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ORIGINAL ARTICLE

Physical Endurance Enhancing Capacity of *Withania somnifera* Root Powder Post-water Extraction in Mice

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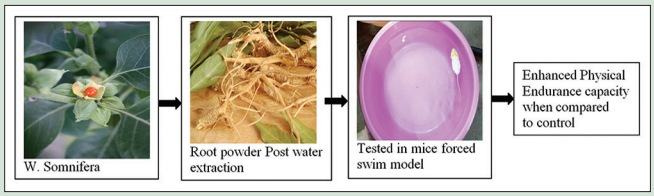
ABSTRACT

Background: *Withania somnifera* (WS), Indian ginseng/ashwagandha, is a common household plant. Its commercially available aqueous or ethanolic root powder extract containing the active substance withanolides is known to enhance physical endurance (stamina). The potency of crude root powders, especially post-extraction, is unknown. **Objectives:** The aim of this study was to compare the efficacy of crude root powder of WS post-extraction with commercial root preparation in enhancing the physical endurance of mice using forced swim endurance model. **Materials and Methods:** Male Swiss albino mice, 6–8 weeks old with mean (standard deviation) weight of 35.17 (3.36) g, were randomized into three groups of six animals each: control – carboxymethyl cellulose 0.5%, standard – commercial root preparation of WS (100 mg/kg), and test – crude root powder post-extraction (100 mg/kg). Drugs were administered per oral, once daily, for 7 days. On the 8th day, animals were allowed to swim till exhaustion in a propylene tub of dimension 25 cm radius and 30 cm height, with water level at 15 cm. The end point of swim endurance was when the mice near drowned. **Results:** There was no significant difference in body weight of mice between groups. One-way ANOVA between groups for drowning time was significant ($F[2,15] = 12.771$, $P = 0.001$). Tukey's *post hoc* test was significant for test versus control ($P < 0.001$) and standard versus control ($P = 0.047$). However, test versus standard was not statistically significant ($P = 0.069$). **Conclusion:** Hence, we conclude that crude root powder of WS after extraction also possesses physical endurance enhancing property and may be used to enhance the stamina.

Key words: Ashwagandha, forced swim, physical endurance, root extract, *Withania somnifera*

SUMMARY

- Withania somnifera root powder post aqueous extraction enhances the physical endurance capacity of the mice as assessed by the forced swim test. This suggests the presence of certain biologically active ingredients even after extraction.



Abbreviations Used: ANOVA: Analyis of Variance; CI: Confidence Interval; CMC: Carboxy Methyl Cellulose; HPLC: High Performance Liquid Chromatography; MeSH: Medical Subject Heading; SD: Standard Deviation; TLC: Thin Layer Chromatography; WS: Withania somnifera

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INTRODUCTION

Stress is defined as "the functional adaptation of the organism to cope with a changing or challenging environment."[1] It may be physical or psychological, and it exerts influence on a lot of body systems, thereby becoming an important entity in the chronic disease process that is pandemic currently.[2] A successful management of stress is considered an integral part of health-care management system and it forms an important and significant part of the health promotion activities. Multiple studies have proven that mental strain can have a negative impact on the stamina and one's activities.[3] Physical activity, on the other hand, can help tide away psychological distress.[4] With changing lifestyle and increasing competitiveness to excel, stress has become inevitable,[5] but enhancing stamina to improve physical activity is a viable option. Currently, there is a need for searching novel, effective, and safer alternatives to existing synthetic drugs for enhancing physical endurance.

Withania somnifera (WS) is a perennial shrub belonging to the family of Solanaceae. It is also referred to as the Indian ginseng, winter cherry, or ashwagandha. Various preparations from roots of WS is already proven to enhance physical endurance.[6] Currently, after obtaining the extract from the root, the remnant is discarded. However, we hypothesize that the remnant will have certain residual active ingredient post-extraction and will, therefore, exert some biological activity. In the present study, we aim to evaluate the effect of remnant root powder postaqueous extraction in enhancing physical endurance in mice using forced swim test. To the best of our knowledge, (PubMed search using MeSH terms [Withania OR ashwagandha] AND root), our experiment is the first to test botanical powder of WS root post-water extraction for its ability to enhance physical endurance, in comparison to the commercial WS root preparations available in the market.

MATERIALS AND METHODS

Animals

Inbred male Swiss albino mice that were 6–8 weeks old were utilized for the experiment. They were housed in the animal house at a temperature of 23°C ± 2°C and humidity of 40%–70%. The photoperiod was 12 h. The animals were fed standard pellet food and were given tap water

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ad libitum. Three animals were housed in one cage and the floor of the cage was covered with husk.

