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Original Article

Physical Endurance Enhancement of *Withania somnifera* Milk Treated Powder Compared to Commercial Preparation in Mice

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ABSTRACT

Background: The duration one can apply energy to an activity is limited by stamina (endurance). Recent studies using extracts of ashwagandha (Withania) root powder, either aqueous or ethanolic suspension, have demonstrated a significant increase in physical endurance. Milk treatment enhances the potency of herbal preparations. **Objectives:** To evaluate the efficacy of milk treated root powder of ashwagandha as compared to standard preparation, in enhancing the physical endurance in mice, using swim endurance model. **Methods:** Male, Swiss albino mice (31 – 35 g, 6 – 8 weeks old) were randomized into 3 groups of 6 animals each : Control - Carboxy Methyl Cellulose 0.5%, Standard - commercial preparation of ashwagandha (100mg / Kg), test - milk treated ashwagandha root powder (100 mg/ Kg). Study drugs were administered per oral, once daily, for 7 days. On day 8, animals were allowed to swim in a propylene tank of dimension 40 cm x 25 cm x 15 cm, with water level at 25 cm. The animals were allowed to swim till exhaustion. The end point of swim endurance was when the mice near drowned. **Results:** There was a significant increase in the physical endurance in the treatment as compared to control .One way ANOVA ($F(2, 15) = 13.000$, $p = 0.001$), Tukey's post hoc test of milk treated vs standard ($p = 0.207$) however, was not significant. **Conclusion:** Milk treated Withania somnifera was not significantly different as compared to the standard preparation, though it was better than the control.

Key words: Antifatigue activity, Glycowithanolides, Swim endurance, Withania somnifera, Physical endurance.

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INTRODUCTION

Strength and stamina are essential components of a healthy human being. Limited stamina reduces a person's ability to exert, thereby reducing the length of time one can apply energy to an activity (endurance).[1] Hard work and intense exercise leads to the production and accumulation of free radicals, which result in oxidative stress injury to the body.[2]

The quest for safe and effective anti-fatigue methods have drawn the attention of researchers towards traditional and alternative medicines. *Withania somnifera* (WS) also known as ashwagandha is one such medicinal herb.[3] It is also referred to as the 'Indian Ginseng'. It is widely used in Indian system of medicine for musculoskeletal conditions generally as an energy booster and to improve overall health.

The roots are the main portion of the plant used therapeutically.[4] Recent studies in animals have demonstrated a significant increase in swim endurance and reduction in cold restraint stress using either aqueous suspension or ethanolic extracts of ashwagandha root.[5] Researchers used chronic footshock to determine stress induced changes in rat brain frontal cortex and striatum, and found that animals treated with *Withania somnifera* an hour before the foot shock experienced a significantly reduced level of stress.[6] This research confirms the theory that ashwagandha has a significant anti-stress adaptogenic effect. Withania Somnifera is usually consumed in a powdered form with milk, as maximum benefits are observed with this formulation.[7] The aim of the current study is to evaluate the efficacy of milk treated root powder of ashwagandha topped up with cold and hot water extracts of ashwagandha in enhancing the physical endurance in mice.

MATERIALS AND METHODS

Preparation of milk treated powder

Preparation of base powder

2 kgs of intact chopped dry Withania roots were boiled with cow's milk for a period of 4 h. The chopped roots were dried and powdered under aseptic conditions. The baseline withanolide concentration of the root powder without milk treatment was 0.37 (% w/w), as measured by HPLC (High performance liquid chromatography) as per USP

Preparation of cold water extract

5 Kg of withania roots was powdered coarsely and two parts of water was added. The mixture was left undisturbed for a period of 24 h. Later the mixture was squeezed and filtered to obtain the extract.

Preparation of hot water extract

5 Kg of withania roots was powdered coarsely and 16 parts of water was added and the mixture boiled under low flame until the contents were reduced to 1/4th and allowed to cool down. The mixture was filtered aseptically.

Preparation of high potency powder

2 parts of cold water extract was added to 2 Kg of the milk treated powder and then blended. The powder was dried, following which 2 parts of hot water extract was added and subjected to drying. The final component obtained was sent for quality control analysis. The preparation when subjected to chemical analysis revealed a higher concentration of with anolide [Total withanolides (% w/w) 0.7, by HPLC as per USP] to be present in comparison to the control [Total withanolides (% w/w) 0.37, by HPLC as per USP].

