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Toxicity of *Withania somnifera* Root Extract in Rats and Mice

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ABSTRACT

Alcohol extracts from the roots of *W. somnifera* ('Ashwaganda' in Sanskrit) were screened for their acute (24 h) toxicity in conventional Swiss albino mice and subacute toxicity (30 days) in Wistar rats. A single intraperitoneal injection of 1100 mg/kg of the extract in mice did not produce any deaths within 24 h, but small increases led to mortality. The LD_{50} value was calculated as 1260 mg/kg body wt. Subacute toxicity studies with repeated injections of Ashwagandha extract at a dose of 100 mg/kg body wt. (≈ 1/12 LD_{50}) for 30 days in Wistar rats of either sex did not result in any mortality or changes in peripheral blood constituents. However, significant reductions in the weights of spleen, thymus and adrenals were observed in male rats at the end of the experiment. The acid phosphatase content of peripheral blood in both sexes showed a significant increase from control, while other biochemical parameters determined in the study were in the normal range.

INTRODUCTION

Withania somnifera Dunal (Solanaceae), popularly known as Ashwagandha, is a plant of repute in the Indian system of medicine. The root of the plant is reported to have a vast range of therapeutic applications, including cancer treatment (CSIR, 1976; Duke, 1987). The pharmacological activity of the roots is attributed to the presence of several alkaloids (Dhalla *et al.*, 1961; Schwarting *et al.*, 1963), a steroidal lactone withaferin. A, withanoides (Sethi *et al.*, 1972; Sankara Subramanian *et al.*, 1977) and a few flavonoids (Hazeena Begum *et al.*, 1982). Withaferin A is responsible for the plant's bacteriostatic and antitumor properties (Asthana and Raina, 1989).

Preliminary screening studies in our laboratory using a 95% ethanol root extract of this plant have shown effective antitumor activity against a mouse transplantable tumor, Sarcoma-180 (Uma Devi *et al.*, 1992). However, a literature survey has indicated that no work has been done on the toxicity of the extract. This is essential if the extract is to be used for human application, where a safe dose needs to be selected. Therefore, the present study was initiated to analyse the acute and subacute toxicity of a 95% ethanol root extract of Ashwagandha in laboratory animals.

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MATERIALS AND METHODS

Plant material

Ashwagandha, the commercial drug consisting of the dried root powder of the plant, was purchased from a local Ayurvedic drug house. It is prepared from plants cultivated for medicinal purposes by Vaipa Pharmaceuticals Pvt. Ltd., Gujarat, India.

Extraction

Routine extraction of the root powder was done with 95% ethanol at 60-80°C in a Soxhlet apparatus (Suffness and Douros, 1979). The solvent was evaporated under reduced pressure to obtain crude extract (yield, ≈ 10%). Thirty percent polyethylene glycol (PEG 400, BDH, England) was used for the preparation of suspensions of the extract.

Acute toxicity studies

Adult random bred Swiss albino mice (6-8 weeks old) of either sex weighing 25-30 g were bought locally. They were housed in polypropylene cages with free access to water and food, and maintained under laboratory conditions for 2 weeks for acclimatisation. Food and water were withdrawn 18 h before injection of the extract.

The acute toxicity was determined in fasting albino mice as described by Turner (1965). The mice were divided into 6 different groups. Each group contained 10-15 animals. Group 1 served as control and received 0.2 ml of 30% PEG 400. Groups 2-6 received 1100, 1200, 1300, 1400 or 1500 mg/kg of Ashwagandha extract. Preliminary studies in our laboratory did not produce any toxic effect in mice in doses up to 1000 mg/kg. Therefore, 1100 mg/kg was selected as the lowest dose in this experiment. All the injections were done intraperitoneally (ip). The animals were observed continuously for the first 2 h and then for 6 h for any toxic symptoms. Finally, the number of survivors was noted after 24 h. The observed percentage mortality was converted into probit values by referring to the appropriate table and the values thus obtained were plotted against log dose. Before plotting, the percentage dead for 0 and 100 were corrected (Table 1). The LD_{50} value was calculated from the graph corresponding to probit value 5 (Fig. 1) (Miller and Tainter, 1944).

