Indian Journal of Clinical Biochemistry, 2001, 16(2), 195-198

A BIOLOGICALLY ACTIVE CONSTITUENT OF WITHANIA SOMNIFERA (ASHWAGANDHA) WITH ANTI-STRESS ACTIVITY

Parvinder Kaur, Sheenu Mathur, Meenakshi Sharma, Manisha Tiwari, K. K. Srivastava and Ramesh Chandra***

Dr. B.R. Ambedkar Center for Biomedical Research, University of Delhi, Delhi-110007. **Defence Institute of Physiology and Allied Sciences, Lucknow Road, Timarpur, Delhi-110 054

ABSTRACT

In Ayurvedic medicine, *Withania somnifera* (Ashwagandha) is well known for its anti-stress activity. A passive rat experimental model, where the animals are subjected to multiple stress of cold, hypoxia, restraint (C-H-R) has been developed to evaluate adaptogenic properties of various fractions of *W. somnifera root* extracts. We have carried out extraction of roots of *W. somnifera* with water and further isolated one of the active constituents called compound X and also tested its anti-stress activity in C-H-R model. The effect of administration of *W. somnifera* water suspension (360 mg/Kg bw) and compound X (20mg/Kg bw) on the fall and recovery of colonic temperature was noted. There was an increase of ~38% and ~54% in the time taken to attain T_{rec} 23°C by rats given a single dose of fresh aqueous suspension and biologically active constituent (Compound X) respectively, where as decrease in the recovery time to attain T_{rec} 37°C is ~13% and ~33% respectively, as compared to control group. It is clear that rats treated with the fresh aqueous suspension and compound X of *Withania somnifera*, could withstand the multiple stress of C-H-R better than control group.

KEY WORDS : *Withania somnifera*, anti-stress, cold- hypoxia-restrainted model.

INTRODUCTION

Since ancient times, man has aspired to find remedies to combat old age and disease. Ayurveda, one of the oldest systems of medicine, has utilized the plant *Withania somnifera* (Ashwagandha) as a remedy for several ailments including debility, rheumatism, dyspesia, consumption and symptoms related with old age and chronic illness. The plant has been used in treating syphilis and a decoction of the root bark is administered in Asthma (1). In the Ayurvedic system of medicine, leaves of *Withania somnifera* have been found to be useful in a number of inflammatory conditions, while the roots have been used for the treatment of rheumatism (2).

Investigations on the active chemical constituents of the plant led to the isolation of Withaferin A (Fig. 1.) (3). This compound represented the first member of a new class of phytosteroids, the withanolides. Later the occurrence of O-glycosylated withanolides, named sitoindosides (Fig. 2.), was reported in extracts from the roots of several cultivated varieties of Withania somnifera (4). Withanolides are a group of naturally occurring C_{28} steroids built on an ergostane –type skeleton in which C-22 and C-26 are appropriately oxidized to form a δ-lactone ring. About 50 withanolides have been isolated from various solanaceous plants belonging to genera *Withania, Acnisus, Physalis, Jaborosa, Nicanara, Datura* etc in which these steroids occur mainly in leaves. These compounds possess interesting biological activity. As a result, intensive research is underway on the plants containing withanolides with a view to isolating new and more potent drugs of this series. An important active principle of *Withania somnifera* is Withaferin A (4β, 27- dihydroxy-1-oxo-5β, 6β-epoxy-22R - witha-2, 24-dienolide) (5)

***Author for correspondence:**
Prof. Ramesh Chandra, at above address

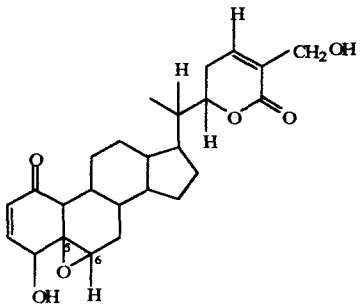
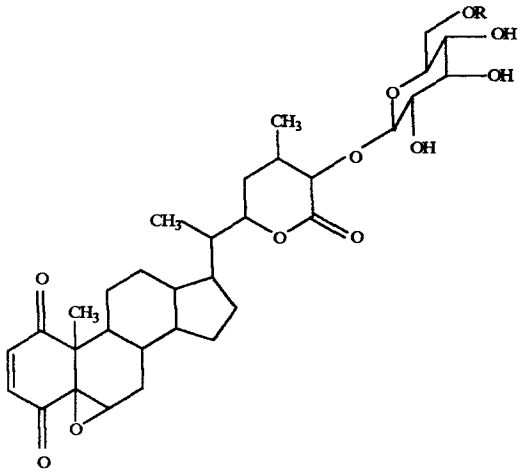