Animals

Male, Swiss albino mice (Mus musculus) (31 – 35 g, 6 – 8 weeks old) which were inbred, were used in the present study with prior approval from Institutional animal ethics committee (IAEC/ NR – PCL/04/11.16). Animals were housed under standard conditions (23°± 2 °C, 40%–70% relative humidity and 12 h photo period) and were maintained on standard rodent pellet diet and water *ad libitum*.

Preparation of the drug solution

100 mg of the test substance was added to 10 ml of CMC (Carboxy Methyl Cellulose), the mixture was then homogenised using a magnetic stirrer for a period of 5 min. The concentration of the final solution was 10 mg/ml. The dose to be administered in the mice was determined to be 100 mg/ kg/ day. The weight of mice in the current experiment ranged from 31 – 35g, thereby requiring 3.5 mg /day. A volume of 0.3ml from the preparation was administered for each mice once daily through oral gavage.

Experimental design

Animals were randomized into 3 groups. Each group comprised of 6 animals. They were randomised as follows:

Group 1: Control - Carboxy Methyl Cellulose (CMC- 0.5%) – 0.5 g / 100ml was administered

Group 2: Standard (commercial preparation of ashwagandha) (100mg / Kg)

Group 3: Milk treated *Withania somnifera* root powder (100 mg/ Kg)

Test, control and standard preparations were administered to mice via oral gavage once daily for a period of 7 days. On day 8, animals were allowed to swim in a propylene tank of dimension 40 cm* 25 cm* 15 cm, filled with water to a height of 25 cm. The animals were allowed to swim till exhaustion. Care was taken so that the tail of the animal did not come in contact with the base of the tank, as this would help the mice in balancing and maintaining its head above the surface of the water. The end point of swim endurance was considered when the mice nearly drowned (drop its head 3 times below the level of water).

Statistical analysis

All values are expressed as mean ± SEM. The difference in mean swimming time between groups was analysed by one way ANOVA. Tukey's post hoc test was applied to determine which two groups differed significantly, p – value < 0.05 was considered for significance.

RESULTS

The final body weight of the animals in each group varied as follows, vehicle control group

(34.17±0.54) g, *Withania somnifera* powder group (35.17 ± 1.47) g and *Withania somnifera* milk treated powder group (35. 17 ± 0.79) g. 18 animals were subjected to force swim test on day 8. (Table 1)

Mean swim time difference between groups as analyzed by ANOVA (F (2, 15) = 13.000, p = 0.001) was significant. Tukey's post hoc analysis revealed a significant increase in the swim time between the control and test substance (p = 0.001). A significant increase in the swim time was noted when control was compared with commercial ashwagandha powder (p = 0.014). However the difference in swim time between the standard and test substance was not statistically significant (p=0.207). (Figure 1)

Table 1: Mean swimming time of mice treated with vehicle, WS powder, WS milk treated powder.

Group	Mean Swim time (mins.)± SEM
Vehicle control	136.22 ± 3.94
WS powder	210.30 ± 12.79
WS milk treated powder	251.20 ± 24.59

Values are expressed as mean ± SEM; n=6

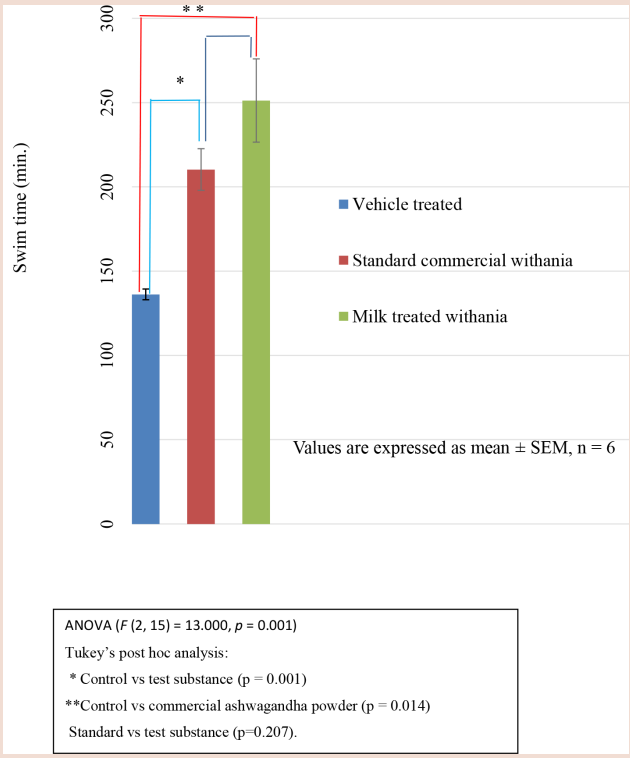

Figure 1: Mean swimming time of mice treated with vehicle, WS powder, WS milk treated powder.

