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Research Paper

A Study on the Effects of Some Medicinal Plants, Specifically Ashwagandha (*Withania somnifera*), on Activity, Autonomic Reactivity, Escape/Avoidance Learning and Response Rate in Albino Rats

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ABSTRACT

The present study investigated the effects of long term administration of crude powder and liquid tonic forms of ashwagandha (*Withania somnifera*) on behaviour in albino rats. The behavioural measures included general motor activity (measured in Revolving Wheel Apparatus), autonomic reactivity (measured in Open Field Test Apparatus), response latency (measured in Two-way Shuttle Box) and response rate (measured in Skinner Box). A 3g/kg treatment of the crude powder of ashwagandha through food did not show any significant effect on any of the behavioural measures. Similar results were obtained for 2ml/kg treatment of the tonic. Another experiment was conducted to compare the effects of ashwagandha, mandukaparni (*Centella asiatica*), jyotishmati (*Celastrus paniculatus*), vacha (*Acorus calamus*) and shankhpushpi (*Convolvulus pluricaulis*). Jyotishmati treated subjects had lower general activity scores as compared to other groups. Autonomic reactivity did not differ among the various drug treated groups. Lower response latency in jyotishmati treated group indicated faster learning than in other groups. Jyotishmati and vacha treatment also resulted in better response strength as compared to the other treatments. It is concluded that the crude forms of plant medicines often used by people may not be much effective in most cases.

Keywords: *Ashwagandha, Activity, Reactivity, Escape/avoidance learning, Response rate*

Various parts of plants have been used as medicines for thousands of years as indigenous practices as well as fully developed medical systems such as Ayurveda and Unani. Ayurveda, an ancient Indian medical system, uses a holistic approach towards life to achieve physical, psychological, social and spiritual well being (Patwardhan et al., 2005). In India, several plants have been known not only to treat various ailments but also to positively influence behaviour such as activity, emotionality, learning, etc. through their stimulatory effects on central nervous system (CNS). "CNS stimulants are those classes of drugs that stimulate the brain, speeding up both mental and physiological processes and

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having sympathomimetic effects. They are widely used either as prescription medications or as performance improving or recreational drugs" (Agarwal et al., 2020, P. 208). Some of the well-known herbs that act as CNS stimulants affecting various cognitive processes include vacha (*Acorus calamus*), shankhpushpi (*Convolvulus pluricaulis*), mandukaparni (*Centella asiatica*), brahmi (*Bacopa monnieri*), malkangni or jyotishmati (*Celastrus paniculatus*) and ashwagandha (*Withania somnifera*) (Sharma, 1987).

Although traditionally, the ayurvedic knowledge was passed on by practitioners to their disciples, a lot of systematic research on medicinal plants has been taken up over the past several decades. The CNS stimulants have been experimentally tested for their effects on behavioural performance mostly using animal subjects under controlled laboratory conditions. However, the results have not always been without contradictions regarding the expected outcomes of drug administration. An eight-day successive administration of brahmi rasayana made from six medicinal plants to rats improved learning and memory and reversed experimentally induced amnesia (Joshi & Parle, 2006). However, in another study, brahmi did not affect the rate of learning but significantly improved retention of newly acquired information in human subjects (Roodenrys et al., 2002). In behavioural studies, another widely tested plant medicine has been shankhpushpi which significantly improved learning and memory, and reversed amnesia induced by scopolamine in rats (Nahata et al., 2008). Shankhpushpi comes from four sources including *Evolvulus alsinoides* (EA), *Clitorea ternata* (CT), *Convolvulus pluricaulis* (CP) and *Canscora decussata* (CD), and it was found that EA had higher neuropharmacological effect measured in memory retrieval in pole climbing and Morris water maze tasks in rats as compared to the other three varieties (Sethiya et al., 2018). Vacha increased acquisition recalling and spatial recognition in male rats tested through Y maze and shuttle box test models (Naderi et al., 2010), Although spontaneous motor activity was not affected, an extract of vacha caused significant inhibition of conditioned avoidance response in rats (Vohora et al. 1990). Likewise, jyotishmati significantly improved the retention ability in drug administered rats compared with saline treated controls (Nalini et al. 1995). Though Veerendra Kumar and Gupta (2002) and Rao et al. (2005) reported that Mandukaparni improved neuronal morphology and learning performance and enhanced memory retention in rats and mice, an exhaustive review based on meta-analysis by Puttarak et al. (2017) found no strong evidence that it improved cognitive functions. It can, therefore, be seen that not all studies are in agreement regarding effects of medicinal plants on cognitive function and more research in this is warranted.