Experimental design

Eighteen animals were randomized into three groups of six animals each. Group 1 was the control group and received the vehicle carboxymethyl cellulose (CMC) 0.5%. Group 2 was the standard group and received WS commercial root preparation in CMC 0.5% vehicle. Group 3 was the test group that received the test substance WS root powder post-extraction in 0.5% CMC.

Manufacture of standard (commercial) preparation

WS roots were cleaned and sorted. They were then subjected to grinding and were passed through size number 40 mesh. The material that remained above the mesh was reground till <5% remained above the mesh. The material was packed in high-density polyethylene bags and sent for steam sterilization. The material was dried such that the moisture content remained ±1% of the moisture of input material. This was followed by delumping, milling, sieving, and magnetic separation of contaminants. The material was then subjected to blending in 500 kg/1 MT double cone blender. Finally, they were packed in single ply laminated polycovers (polyethylene 100 micron and polyester 12 micron) and passed through metal detectors.

Manufacture of test substance

The roots of WS were cleaned and sorted. This was then ground into coarse particles and passed through size number 10 mesh. Hot water at 75°C–85°C was allowed to circulate over the coarse particles for 3 h. This process is known as water/aqueous extraction which was performed using a commercial scale extractor (Miscella SS316). The extraction process was repeated three times. The remnant is the spent root post-extraction. These roots were finely ground, steam sterilized, and spray dried. After delumping, milling, sieving, magnetic separation of contaminants, and blending as explained in the previous section, it was packed in ply laminated polycovers.

Authentication of drug

The herbal raw material used for preparing the test drugs (the standardized commercial powder and the post-extraction powder) was identified by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The TLC profile of the ashwagandha powder was compared with the profile of the botanical reference standard of ashwagandha root. The identity of ashwagandha was also confirmed by checking the presence of withanolides in the powders using HPLC. The total withanolides (%w/w) in the standard and the test substance were 0.37 and 0.29, respectively.

Preparation of solution of test substances

Almost 0.5% solution of CMC was first prepared and homogenized using a magnetic stirrer. To 10 ml of CMC solution, 100 mg of the test substance was added and the mixture was again homogenized using a magnetic stirrer for at least 10 min. Thus, the final concentration of the solution formed was 10 mg/ml. Solutions of commercial preparation and the post-extraction root powder were prepared separately in a similar manner.

Dose calculation

The required dose based on other studies was 100 mg/kg/day of WS commercial preparation of root powder.[7] Thus, for a 35 g animal, the required dosage will be 3.5 mg/day. The solution which we prepared was at a concentration of 10 mg/ml and so the volume of prepared solution required for a 35 g animal was 0.35 ml.

Dosing

The animals were allowed to acclimatize to the laboratory environment for 1 day. Test substances were administered to the animals once daily through oral gavage starting from the 2nd day. This was repeated for 7 days. After 1 week of dosing, the animals were subjected to forced swim test on the 8th day.

Swim endurance test

We used plastic tubs of dimensions 25 cm radius and height 30 cm. Water was filled to a height of 15 cm so that the tail of the animal never touched the bottom of the tank. It was also ensured that the water level was well below the brim of the tank so that the animal could not jump out of the tank. Animals were placed individually in each of the tanks. They were allowed to swim till exhaustion. The end point was recorded when the animal started to drown which was identified by the snout of the animal dipping briefly below the water level. The time taken in seconds was noted and the animals were immediately rescued from drowning and placed in a warmer till they were dry.

Ethics

Ethics approval was obtained from the Institutional Animal Ethics Committee vide reference number IAEC/NR – PCL/04/11.16. We followed the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines throughout experimental procedure for animal care and handling.

Statistical analysis

The weight of the mice and swim time in each group was summarized as mean and standard deviation (standard deviation [SD]). One-way ANOVA was used to test for difference in mean swim time across the three groups. Tukey's *post hoc* test was used to identify the mean difference between all three pairs of groups. $P < 0.05$ was considered statistically significant.