Subacute toxicity studies

Animals

Six to eight week old albino rats (Wistar strain) of either sex, weighing 120-150 g, were purchased from a local animal dealer. They were housed two per cage in elevated wire mesh cages and maintained under laboratory conditions for two weeks with free access to food and water, for acclimatization prior to initiation of treatments.

Experimental design

The animals were divided into two groups of 12 animals each (6 males and 6 females). The first group which served as control was injected ip with 0.5 ml of 30% polyethylene glycol and the second group was treated with Ashwagandha extract ip at a dose of 100 mg/kg for 30 days. All the rats were observed for physiological and behavioral responses and mortality. Food consumption and water intake were checked daily. Body weights were recorded at the beginning and twice weekly throughout the study.

Parameters

On the 31st day of the experiment, the animals were anaesthetized with ether and blood samples were collected by heart puncture in sterilized heparinized and dry non-heparinized tubes. The heparinized blood was used for hematological evaluation which included total RBC, WBC, differential leukocyte counts and hemoglobin estimations (Wintrobe, 1961). The non-heparinized blood was allowed to coagulate, then centrifuged, and the serum was separated. Serum was analysed for alkaline and acid phosphatases (Moss, 1984; Pierre and Spillman, 1984), alanine transaminase (ALT), and aspartate transaminase (AST) (Varley *et al.*, 1980). All the chemicals used were of analytical grade from Sigma (USA).

Organs (liver, lung, heart, thymus, spleen, adrenals, testes and uterus) were removed and weighed immediately. A part of the liver was processed for estimation of DNA (Burton, 1956), RNA (Ceriotti, 1955) and total protein (Lowry *et al.*, 1951). Acid and alkaline phosphatases were also estimated as above. Pieces of organs, other than lung and heart, were fixed in Bouin's fixative and processed

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routinely for histological examination after staining with haematoxylene and eosin. The slides were observed microscopically for any pathological lesions.

Statistical analysis

Student's 't'-test was employed to analyze the results. Differences below the probability level 0.05 were considered statistically significant.

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RESULTS

In this study, mice showed relatively high tolerance to Ashwagandha. No acute mortality was observed at 1100 mg/kg, but with a further 100 mg increment in the dose, there was a sharp increase in the death rate. No animals survived after an injection of 1500 mg/kg (Table 1). The LD_{50} of the extract was found to be 1260 mg/kg (Fig. 1).

In the subacute toxicity study, there were no deaths during the treatment period, either in the control or in the treated groups. No significant differences in food and water consumption from the controls were found in either sex. There

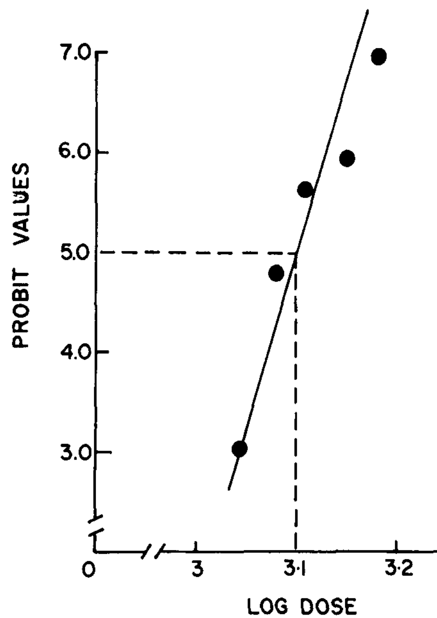

Fig. 1. Twenty four hour mortality data plotted as probit values *vs* log dose (from Table 1). The LD_{50} was found to be 1260 mg/kg. body wt.

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Table 1. Acute toxicity (24 h mortality) of Ashwagandha in Swiss albino mice.