Ashwagandha has been widely used in India as a traditional medicine for many ailments, and for an overall well-being. Rayees and Malik (2017) has provided an exhaustive review on anti-stress, anti-cancer, anti-inflammatory, anti-oxidant, cardio protective activity and immune modulatory activity properties, and chemical characterization visa-vis pharmacological activities of ashwagandha. The effects of ashwagandha on central nervous system are reviewed by Singh et al. (2011). Dutta et al. (2019) reported the properties of *Withania somnifera* extracts that contain diverse components including Withaferin A that show inhibitory properties against many cancers, and they also revealed their mechanism of action and pathways involved. Whereas in most studies, the plant-based drug administration has been for a period of few days and to the adult animals, the present study was designed to administer Ashwagandha to rats immediately after weaning at the age of 21 days and continued for a period of 62 days. Since plant-based drugs are often used in crude form, the main aim of this study was to determine whether crude form is also effective. For a comparative analysis of the effect of different drugs, the study included four more medicinal plants than ashwagandha. The animals were tested on a variety of behavioural measures including general motor activity, emotionality, learning and response strength.

METHODOLOGY

Experimental Design and Procedure

The study was carried to investigate primarily the effect of ashwagandha using albino rats of the Wistar strain bred and raised in the Biopsychology Laboratory of University of Mysore. On the basis of advice from practitioners of Ayurvedic medicine, it was decided to feed crude powder of ashwagandha mixed in the pasted food pallets with a dosage level of 3g/kg of body weight. A commercially available ashwagandha tonic was also administered with a dosage level of 2ml/kg of body weight. For a comparative analysis of the effects of various drugs, mandukaparni, vacha, sankhpushpi and jytoshmati were also administered with a dosage level of 3mg/kg of crude powder mixed in food. The control group animals were given the same food without drugs. Table 1 provides the details of the number of animals used in various experiments. The drug administration in each experiment was started at the age of 21 days and continued for a period of 62 days.

Behavioural Measurements

Starting from the age of 83 days, the animals were tested for the following behavioural measures:

- **General motor activity**: The general activity was tested in the Revolving Wheel Test Apparatus for half an hour each day for two days. The wheel revolutions automatically recorded by a recorder were taken as the general activity score of the subject. Higher scores indicate stimulating effects of the drug.
- **Autonomic reactivity**: Autonomic reactivity was tested in the Open Field Test Apparatus for a period of two minutes each day for five days. The mean number of faecal boluses deposited by the animal over five days was taken as the reactivity score. Higher scores indicate a weaker autonomic nervous system.
- **Escape/avoidance learning**: Learning was measured in a Two-Way Shuttle Box with a buzzer as the conditioned stimulus (CS) and a mild foot shock as the unconditioned stimulus (UCS). The CS was presented and if the animal did not escape to the other chamber for a period of five seconds, foot shock as administered for a maximum period of 5 seconds. The maximum response latency, i.e., the time between the onset of the CS and the escape response by the animal which is a measure of learning, therefore, could be 10 seconds. An inter-trial interval of 20 seconds was maintained. An animal was 20 given training trials each day for two days, and tested for response latency for the next two days with 20 trials each day. The mean response latency of 40 trials was taken as the learning score. Smaller the response latency, faster is the learning.
- **Response rate**: Response rate, or response strength, was measured in a standard Skinner Box. An animal, deprived of food for 48 hours, was released in the box for a period of 20 minutes each day for three days. The total number of bar presses was taken as the response rate.

Data Analysis

The scores for each behavioural measure in experiments one and two were subjected to factorial ANOVA with groups (experimental and control), sex (male and female) and group x sex interaction as the independent variables and a behaviour score as the dependent variable. In experiment three, scores on each behavioural measures were subjected to one-way ANOVA to compare mean scores of five drug administered and one control groups. If any F was found significant, Sheffe's post hoc test was applied for pair-wise comparison among groups.

