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Short Communication

Low dose effects of a *Withania somnifera* extract on altered marble burying behavior in stressed mice

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ABSTRACT

Aim: *Withania somnifera* root (WSR) extracts are often used in traditionally known Indian systems of medicine for prevention and cure of psychosomatic disorders. The reported experiment was designed to test whether low daily oral doses of such extracts are also effective in suppressing marble burying behavior in stressed mice or not. **Materials and Methods:** Groups of mice treated with 10, 20, or 40 mg/kg daily oral doses of WSR were subjected to a foot shock stress-induced hyperthermia test on the 1st, 5th, 7th, and 10th day of the experiment. On the 11th and 12th treatment days, they were subjected to marble burying tests. Stress response suppressing effects of low dose WSR were estimated by its effects on body weight and basal core temperature of animals during the course of the experiment. **Results:** Alterations in bodyweight and basal core temperature triggered by repeated exposures to foot shock stress were absent even in the 10 mg/kg/day WSR treated group, whereas the effectiveness of the extract in foot shock stress-induced hyperthermia and marble burying tests increased with its increasing daily dose. **Conclusion:** Marble burying test in stressed mice is well suited for identifying bioactive constituents of *W. somnifera* like medicinal plants with adaptogenic, anxiolytic and antidepressant activities, or for quantifying pharmacological interactions between them.

KEY WORDS: Bioassay, foot shock stress, marble burying test, thermoregulation, treatment regimen, *Withania somnifera*

INTRODUCTION

Withania somnifera is a psychoactive medicinal plant often used in traditionally known systems of medicine in India and other countries [1-4]. Modern herbal researchers and practitioners considered it to be an adaptogenic plant useful for treatments of chronic diseases caused by, or associated with, exaggerated anxiety and other nervous system disorders [5-8]. However, as yet the questions concerning bioactive constituents of its medicinally used extracts, or on their therapeutically interesting doses and dosing regimen, still remain open [4,9].

Recent observations made during our exploratory dose-finding studies have revealed that fairly low daily oral dose of *W. somnifera* extracts increases stress resistance in laboratory rodents, and indicated that their anxiolytics or antidepressants like effectiveness in stressed mice increases with increasing number of treatment days [10,11]. Similar was also the observations made with several plant metabolites encountered in *W. somnifera* and other adaptogenic herbs [12-15]. In this communication, the results of an experiment suggesting that marble burying test in stressed mice could be helpful for better understanding of low dose pharmacology of *W. somnifera* like adaptogenic herbs and of their bioactive constituents are summarized and briefly discussed in light of our current knowledge on medicinal phytochemistry and pharmacology of the plant.

MATERIALS AND METHODS

Animals and the Extract Used

Experimentally inexperienced Wistar Albino male mice (25 ± 5 g) were procured from Central Animal House of Institute of Medical Sciences, Banaras Hindu University,

Varanasi (Registration number: 542/AB/CPCSEA). They were group-housed at an ambient condition (25°C ± 1°C and relative humidity 50% ± 10%) and in 12:12 h light/dark cycle with free accesses to food and water. Ethical clearance for animal experimental work was obtained from the Central Animal Ethical Committee of the University (Dean/2014/CAECI/604, dated 30/05/2014) before the commencement of experiments.

The medicinally used and pharmaceutically well standardized *W. somnifera* root (WSR) extract together with its analytical details was generously supplied by Natural Remedies Private Limited, Bengaluru, Karnataka, India. A total withanolides content of the extract was 2.7% (w/w).

Animal Grouping and Drug Treatments

A total of 30 randomly selected mice were allotted to five experimental groups consisting of six animals each. One of them serving as a non-stressed control group was not subjected to foot shock stress-induced hyperthermia tests while all others were subjected to the test on the 1st, 5th, 7th, and 10th days of the experiment. The non-stressed and stressed control groups were treated once daily with 10 ml/kg of 0.3% aqueous carboxymethyl cellulose (CMC, Central Drug House, New Delhi, India) for 12 consecutive days, and the three stressed ones were similarly treated with 10, or 20 or 40 mg/kg daily oral doses of WSR suspended in the vehicle. On all test days, body weights and basal core temperatures of all animals were recorded before the day's treatment, and all tests were performed 1 h thereafter.