Group	Dose mg/kg	Log dose	Dead/ Total	Dead%	Corrected%	Probit Value
1	1100	3.04	0/10	0.00	2.5	3.04
2	1200	3.08	5/12	41.66	41.66	4.79
3	1300	3.11	11/15	73.33	73.33	5.63
4	1400	3.15	10/12	83.33	83.33	5.97
5	1500	3.18	10/10	100.00	97.50	6.96

Corrected formula: for the 0% dead: 100 (0.25/n)
For the 100% dead: 100 (n-0.25/n).

was no change in general behavior or other physiological activities of the animals. The control and drug treated groups showed normal increases in body wt. (32% and 25%, respectively, which were not significantly different from each other) (Table 2). However, the male rats showed significant ($P<0.05$) decreases in the weights of spleen, adrenals and thymus after 30 days of treatment, whereas the weights of other organs did not vary from the control. None of the organs in the female rats showed any significant changes in weight after Ashwagandha treatment (Table 2).

Hematological analysis (Table 3) showed an increase in RBC counts and percentage of hemoglobin, although only the latter was statistically significant compared to the control. There was no change in serum alkaline phosphatase, ALT or AST levels compared to the control. However, a statistically significant increase was observed in blood acid phosphatase activity (Table 4). None of the parameters analysed in the liver showed any significant variation from the control value (Table 5). There were no sex biased differences in any of these parameters and therefore the average values from both sexes are presented.

Histopathological examination of the organs did not reveal any gross pathological lesions in either sex.

DISCUSSION

The high tolerance to Ashwagandha extract by the mouse is indicated by the finding that no acute lethality is produced even at a dose as high as 1100 mg/kg body wt., although small increases in dose above this level produced a substantial increase in mortality. Thus, the animals are able to tolerate well over 85% of the LD_{50} (1259 mg/kg body wt.) without suffering any acute toxicity. Purified withaferin A is less tolerated by mice, showing an approximate LD_{50} of 54 mg/kg body wt. ip (Shohat *et al.*, 1970). The crude extract of the roots, as used in the present study, is reported to contain two withanolides, withaferin A and withanone,

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Table 2. Changes in body weight and organ weight to body weight ratios in albino rats (Wistar strain) after treatment with Ashwagandha extract for 30 days. Results are expressed as g/100g body weight as the mean ± S.D. of 6 animals.

	Control	Extract (100 mg/kg)
Body weight		
Initial	134.88 ± 28.68	133.16 ± 19.94
Final	179.33 ± 41.15	166.35 ± 29.08
% Increase	31.91 ± 9.25	24.78 ± 4.59
Liver		
Male	3.949 ± 0.165	3.695 ± 0.165
Female	3.797 ± 0.571	3.741 ± 0.136
Spleen		
Male	0.429 ± 0.042	0.365 ± 0.007*
Female	0.465 ± 0.104	0.395 ± 0.052
Adrenals		
Male	0.025 ± 0.007	0.016 ± 0.002**
Female	0.034 ± 0.002	0.025 ± 0.016
Thymus		
Male	0.168 ± 0.055	0.074 ± 0.008*
Female	0.177 ± 0.020	0.161 ± 0.016
Kidneys		
Male	0.784 ± 0.037	0.779 ± 0.012
Female	0.722 ± 0.088	0.661 ± 0.038
Lungs		
Male	0.557 ± 0.651	0.611 ± 0.063
Female	0.651 ± 0.590	0.667 ± 0.029
Heart		
Male	0.326 ± 0.025	0.304 ± 0.005
Female	0.337 ± 0.018	0.335 ± 0.019
Testes	1.248 ± 0.076	1.258 ± 0.013
Uterus	0.220 ± 0.033	0.209 ± 0.025

*P<0.005, **P<0.05 Compared to control group.

of which the former is the major component (Sethi, 1978). The extract also contains heterogeneous alkaloids and flavonoids (Dhalla *et al.*, 1961; Hazeena Begum *et al.*, 1982), which may have toxic effects in mice, as oral administration of the extract produced diarrhoea and vomiting, probably caused by the direct irritant effect of the alkaloids (CSIR, 1976). However, some other components like reducing sugars (CSIR, 1976) may serve to reduce the toxicity of the alka-

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Table 3. Peripheral blood changes in rats observed after treatment with Ashwagandha for 30 days. Values expressed are the means ± S.D. of data from 12 animals.