RESULTS

Experiment 1: 3g/kg drug administration

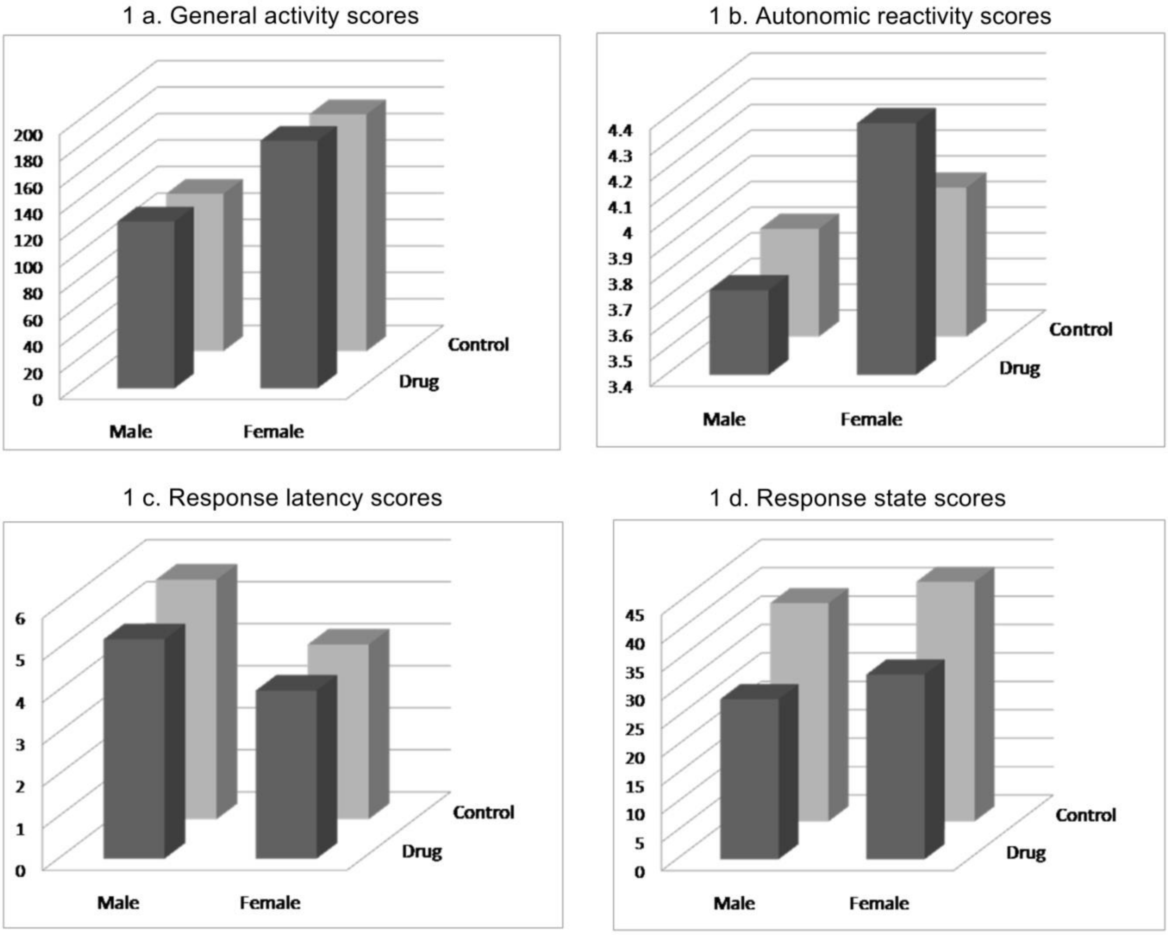

Fig. 1: Score on the effect of 3g/kg ashwagandha treatment on general motor activity (1a), autonomic reactivity (1b), response latency (1c) and response rate (1d)

