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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.

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.

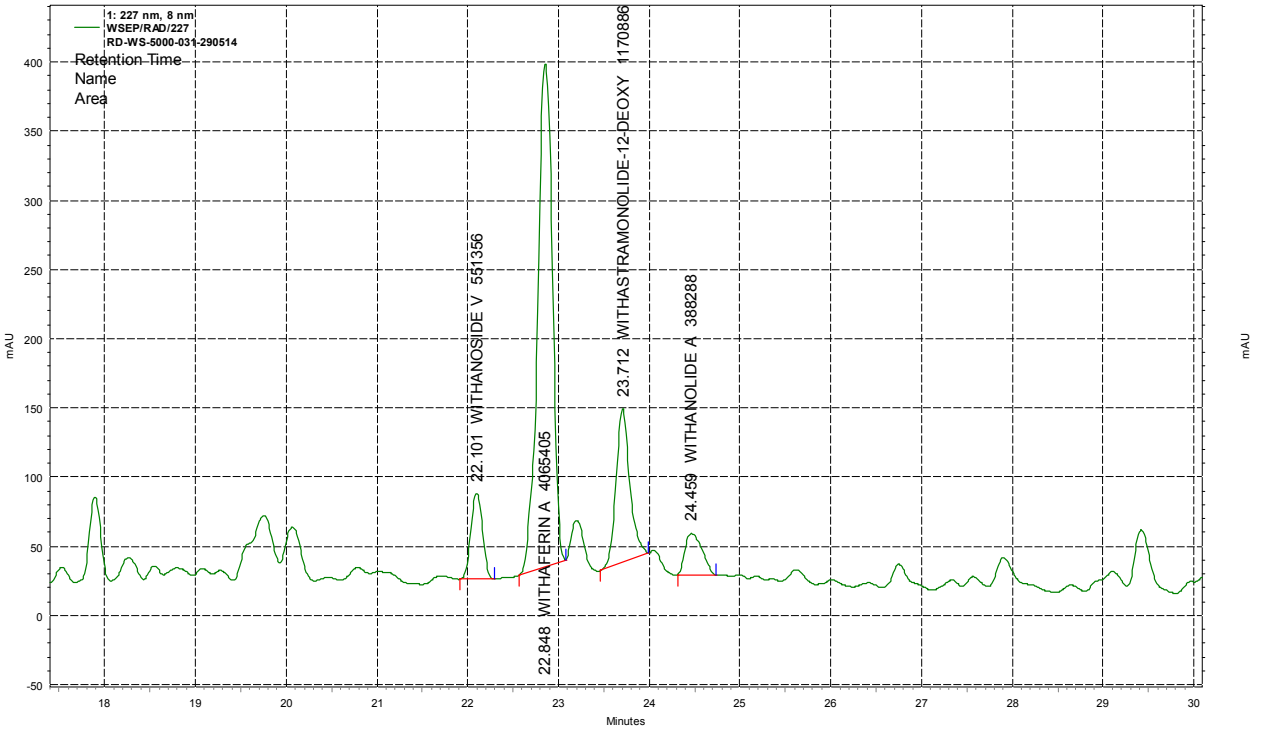

Fig. 1. Chromatogram of *Withania somnifera* extract.