Treatment	RBC ($10^6/\mu l$)	WBC ($10^3/\mu l$)	Hb (g/dl)	Differential count of leucocytes % Neutro-phils	Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils
Control	6.68 ± 0.96	4.416 ± 0.80	14.29 ± 0.95	21.01 ± 5.17	77.66 ± 5.57	1.00 ± 0.894	0.30 ± 0.51	--
Extract (100 mg/kg)	6.91 ± 0.23	3.66 ± 0.37	16.25* ± 0.88	24.00 ± 3.22	71.66 ± 4.22	0.80 ± 0.05	0.30 ± 0.89	--

*P<0.005

Table 4. Serum activities of different enzymes in rats treated ip with Ashwagandha for 30 days. Values expressed are the means ± S.D. of data from 12 animals.

Treatment	Alkaline Phosphatase (U/L)	Acid Phosphatase (U/L)	AST (U/L)	ALT (U/L)
Control	61.97 ± 15.58	26.11 ± 10.22	66.00 ± 7.66	54.16 ± 8.55
Extract (100 mg/kg)	61.25 ± 21.71	38.47* ± 6.73	61.76 ± 5.06	49.50 ± 6.22

*P<0.05

loids. This is a basic principle in the use of crude plant products in Ayurvedic practice.

Repeated injection of Ashwagandha extract at a dose of 100 mg/kg body wt. (≈ 1/12 LD_{50}) for 30 days in Wistar rats of either sex did not result in any mortality. The extract did not have any adverse effect on the normal growth of the animals (Table 1), indicating that the drug tolerance can be increased by fractionated dose administration over an extended period. The crude drug has been reported to produce anabolic effects, enhancing the synthesis of certain modulator proteins in rat liver (Anbalagan and Sadique, 1980) and increasing body weight in humans (Singh and Malviya, 1978), and is also reputed to have

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Table 5. DNA, RNA, total protein, acid and alkaline phosphatase activities in the livers of rats after Ashwagandha treatments. [Mean ± 1 S.E.].

Treatments*	DNA (µg/mg tissue)	RNA (µg/mg tissue)	Protein (µg/mg tissue)	Alkaline Phosphatase (µM/min/mg protein)	Acid Phosphatase (µM/min/mg protein)
Control	2.32 ± 0.01	7.56 ± 0.07	158.50 ± 5.07	0.609 ± 0.02 x 10^{-3}	8.29 ± 0.38 x 10^{-3}
Ashwagandha 100 mg/kg for 30 days	2.31** ± 0.01	7.50** ± 0.17	165.05** ± 8.01	0.589** ± 0.02 x 10^{-3}	10.09** ± 0.81x10^{-3}

* Nonsignificant from control
** 12 animals were used in each group.

antiaging effects (Kuppurajan *et al.*, 1980). The crude extract contains free amino acids such as aspartic acid, glutamic acid, glycine, alanine, proline, cystine and tyrosine along with tryptophan, an essential aminoacid (CSIR, 1976), which may be responsible for the observed anabolic effects in mice. The weight of the treated animals remained within the normal range, as also did most of the organ weights (Table 2). The role of some components of the extract in reducing stress related changes in the alimentary system (e.g., Sitointocides, Bhattacharya *et al.*, 1987; Ghosal, *et al.*, 1988) may also contribute to the above effect. However, male animals showed significant changes in the weights of adrenals, thymus and spleen; this finding cannot be explained at present. A significant increase in hemoglobin level and a nonsignificant increase in RBC were observed in both sexes. The high iron content of Ashwagandha (Kuppurajan *et al.*, 1980) may account for some of the increase in the hemoglobin level. Of the enzymes studied, only acid phosphatase showed a significant increase. This may be related to macrophage activity, as Shohat and Joshua (1971) have reported that injection of withaferin A resulted in an increase of macrophage acid phosphatase. However, this needs to be further investigated.

The present study shows that the tolerance dose of the extract is much higher than the effective antitumor dose in mouse (Uma Devi *et al.*, 1992).

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