There was no significant difference in the scores on general motor activity between the groups (F=0.05; df 1,44; p=.83), sex (F=3.17; df 1,44; p=.08) and group x sex interaction was also nonsignificant (F=0.01; df 1,44; p=.98) (Fig. 1a). Scores on autonomic reactivity also did not differ between the groups (F=0.08; df 1,44; p=.78), sex (F=0.53; df 1,44; p=.47) and group x sex interaction was also not significant (F=0.18; df 1,44; p=.67) (Fig. 1b). The groups did not differ in their scores on response latency (F=0.59; df 1,44; p=.45); females were faster learners than males (F=10.81; df 1,44; p=.01) with groups x sex interaction being nonsignificant (F=0.15; df 1,44; p=.70) (Fig. 1c). Neither groups (F=0.58; df 1,44; p=.45) nor sex (F=0.10; df 1,44; p=.76) differed in their scores on response rate with interaction between groups and sex also being nonsignificant (F=0; df 1,44; p=.99) (Fig. 1d).

Experiment 2: 2ml/kg drug administration

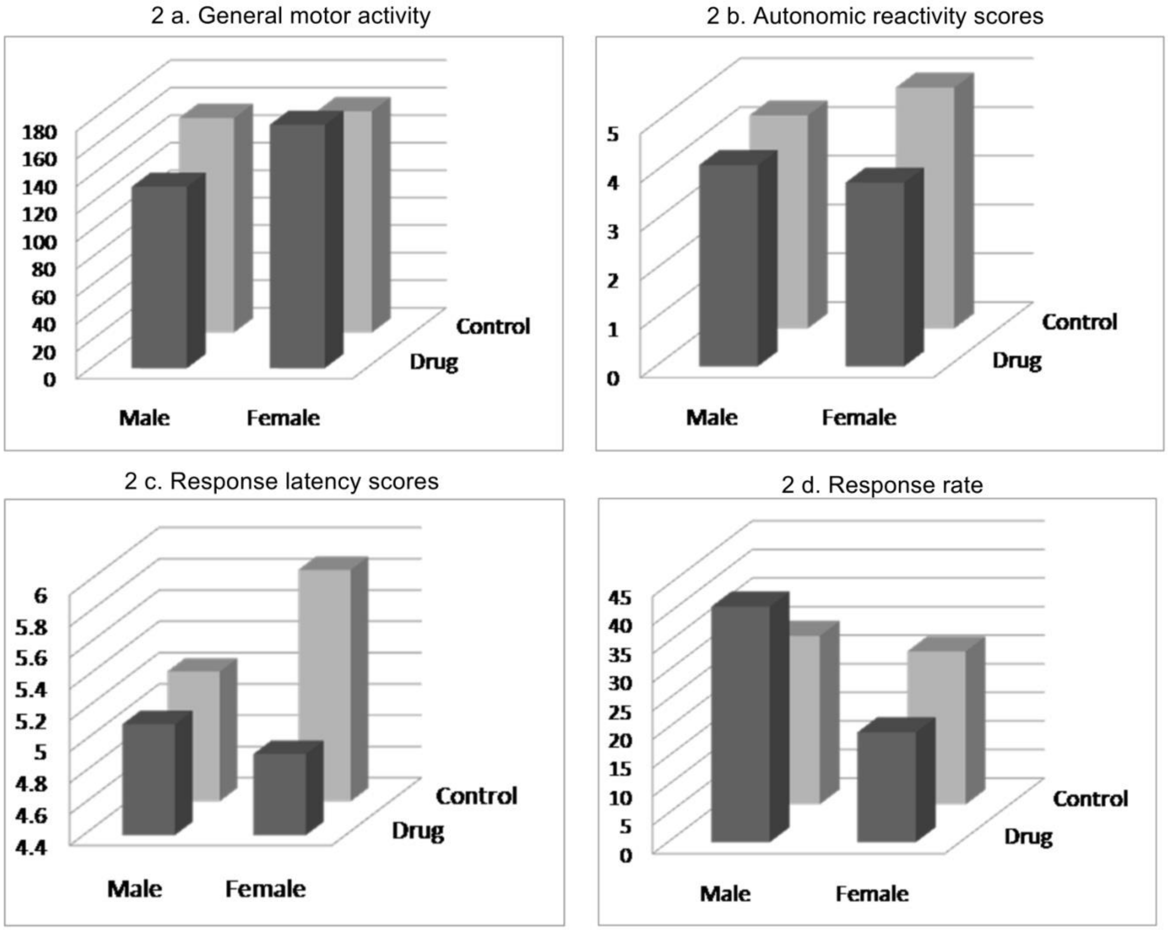

Fig. 2: Score on the effect of 2ml/kg ashwagandha treatment on general motor activity (2a), autonomic reactivity (2b), response latency (2c) and response rate (2d)

