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SHORT COMMUNICATION

Adrenocorticosterone Alterations in Male, Albino Mice Treated with *Trichopus zeylanicus*, *Withania somnifera* and *Panax ginseng* Preparations

Anubha Singh, Ekta Saxena and K. K. Bhutani*

Department of Natural Products, National Institute of Pharmaceutical Education and Research (NIPER), Sector 67, S.A.S. Nagar (Mohali), Near Chandigarh - 160062, India

The levels of corticosterone were estimated by the HPLC method in the adrenal glands of stressed (5 h constant swimming) male albino mice treated with *Trichopus zeylanicus, Withania somnifera* and *Panax ginseng* preparations and compared with non-treated stressed and normal controls. The treatments increased the corticosterone levels in all the groups. The physical endurance (increased survival time) of swimming mice also increased in all the treated groups, except in the group treated with *Withania somnifera* powder (500 mg/kg, p.o.).

Keywords: HPLC; corticosterone; *Trichopus zeylanicus*; *Panax ginseng*; *Withania somnifera*; antistress activity.

INTRODUCTION

We used *Withania somnifera, Trichopus zeylanicus* and *Panax ginseng* preparations for the evaluation of antistress activity by a conventional swimming endurance test and estimated adrenal corticosterone levels by the HPLC method. The previous method reported for the measurement of adrenal and plasma corticosterone is mainly the photofluorimetric method (Zenker and Bernstein, 1958) but some corticosterone measurements in plasma have been done by the [^{125}I] radioimmunoassay (Coat-A-count rat corticosterone method) (Sharma *et al.*, 1996), and the paper chromatography method (Bush B-5 solvent system Whatman no. 2 paper) by a competitive protein binding method using 5% dog plasma (Boulfekhar and Brudieux, 1980). The HPLC method for the estimation of adrenocorticosterone was used and is reported for the first time to the best of our knowledge.

The roots of *Withania somnifera* Dun (Ashwagandha) are widely regarded as 'Indian Ginseng' by practitioners of the traditional Indian system of medicine (Kiritikar and Basu, 1987). The plant (herb) has a vast range of applications in the treatment of different physiological disorders and is useful in diverse pathological states giving rise to the possibility that the drug might act by inducing a SNIR in the organism (Brekhman and Dardiymov, 1969). The putative antistress activity of the hydroalcohol extract of *Withania somnifera* roots has been evaluated (Bhattacharya *et al.*, 1987).

Trichopus zeylanicus is a rare herbaceous, rhizomatous wild plant which is endemic to the Agasthyr Hills of Western Ghats (Kerala) in India. The unripe fruits of *Trichopus zeylanicus* are said to be a highly rejuvenative tonic comparable to 'Ginseng' and are used by the Kani tribe of Kerala to avoid fatigue (Pushpangadan *et al.*, 1988). The seeds have been shown to possess antistress properties as tested in rats and mice using a variety of stress models such as milk induced leucocytosis, restraint ulceration, cold stress ulceration, aspirin induced ulceration and gastric antisecretory activity (Sharma *et al.*, 1989).

Trichopus zeylanicus and *Withania somnifera* in comparison with *Panax ginseng* are considered as antistress drugs although there is no fixed protocol to evaluate antistress activity as such. Normally a battery of tests is used, employing widely differing stress situations, with the rationale that an antistress agent will attenuate the stress induced response, irrespective of the nature of the stressor used (Selye, 1973).

MATERIALS AND METHODS

Plant material and extraction. The root powder of *P. ginseng* was obtained from Ginsec^{R} capsules (Duphar Interfran Ltd., India) and Aswagandha churna (Dabur India Ltd., India) was used as the source of W. *somnifera* powder. In the present study these plant preparations were administered for the evaluation of antistress activity by the swimming endurance test with simultaneous measurement of the levels of adrenal corticosterone by the HPLC method. For preparation of the extracts of *W. somnifera* and *T. zeylanicus*, the roots and aerial parts of these two plants were collected from the NIPER Nursery. The plant materials were shade dried and powdered. The

* Correspondence to: Dr K. K. Bhutani, Department of Natural Products, National Institute of Pharmaceutical, Education and Research, Sector 67, SAS Nagar, Near Chandigarh - 160062, India.

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powders obtained were extracted with 70% ethanol by soxhlation. The extracts were evacuated to dryness under vacuum and made into a suspension in a 50% sucrose solution. The commercial *P. ginseng* and *W. somnifera* powder were also made into a suspension in 50% sucrose solution.

Animals. Experiments were carried out in randomly selected animals (male Swiss albino mice), weighing 25–30 g. The mice were procured from the animal house of our institute in healthy condition. They were maintained at 25° ± 2° C and fed standard pellet diet (Lipton India Ltd.) and tap water *ad libitum*. The animals were kept in a low stress environment and an enclosed door provided a substantial amount of sound proofing. Stressful cage motion, frequent cage handling and other uncontrolled stress-induced practices were avoided.