Foot Shock Stress Induced Hyperthermia Test

The test procedure described elsewhere in details [10,12] were strictly followed. In short, five consecutive electric foot shocks (2 mA, 50 Hz of 2 ms duration) were delivered to each individual animal of a stressed group, and its core temperature was recorded again 10 min after it was placed back to its home cage. The difference between this temperature and its basal core temperatures recorded on the test day was used as an index for stress-triggered hyperthermia. On all test days, the animals of the non-stressed control group were also placed individually for 1 min in the stress chamber, but no foot shocks were given them. Otherwise, all animals of all groups were handled similarly.

Marble Burying Test

On the 11th day, an individual animal of a group was placed in a test cage (30 cm × 23 cm) where 12 glass marbles (color and size of marbles were kept constant) were evenly spaced for standard marble burying condition. On the 12th day, they were tested similarly, in the two-zone marble burying condition, whereupon 8 glass marbles were evenly spaced only on one-half of the box. After 15 min (standard condition) or 30 min (two-zone condition) of exposure, the number of marbles, at least, two-thirds covers by husk was counted [16].

Statistical Analysis

Mean ± standard error of mean was calculated for the observed values in each experimental group. A statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Student - Newman–Keuls multiple comparison tests and two-way ANOVA followed by Bonferroni *post hoc* test unless otherwise stated. GraphPad Prism - 5 (GraphPad software Inc., La Jolla, California, USA) was used for statistical analysis. A $P < 0.05$ was considered as statistically significant.

RESULTS

As expected from the observation made during our earlier studies with *W. somnifera* and other herbal extracts [10-13], mean body weight of the stressed control group from the 5th day onward of the experiment decreased continuously, whereas that of the unstressed control group continued to increase. On all observational days, no statistically significant differences between the mean body weights of none of the foot shock stressed and WSR treated groups and the non-stressed control group were observed (results not shown). Moderate but significant elevation in the mean basal core temperature of the stressed control group observed during the course of the experiment was also completely suppressed by repeated daily 10 mg/kg and the higher daily oral doses of WSR [Figure 1a].

Results of the foot shock stress triggered hyperthermia test summarized in Figure 1b revealed that even a single oral 40 mg/kg WSR dose is effective in suppressing stress induced hyperthermic response. The effectiveness of all tested doses of the extract in this test increased not only with its increasing dose but also with increasing number of treatment days. These observations reaffirm that biological processes and mechanisms regulating stress triggered hyperthermic responses are involved in the modes of action of WSR.

Results of the marble burying tests are summarized in Figure 2a and b. The mean number of marbles buried by the stressed control group in both version of the test were higher than the corresponding values of the non-stressed group, but statistically significant differences between the two groups were observed in

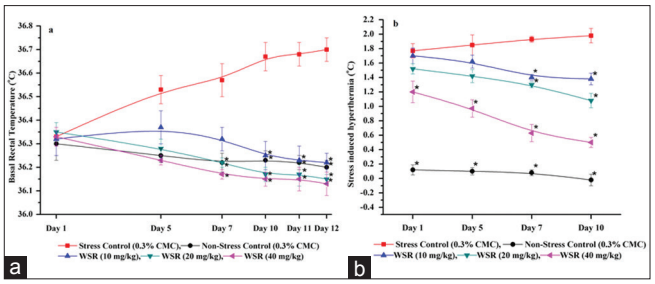

Figure 1: Effect of WSR extract on (a) basal rectal temperature and (b) stress induced hyperthermia in male mice. WSR: *Withania somnifera* root extract, CMC: Carboxymethyl cellulose, SEM: Standard error of the mean . Values are mean±SEM ($n = 6$). *denotes statistically significant difference (two-way analysis of variance followed by Bonferroni *post hoc* test) relative to the corresponding values of the stress control group (*$P < 0.05$)