There was no significant difference in the scores on general motor activity between the groups (F=0.02; df 1,44; p=.89), sex (F=0.74; df 1,44; p=.39) and group x sex interaction was also nonsignificant (F=0.49; df 1,44; p=.49) (Fig. 2a). Scores on autonomic reactivity also did not differ between the groups (F=2.48; df 1,44; p=.12), sex (F=0.06; df 1,44; p=.81) and group x sex interaction was also not significant (F=1.11; df 1,44; p=.28) (Fig. 2b). The groups did not differ in their scores on response latency (F=1.50; df 1,44; p=.23), sex (F=0; df 1,44; p=.92) with groups x sex interaction being significant (F=4.97; df 1,44; p=.03) where drug administered females were faster learners than control females with no difference between the males in two groups (Fig. 2c). Neither groups (F=0.05; df 1,44; p=.82) nor sex (F=1.85; df 1,44; p=.18) differed in their scores on response rate with interaction between groups and sex also being nonsignificant (F=1.13; df 1,44; p=.29) (Fig. 2d).

Experiment 3: Comparison under different drug treatments

The general motor activity scores of subjects treated with different drugs differed significantly (F=2.27; df 5,128; p=.05) (Fig. 3a). Sheffe's post hoc tests revealed that the activity score of jyotishmati treated animals was smaller than the scores in all other groups. Groups did not differ in their autonomic reactivity scores (F=1.71; df 5,128; p=.14) (Fig 3b). The response latency scores of groups differed significantly (F=2.67; df 5,128; p=.05)

(Fig.3c). The post hos tests revealed that the score only in jyotishmati treated group was smaller, indicating faster learning, than in all other groups. The scores on response rate also differed significantly among the groups (F=2.76; df 5,128; p=,05) (Fig. 3d). Post hoc tests revealed that the response rate scores were significantly higher in vacha and jyotishmati treated groups, indicating better response strength, than other groups.