The animals were divided in to nine groups of six mice each and pretreated with drugs. The drugs used for the evaluation of antistress activity were *W. somnifera* commercial root powder as well as extract, an extract of *T. zeylanicus* and a commercial sample of *P. ginseng*. The drugs were administered orally with vehicle (50% sucrose solution) in different doses (250 and 500 mg/kg;p.o.) for 6 days. On day 7 the drugs were given 1 h before the animals were subjected to endurance and constant swimming tests. Only one dose (250 mg/kg;p.o.) of the commercial root powder of *P. ginseng* was used. The animals of swimmer and non-swimmer groups were taken as control and they were treated with vehicle.

Swimming endurance test. The swimming test was carried out in plastic tubs (size 55 × 34 × 20 cm) partitioned by two glass plates in three compartments. The temperature of the water was maintained at 25° ± 2° C. Two groups of animals were kept as controls, one as non-swimmer and the second as swimmer and the other seven groups were treated with drugs. The mice were subjected to swimming test a 1 h after the last dose. They were allowed to swim until exhausted in separate compartments. The mean swimming time for each group was calculated.

Adrenal function test after 5 h constant swimming. The protocol for the selection of groups of animals and drug treatment was the same as for the swimming endurance test. The animals were allowed to swim easily and were killed after 5 h swimming. Their adrenal glands were decapsulated, weighed and subjected to solvent extraction with chloroform. The extract obtained was used for HPLC analysis to determine corticosterone content.

Statistical analysis. Mean values of groups were expressed as mean ± SEM. The results were statistically analysed by Student's *t*-test.

Assay procedure for adrenal corticosterone. Decapsulated pooled adrenals of control and treated animals were weighed and homogenized for 5 min in triple-distilled water. The extractions of corticosterone from homogenized adrenal tissues were carried out with chloroform AR (3 × 5 mL). The chloroform layer was separated, dried over sodium sulphate, and evacuated to dryness *in vacuo*. The dried extracts were taken in HPLC grade chloroform and the concentrations were uniformally adjusted to 1 mg/0.5 mL. The samples were filtered with a 0.22 μm nylon filter and subjected to RPHPLC analysis. The chromatographic system (Waters) consisted of a HPLC pump 600E, Photodiode Array detector 996, HPLC column thermostat (27° C), 4 Channel online degasser, Rheodyne injector with 20μl loop and software Millenium V 2.10. The column used was a Bonda Pak C_{18}(4 μm, 250 × 4.6 mm). HPLC grade (Merck, India) solvents were filtered by using a Millipore 0.45 μm filter system. The mobile phase consisted of H_2O: CH_3OH:THF (69:08:23), flowing through the column at a constant flow rate of 0.8 mL/min isocratically. The column eluents were monitored by a PDA detector set between 210 nm and 310 nm. Under the chromatographic conditions described above, corticosterone, obtained from Steraloids Inc. USA, was used as a standard. A stock solution of standard sample was prepared in chloroform at a concentration of 1 mg/mL. The standard sample was eluted through the column at an RT of 28.9 min and a wavelength of 248 nm. The accuracy and reproducibility of method was investigated by replicate analysis. The corticosterone peaks in chromatograms of biological samples were identified by comparison of their retention times with that of pure standard sample and further confirmed by peak enrichment with the same. The quantification was achieved by plotting a standard graph of the peak areas of standard corticosterone in different concentrations (5 ng–10 ug) and obtaining the values of the test samples from the same. Each sample was injected three times and the mean value of the peak area was taken for calculation. The average percent relative standard deviation in the test samples was in the range 0.3–1.5.

Table 1. Effect of various drugs on swimming performance in albino mice

	Treatment group	Dose (mg/kg; p.o.)	Number of mice	Mean duration of swimming (min ± SE)
1	Control (Non-swimmer)	Vehicle	6	—
2	Control (Swimmer)	Vehicle	6	240.83 ± 3.51
3	*W. somnifera* (Alc. ext. of whole plant)	250	6	380 ± 3.42[a]
4	*W. somnifera* (Alc. ext. of whole plant)	500	6	359.2 ± 3.27[a]
5	*W. somnifera* (Root powder)	250	6	360.67 ± 19.3[a]
6	*W. somnifera* (Root powder)	500	6	195.83 ± 3.51
7	*T. zeylanicus* (Alc. ext. of whole plant)	250	6	275 ± 2.58[a]
8	*T. zeylanicus* (Alc. ext. of whole plant)	500	6	312.5 ± 2.50[a]
9	*P. ginseng* (Root powder)	250	6	370.2 ± 21.49[a]

[a] Significant ($p < 0.001$) increase in swimming time compared with control group (swimmer).