DISCUSSION

Withania somnifera (WS) has been held in high esteem in Ayurveda because of its rejuvenative and tonic effects that are reminiscent of Asian ginseng. These similarities have made Ashwagandha to be referred as Indian Ginseng. In Indian medicine / Ayurveda, it has been traditionally used for lack of libido, fatigue, recovery from prolonged illness, mental problems and as a rasayana (rejuvenator).[8] The present study evaluated the physical endurance enhancing property of milk treated Withania root powder in comparison to the commercial preparation of Withania root powder among mice. The swim endurance test is a commonly used experimental model for evaluating this parameter. Experiments utilising swim endurance model and *Withania Somnifera* (100 mg/kg, p.o) as the reference standard have been conducted to demonstrate Antistress and immunomodulatory activity of other natural products.[9] To the best of our knowledge the milk treated *Withania* preparation topped with cold and hot water extract is probably the first of its kind to be evaluated.

This study was conducted to demonstrate an improvement in the physical endurance in mice when given milk treated *Withania somnifera* in a dose of 100 mg / Kg. The preparation did demonstrate a significant increase in swim time (mean difference - 114.98 min.) as compared to the control group (CMC - 0.5%). There was an increase in the swim time as compared to the commercial preparation (root powder) (mean difference – 40.9 min) but this was not significant.

This study was a preliminary analysis using a single model. However, larger studies which include evaluation of biochemical parameters known to indicate reduced stress and improve physical endurance can be conducted for conclusive results. This will aid in detection of other beneficial changes brought about by milk treated preparation. Much of Ashwaganda's pharmacological activity has been attributed to two main withanolides, withaferin A and D.[10] The preparation when subjected to

chemical analysis revealed a higher concentration of withanolide [Total withanolides (% w/w) 0.7, by HPLC as per USP] to be present in comparison to the control [Total withanolides (% w/w) 0.37, by HPLC as per USP] even though this study did not demonstrate statistically significant effect of this higher concentration on physical endurance.

CONCLUSION

The preliminary conclusion from the study is that milk treatment of ashwagandha roots along with topping up with cold and hot water extracts of ashwagandha does not increase the physical endurance enhancing activity, even though the withanolide content of the preparation was found to be higher in this preparation.

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CONFLICT OF INTEREST

The authors declare none.

DECLARATION

Study drugs, Infrastructure and personnel support was provided by Natural Remedies, Bangalore, India.

ABBREVIATIONS USED

WS: Withania somnifera; **HPLC:** High performance liquid chromatography; **CMC:** carboxy methyl cellulose.

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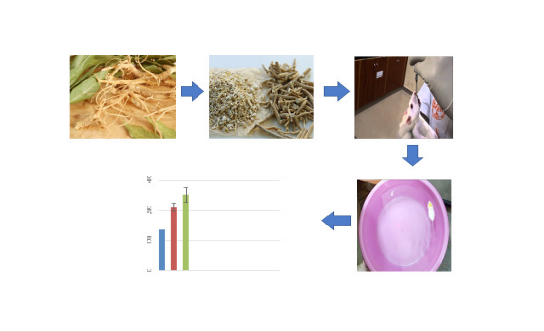
PICTORIAL ABSTRACT



SUMMARY

Withania somnifera (WS) is used in Ayurveda because of its rejuvenative and tonic effects. The present study evaluated the physical endurance enhancing property of milk treated Withania root powder in comparison to the commercial preparation of Withania root powder in mice. Forced swim test model was used. The preparation did demonstrate a significant increase in swim time (mean difference - 114.98 min.) as compared to the control group (CMC - 0.5%). There was an increase in the swim time as compared to the commercial preparation (root powder) (mean difference – 40.9 min) however, this was not significant. The preparation also showed high concentration of Withanolides which was quantified using HPLC.

ABOUT AUTHOR

Dr. Pradhyumna M Completed his MBBS in Kempegowda Institute of Medical Sciences, Bangalore and is currently pursuing his Post Graduation in Pharmacology, St. John's Medical College, Bangalore, Karnataka. He has presented the current study at AIGJCONIPS, Indira Gandhi Institute of Medical Sciences, Patna, Bihar 2017. He also has received first prize for best oral presentation at the National Conference on GCP, Bioethics and strategies to conduct Clinical Trials, Trichy, Tamil Nadu.

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