RESULTS

We used a total of 18 animals whose mean (SD) weight was 32.61 (3.85) g. The mean weight in grams and swim time in seconds in each of the three groups are depicted in Table 1. Difference in mean swim time across the three groups was significant (F[2,15] =12.771, $P = 0.001$). Tukey's *post hoc* test was conducted; the results are shown in Table 2. The mean difference between test group and control group was 113.67

Table 1: Mean weight and swim time of mice in three groups

Groups	Mean±SD	
	Weight (g)	Swim time (s)
Control	32.17±2.93	151.00±17.23
Standard	31.17±2.99	210.17±31.61
Test	34.50±5.09	264.67±57.09

SD: Standard deviation

Table 2: Tukey's *post hoc* test for swim time

Groups	Mean difference (s)	95% CI	*P*
Test versus control	113.67	55.23-172.10	<0.001
Test versus standard	54.50	−3.94-112.94	0.069
Standard versus control	59.17	0.73-117.60	0.047

SE=22.50. SE: Standard error; CI: Confidence interval

s (95% confidence interval [CI]: 55.23, 172.10) which was statistically significant ($P < 0.001$). The mean difference in swim time between the test group and the standard group was not significant (54. 50; 95% CI: −3.94, 112.94; $P = 0.069$).

DISCUSSION

Phytopharmacology is a branch of pharmacology that deals with the study of pharmaceutical properties of natural plant derivatives. Recently, more attention is being given to plant products as they are natural and presumably less toxic than the synthetic drugs.[8] WS has been used widely used in Ayurveda generally as an energy booster to improve the overall health and in musculoskeletal conditions such as rheumatism and arthritis and as a calming agent.[9] WS root extracts and crude powders are currently being manufactured. During the manufacture of the root extract, the remnant root post-extraction is discarded.[6]

Forced swim model in rodents is one of the best and the most commonly used animal models to test for physical endurance and antidepressive effects. Previous studies with ethanolic extract of WS at 100 mg/kg twice daily orally on days 1, 4, and 7 showed reduction in the blood urea nitrogen levels, lactic acid, and adrenal hypertrophy that normally increase with stress in rats, thus demonstrating physical endurance enhancing capacity. There was also no change in the weight of thymus gland and hyperglycemia.[10] Another study with aqueous suspension of WS root powder at 100 mg/kg orally for 7 days indicated better stress tolerance in rats in swimming test in cold water. The plasma corticosterone level in the stress alone arm was 107.28 μg/dl while that in the stress and drug arm was 99.77 μg/dl. Similarly, the total swimming time was 5.30 ± 0.24 min and 8.9 ± 0.5 min, respectively. It also increased the avidity index and phagocytic index in test animals.[7] Another similar study conducted in mice with aqueous suspension of the root powder demonstrated that there was doubling of swimming time in the test group when compared to the control group. The mean swim time was 385 min in the control group whereas it was 740 in the test group.[11] The mice used in our study were not significantly different from each other in terms of their age, sex, and body weight which are the most important factors affecting stamina. The findings of our study are also in line with the findings of the above-mentioned studies.[7,10,11] The animals in the test group swam for a much longer time when compared to those in the control group. This suggests that the remnant root material after extraction also could be effectively used as an energy booster after extraction process.

Laboratory analysis has identified certain biologically active constituents in WS root, namely, the alkaloids (isopellertierine and anferine) and the steroidal lactones (withanolides, withaferins, and glycowithanolides). However, among these, the withanolides are believed to be responsible for the extraordinary medicinal value.[12] In our study, the mean difference in swim time between the commercial preparation and the root powder post-extraction is almost the same with no significant difference. This suggests that the remnant root material postwithanolide extraction also had some effects on physical endurance. This effect may be due to the presence of other active constituents in WS which needs further evaluation. Recent experimental studies using HPLC have identified few more active constituents such as withanamides, steroidal saponins, lignanamides, and tyramine derivatives[13] and certain active secondary metabolites such as anthocyanins, carotenoids, flavonoids, and tannins.[14] However, the biological activity of these active moieties is yet to be explored.[13]

Our study had few limitations. It was a smaller study with only six animals in each group. Biochemical markers of stress could have been measured to corroborate our findings. We used roots postaqueous extraction, and hence, the findings cannot be generalized to those root powders which are subjected to alcoholic extraction.

We conclude that the remnant root powder post-extraction also has physical endurance enhancing capacity in mice similar to the commercial preparation. However, larger studies are warranted to confirm and reproduce the findings and identify any other potential active ingredient besides withanolides. Biochemical parameters of stress should be measured to corroborate with the clinical parameters. These findings will have an economic impact both to the farmers cultivating WS and the industries processing WS as the root material of WS after extraction that is discarded currently can also be utilized.

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Conflicts of interest

There are no conflicts of interest.

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