Table 2. Effect of various drugs on weight of adrenal glands after physical stress (5 h easy swimming)

	Treatment group	Dose mg/kg; p.o.	Number of mice	Adrenal glands weight(mg) mean ± SE
1	Control (Non-swimmer)	Vehicle	6	6.0 ± 0.826
2	Control (Swimmer)	Vehicle	6	7.5 ± 0.413
3	*W. somnifera* (Alc. ext. of whole plant)	250	6	6.5 ± 0.597
4	*W. somnifera* (Alc. ext. of whole plant)	500	6	6.67 ± 0.73
5	*W. somnifera* (Root powder)	250	5	5.20 ± 0.489[a]
6	*W. somnifera* (Root powder)	500	6	7.17 ± 1.039
7	*T. zeylanicus* (Alc. ext. of whole plant)	250	6	6.8 ± 0.631
8	*T. zeylanicus* (Alc. ext. of whole plant)	500	6	5.5 ± 0.413[a]
9	*P. ginseng* (Root powder)	250	6	5.93 ± 1.22

[a] Significant ($p < 0.001$) decrease in adrenal weight (mg) compared with control group (swimmer).

RESULTS AND DISCUSSION

Considering the lack of an acceptable test protocol for screening putative antistress agents, the test drugs, namely alcohol extracts of *T. zeylanicus* and *W. somnifera* and commercially available powders of *P. ginseng* and *W. somnifera*, were subjected to two types of investigation, one based on classical work and another on the measurement of the alteration of adrenocorticosterone content in the animals. In the first experiment, the animals treated with test drugs were allowed to forced swim until exhausted, along with the non-treated control group. The animals treated with test drugs showed significantly ($P < 0.001$) greater endurance as judged by a marked increase in the duration of swimming performance (survival time). The *W. somnifera* powder treated animals (500 mg/kg/p.o.), however, registered a decrease in swimming time compared with the control group and thereby showed negative endurance (Table 1).

In the second experiment the animals of both treated and non-treated groups were allowed to swim easily for a fixed duration (5 h), killed and their adrenals extracted. Adrenal enlargement was noticed in the non-treated swimmer group of animals compared with the non-swimmer control group. The test drug treatments inhibited the increase in adrenal weights in all the groups. However, a significant change in adrenal shrinkage (decrease in weights) was observed in the animals treated with *W. somnifera* powder (250 mg dose) and *T. zeylanicus* alcoholic extract (500 mg dose).

Adrenal enlargement is a phenomenon resulting from stress (Selye, 1936), and an agent which can arrest this stress-induced pathological change is termed an antistress agent. In the present study, all the treated animals showed decreased adrenal weights to some degree compared with the stressed animals (Table 2).

To shed better light, we undertook a measurement of the concentration of corticosterone from the adrenal glands. The homogenized tissues were extracted with chloroform, filtered and dried. The biological extracts were subjected to RPHPLC. This experiment demonstrated that administration of antistress drugs leads to glucocorticoid hyperactivity with a significant increase in adrenal concentrations of corticosterone in the adult male albino mice. The basal concentration of corticosterone obtained from control mice (non-swimmers) was 11.30 ng/100 mg of adrenal tissue and was found to be increased to 50.87 ng/100 mg of adrenal tissue in the animals of the swimmer control group. All the treatments with drugs led to a significant elevation in the concentrations of corticosterone compared with basal values of the non-swimmer and swimmer groups (Table 3). Moreover, the elevation in the concentration of corticosterone was also higher when 500 mg/kg doses were used compared with 250 mg/kg doses.

The regulatory mechanisms of endogenous glucocorticoids are being revised as they not only suppress, but also direct and enhance, immune functions (Wilckens and De Rijk, 1997). Therefore, the data generated on the elevation of levels of adrenal corticosterone with test drugs are useful and it is proposed to compare them with cytokine production in future studies.

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Table 3. Concentration of corticosterone per 100 mg of adrenal tissue after treatment with various drugs

	Treatment group	Dose (mg/kg; p.o.)	Corticosterone concentration (ng)
1	Control (Non-swimmer)	Vehicle	11.36
2	Control (Swimmer)	Vehicle	50.87
3	*W. somnifera* (Alc. ext. of whole plant)	250	123.09
4	*W. somnifera* (Alc. ext. of whole plant)	500	168.09
5	*W. somnifera* (Root powder)	250	111.68
6	*W. somnifera* (Root powder)	500	229.15
7	*T. zeylanicus* (Alc. ext. of whole plant)	250	117.34
8	*T. zeylanicus* (Alc. ext. of whole plant)	500	138.15
9	*P. ginseng* (Root powder)	250	137.00

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