0.78	10.01 $\pm$ 0.92
Total RBC count (millions/mm ³)	4.83 $\pm$ 0.36	4.56 $\pm$ 0.57	4.62 $\pm$ 0.54	4.57 $\pm$ 0.65	4.38 $\pm$ 0.33	4.50 $\pm$ 0.34	4.38 $\pm$ 0.32	6.08 $\pm$ 0.19	4.35 $\pm$ 0.40	4.62 $\pm$ 0.53	5.48 $\pm$ 0.49	4.61 $\pm$ 0.32
Serum creatinine (mg/dL)	0.74 $\pm$ 0.06	0.74 $\pm$ 0.06	0.59 $\pm$ 0.08	0.68 $\pm$ 0.05	0.47 $\pm$ 0.03	0.42 $\pm$ 0.08	0.58 $\pm$ 0.08	0.34 $\pm$ 0.05	0.58 $\pm$ 0.05	0.56 $\pm$ 0.04	0.78 $\pm$ 0.05	0.67 $\pm$ 0.05
SGOT (U/L)	92.80 $\pm$ 2.48	98.20 $\pm$ 6.70	122.20 $\pm$ 14.05	116.20 $\pm$ 7.26	137.20 $\pm$ 10.20	136.80 $\pm$ 7.39	115.60 $\pm$ 7.82	115.60 $\pm$ 9.65	165.00 $\pm$ 12.90	161.40 $\pm$ 5.37	130.00 $\pm$ 15.10	150.00 $\pm$ 9.35
SGPT (U/L)	40.80 $\pm$ 2.84	32.20 $\pm$ 1.93	28.40 $\pm$ 4.02	31.60 $\pm$ 4.68	49.40 $\pm$ 2.98	42.80 $\pm$ 1.85	29.20 $\pm$ 4.26**	30.40 $\pm$ 4.09**	48.60 $\pm$ 3.72	49.20 $\pm$ 4.08	36.20 $\pm$ 4.99**	35.60 $\pm$ 5.56**
Serum total cholesterol (mg/dL)	100.92 $\pm$ 11.05	136.94 $\pm$ 21.93	110.57 $\pm$ 8.19	127.42 $\pm$ 10.70	137.01 $\pm$ 8.50	172.88 $\pm$ 18.75	188.90 $\pm$ 21.38	165.33 $\pm$ 11.97	111.44 $\pm$ 11.43	123.81 $\pm$ 3.68	131.03 $\pm$ 23.15	113.04 $\pm$ 6.19
Serum triglycerides (mg/dL)	52.54 $\pm$ 4.85	62.64 $\pm$ 6.68	60.07 $\pm$ 6.03	52.99 $\pm$ 3.32	39.42 $\pm$ 7.20	41.54 $\pm$ 8.74	43.65 $\pm$ 5.61	35.71 $\pm$ 3.22	52.75 $\pm$ 3.66	53.05 $\pm$ 6.28	50.83 $\pm$ 3.80	49.41 $\pm$ 9.37
Serum HDL cholesterol (mg/dL)	41.83 $\pm$ 6.12	62.50 $\pm$ 12.97	57.79 $\pm$ 9.68	36.39 $\pm$ 5.35	79.82 $\pm$ 3.05	110.42 $\pm$ 10.87	112.05 $\pm$ 16.43	93.83 $\pm$ 10.00	69.22 $\pm$ 6.74	60.24 $\pm$ 6.85	82.67 $\pm$ 20.55	69.54 $\pm$ 5.17
Serum LDL cholesterol (mg/dL)	48.58 $\pm$ 11.83	61.91 $\pm$ 11.44	40.77 $\pm$ 13.44	80.43 $\pm$ 8.99	49.31 $\pm$ 8.23	54.16 $\pm$ 6.75	68.12 $\pm$ 12.14	64.36 $\pm$ 1.84	31.66 $\pm$ 5.67	52.96 $\pm$ 8.32	38.20 $\pm$ 8.62	33.61 $\pm$ 2.85

fraction of *W. somnifera* (Tohda et al., 2005) in rats and mice showed that the herb/extract/fractions from *W. somnifera* are well tolerated. Kulkarni and Dhir (2000) reviewed exhaustive literature about the tradition claim, validation of the traditional claim, biological activity, chemistry and safety of *W. somnifera*. In terms of safety and quality, herbal medicines have become important concerns for health authorities and the public. *W. somnifera* is native to India and commercially cultivated in different parts of the country and extensively utilized in the preparation of medicines in Ayurvedic system. It promotes strength and vigour as an aphrodisiac and rejuvenator, in the treatment of rheumatism, inflammation of joints and certain paralytic conditions (Basu and Kirtikar, 1991). *Withania* is a pharmacologically very important genus of flowering plants and is well known for its diverse biological activities. Further, many reviewers emphasized the need for a systematic safety study of this herb. Hence, the present experiment envisaged the chemical marker-based analysis of leaf and root extracts of *W. somnifera* by HPLC and detailed acute and sub-acute toxicity of both the extracts in CF rats. Ronen et al. (2025), suggested that the root of *W. somnifera* (Ashwagandha) demonstrates a favorable safety profile, particularly concerning liver and reproductive toxicity, when compared to other herbal supplements.

Conclusion

Our present experiment concludes that both root and leaf extracts were well tolerated by the experimental animals in either the acute or sub-acute toxicity study.

Acknowledgments

The authors are thankful to the director, CSIR-CIMAP, Lucknow, for financial support.

Author Contribution

Ruchi Kumari: Writing and formatting of the manuscript
Anirban Pal: Design and planning of the experiment
Madan M Gupta: Chemical analysis of the extract
Dayanandan Mani: Preparation of the extract
Debabrata Chanda: Actual experimentation, design of the experiment, writing and formatting of the manuscript

Conflict of Interest

Authors declare no conflict of interest.

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