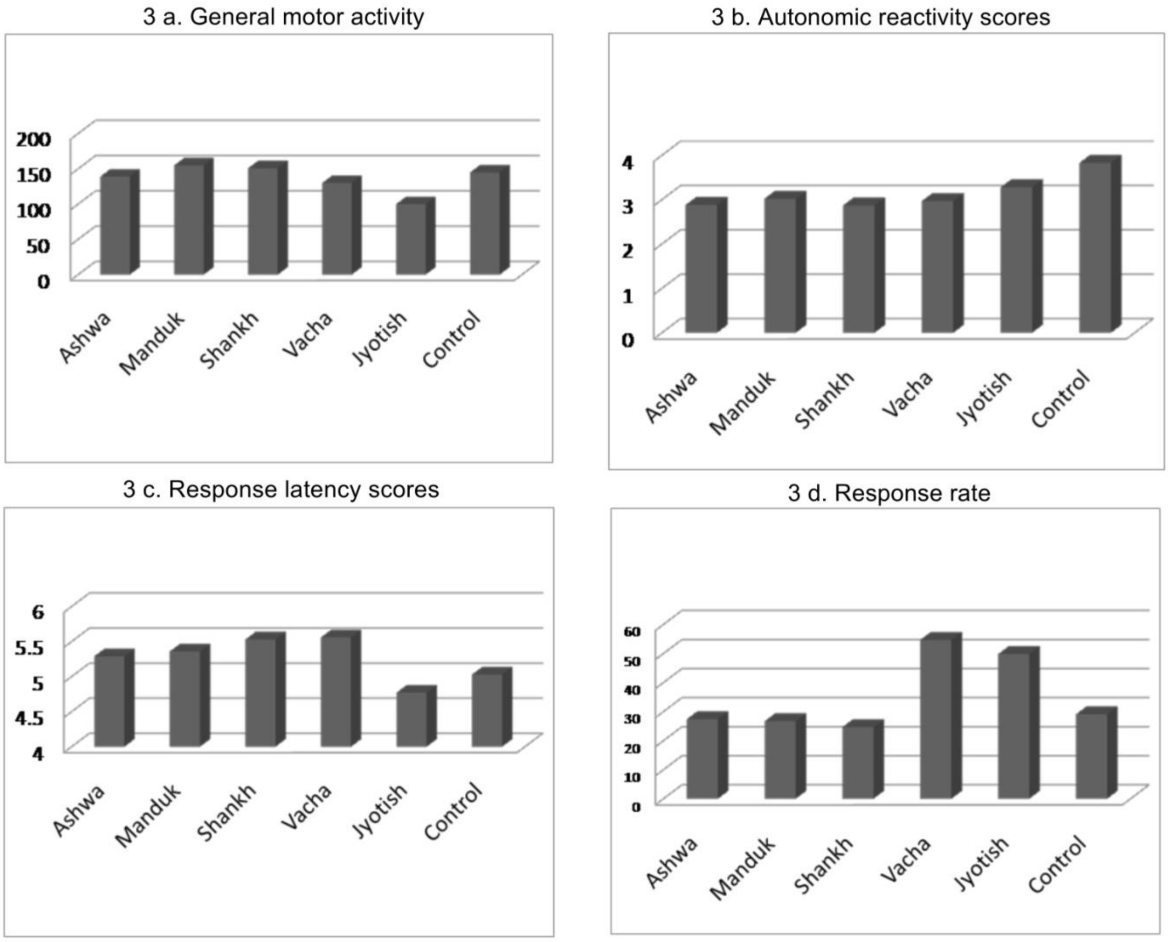

Fig. 3. Score on the effect of different drug treatments on general motor activity (3a), autonomic reactivity (3b), response latency (3c) and response rate (3d)

Table 1: Number of animals used in each experiment

	Experimental group		**Control group**	
Experiment	**Males**	**Females**	**Males**	**Females**
Ashwagandha 3g/kg	12	12	12	12
Ashwagandha 2ml/kg	12	12	12	12
Comparative study				
Ashwagandha	11	11	12	12
Brahmi	11	12		
Shankhpushpi	12	12		
Vacha	11	10		
Jyotishmati	10	10		

DISCUSSION

The behavioural tests used in the present study are standardized and used in most scientific laboratories in the world. These tests are sensitive enough detect effects of drug and chemical administered to rodents. In the present investigation, ashwagandha administered as crude powder, and also in the form of liquid tonic, did not show any effect on general, activity which is a measure of the general motor state of an animal. Autonomic reactivity which indicates the strength of autonomic nervous system was also not affected. Two measures of learning, shuttle box escape/avoidance response latency and Skinner box response rate (strength) scores were not affected by ashwagandha treatment. However, jyotishmati and vacha showed significant effects of general activity and improvement in measures of learning. In the previous studies from the same laboratory, jyotishmati (D'Souza et al. 1992) and vacha (Singh 1989) were found to improve learning in rats.

In several other studies, many positive effects, including on cognition, of ashwagandha have been observed. A 14 day administration of ashwagandha to healthy human subjects aged 20-35 years significantly improved reaction time in five of the six psychomotor performance tests, with no sedative effects (Pingali et al. 2014). A similar 14 day administration of the protein extract of ashwagandha significantly enhanced learning and memory as compared to negative and positive control groups in Wistar albino rats (Shivamurthy et al. 2016). The positive effects of other plant-based medicines employed in the present study are reported earlier in the introduction section. However, it may be noted that in almost all other studies, researchers used various extracts from the plants and tested these for their effects on behaviour. Since most people still use parts of medicinal plants in their crude, powdered or liquid, forms, the present study was carried out with the purpose of testing these crude forms. Though some positive results were obtained, the drugs in crude forms did not show appreciable effects on measures of activity and learning. It is therefore concluded that the crude form of medicinal plants may only have limited uses.

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Conflict of Interest

The author(s) declared no conflict of interest.

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