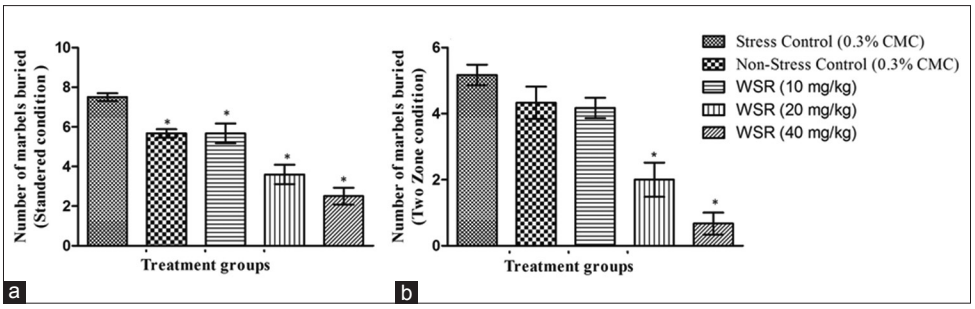

Figure 2: Effect of WSR extract in mice marble burying test: (a) Standard condition on day 11 and (b) Two-zone condition on day 12. WSR: *Withania somnifera* root extract, CMC: Carboxymethyl cellulose, SEM: Standard error of the mean. Values are mean±SEM (n = 6). *denotes statistically significant difference (analysis of variance followed by Student - Newman-Keuls multiple comparison test) relative to the values of the corresponding stress control group (*$P < 0.05$)

the standard version of the test only [Figure 2a]. These numbers for the 10 mg/kg/day WSR treated group in both versions of the test were almost identical to those of corresponding ones for the non-stressed control group, and its dose dependent effects in decreasing these numbers were observed in both version of the test.

DISCUSSION

Reported results reveal that 10 mg/kg daily oral dose of WSR is high enough for completely suppressing the foot shock stress-triggered alterations in body weight, basal core temperature, and marble burying behavior of mice, and that its effectiveness in the foot shock stress-induced hyperthermia and in both versions of the marble burying test increases with its increasing daily dose. It has recently been reported that a single 25 mg/kg intraperitoneally administered doses of WSR possess anxiolytics or antidepressants like activities in marble burying test [17], and that delayed anxiogenic effects of stress in mice are also detectable in this test [18]. This test is often used for assessing behavioral effects of genetic manipulations and drugs in rodents, and predictive validity of stress-induced hyperthermia tests for identifying drug leads against anxiety disorders have also been well established [14-19].

Our observations strongly suggest that standard version of marble burying test is more sensitive than its two zone version for quantifying anxiogenic state in mice triggered by prior exposures to unpredictable foot shock stress, and that this version of the test is well suited for stress response suppressing as well as antidepressants or anxiolytics like effects of fairly low daily oral doses of WSR like herbal extracts. Since, *W. somnifera* extracts devoid of withanolides also possess adaptogenic and other therapeutically interesting bioactivities [20-22], appropriate uses of the bioassay procedure used in the described experiment for identifying their bioactive constituents or for clarifying possible pharmacological interactions between them can be warranted. Such efforts will not only be useful for more rational medicinal uses of *W. somnifera*, but also could lead to novel therapeutic principles and drug leads urgently needed for prevention and cure of mental health problems accompanying, or caused by, stress triggered chronic diseases including cancer and Alzheimer's disease [23-27].

In any case, it remains certain that fairly low daily oral doses of the tested extract is effective in increasing stress resistance and that its slightly higher ones could be useful for treatments of co-morbid depression and anxiety in stressed patients. Since total contents if withanolides in WSR is only 2.7%, it seems reasonable also to assume that phytochemicals other than withanolides are also involved in its observed low dose effects. This inference is well supported by our current knowledge on medicinal phytochemistry and experimental pharmacology of the plant [2,28,29].

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