Fig. 1. Withaferin A



Sitoindoside - IX-H
Sitoindoside - X-Palmitoyl
Fig. 2. Sitoindosides

These compounds have been shown to possess a remarkable range of therapeutic properties, i.e. antistress (6,7), antitumor, antibacterial, antioxidant, immunomodulatory and cognition facilitating effects. Several of these effects have been studied in animal models (8). In our study we have isolated a biologically active compound from the acqueous suspension of roots of Withania somnifera and tested it for its anti-stress activity in C-H-R model (9).

MATERIALS AND METHODS

Chemical and animal model

Dried roots of Withania somnifera were as obatined from Dr. K.K. Srivastava. Defence Institute of Physiology and Allied Sciences (DIPAS), Delhi. The aqueous suspension was obtained by soaking roots of Withania somnifera for 24hrs, the suspension was filtered rotary evaporated and lyophilized. We have isolated one of the active constituents (Compound X) from the aqueous suspension, by column chromatography using silica gel (Grade 'G' CDH). Compound X is observed as a single spot on a TLC plate.

Animals and treatment

18 adult male rats of Wistar strain weighing 125-150g were used in this study. The rats were selected at random from the stock colony maintained in the animal house facility, DR. B.R. Ambedkar center for Biomedical Reseach, University of Delhi. The animals were reared on laboratory chow, fed ad libitum and had free access of water all the time. The room was maintained at 25°C ±2°C with natural day time and no light after 1900 h, until morning.

Animals were divided into 3 groups of 6 each. Each group was fasted for 12hrs before experiment. Group I animals were treated as control and were administered saline orally. Group II animals were orally administered 360mg/ Kg body weight, of the fresh aqueous suspension. Group III Animals were orally administered 20mg/ Kg body weight, of Compound X in water.

Experimental Procedure

Animals were subjected to stress in cold-hypoxia-restraint model (C-H-R)(7). Initially animals were weighed and marked. The single dose of either saline or aqueous suspension or compound X was given about half an hour before keeping the animals in the de-compression chamber.

The colonic temperature of the animals was recorded by inserting rectal probes in their anus. The probe on other end was connected to PC-based isothermex-16 channel telethermometer of range 0°-50°C (Columbus instrument). Colonic temperature of animals were monitored till it reached 35°-37°C. After, the constant colonic temperature was attained, the animals were kept in de-compression chamber, with a temperature of 2°-5°C, atmospheric pressure 426mm

that corresponds to15000 ft above sea level and air flow 2L/min. The temperature was recorded after every 60 sec automatically. When colonic temperature fell down to 23°C, animals were taken out of the de-compression chamber and kept at room temperature 30°-32°C, till colonic temperature recovered back to 37°C. Animals were continuously monitored for any sudden rise or fall in temperature. T_{rec} 23 ° C was taken as a measure of maximum stress that can be induced in the animal, without being fatal.

RESULTS AND DISCUSSION

The effect of administration of W. somnifera aqueous suspension and its compound X on the fall in T_{rec} (colonic temperature) to 23°C and recovery of T_{rec} to 37° C is shown in Table.1.

Table.1. Effect of oral administration of single dose of Withania somnifera aqueous suspension (360 mg/ Kg bw) and of compound X (20mg/Kg bw) on maintenance and recovery of colonic temperature under acute cold restrained and hypoxia stress.

Animals Groups (n=6)	Time to attain T_{rec} 23°C Colonic temperature (Min.)	Time to recover to T_{rec} 37°C () Colonic temperature (Min.)
	(Mean±S.E.)	(Mean±S.E.)
Control	58.6±1.26	180.6±3.98
Aqueous Suspension	80.6±0.299	156.3±0.541
Compound X	90.2±0.987	119.4±1.01

The changes in colonic temperature of cold-hypoxia-restraint (C-H-R) stressed rats of control group during stress and recovery in comparison to those groups fed with either aqueous suspension or compound X of Withania somnifera are shown in Fig. 3. & 4.

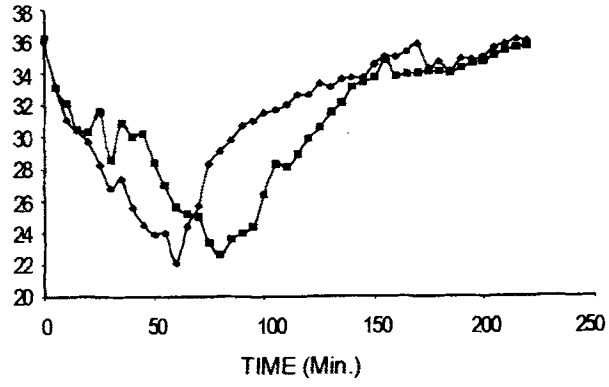


Fig. 3. Effect of aqueous suspension of W.somnifera on Trec. of rats

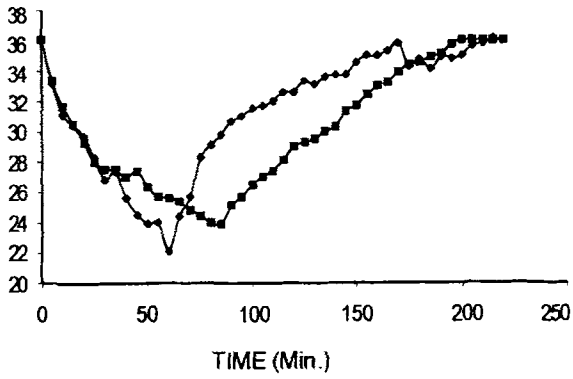


Fig. 4. Effect of Compound X of W. Somnifera on Trec. of rats

When compared to normal rats, there was 37.5% prolongation of the time taken to attain 23°C by rats given single dose of fresh aqueous suspension, whereas there was decrease in the recovery time 13.4%. Compound X isolated from the extract was found to be more active then the crude aqueous suspension, as there was 54% increase in the time taken to attain 23°C by rats given single dose of Compound X and decrease the recovery time was 33% as compared to control group.

It was clear that rats treated with either aqueous suspension or compound X Withania somnifera could withstand the multiple stress of C-H-R better than control rats and Compound X shows better results that the aqueous suspension of Withania somnifera.

Thus, the compound obtained from the aqueous suspension of the powdered roots of the plant, is a promising active principle. A characterization of this compound is presently under progress.

REFERENCES

1. Nadkarni, A.K. (1954) Indian Material Medica 3rd Ed, Popular Book Depot,Bombay, Vol.1, 1292-1294.
2. Bahr, V. and Hansel, R. (1982) Immunomodulating Properties of 5,20α (R) – Dihydroxy - 6α, 7α -epoxy-1-oxo-(5 α)- with α- 2,24-dienolide and Solasodine. J. Med. Plant Res. 44, 32-33.
3. Lavie, D., Glotter, E. and Shvo, Y. (1965) Constituents of Withania somnifera Dun., The structure of Withaferin- Amer. J.Chem. Soc. 30, 7517-7531.
4. Ghosal, S., Kaur, R. and Srivastava, R.S. (1988) Sitoindosides IX and X, new glycowithanolides from Withania somnifera. Ind. J. atural Prod.(Sagar) 4, 12-13.
5. Schliebs, R., Liebmann, A., Bhattacharya, K.S., Kumar, A. Ghosal, S. and Bigl, V. (1997) Systemic administration of defined extracts from Withania somnifera (Indian Ginseng) and Shilajit differentially affects cholinergic but not glutamatergic and Gabaergic markers in rat brain. Neuro. Chem. Int. 30, 181-190.
6. Grandhi, A., Mujumdar, A.M. and Patwardhan, B. (1994) A comparative pharmacological investigation of Ashwagandha and Ginseng. J. Ethnopharmacol. 44, 131-135.
7. Archana, R. and Namasivayam, A. (1999) Antistress effect of Withania somnifera. J. Ethnopharmacol. 64, 91-93.
8. Lavie, D., Glotter, E. and Shvo, Y. (1965) Constituents of *Withania somnifera* Dun. III-the side chain of Withaferin Amer. J. Chem. Soc. Perkin Trans 1, 30, 1775-1778.
9. Ramachandran, U., Divekar, H.M. , Grover, S.K. and Srivastava, K. K. (1990) New experimental model for the evaluation of adaptogenic products. J. Ethanopharmacol. 29, 275-281.