Original Research Article

Oral supplementations of *Bauhinia variegata's* leaf extract has anxiolytic effect and improves locomotory and exploratory behavior of female albino mice

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A b s t r a c t

Present study was designed to investigate the effects of 200 mg/ml solvent/Kg of body weight of *Bauhinia variegata's* leaf extract on neuromuscular coordination, locomotory and exploratory behavior of adult female albino mice. Six week old female albino mice were used as experimental animals. Mice were either orally supplemented with 200 mg/ml/Kg of body weight *Bauhinia variegata's* leaf extract or with saline solution [0.9%] for 7 days. Behavioral observations were made by applying a series of neurological tests [Rota rod, Elevated plus maze, Light and dark box, Open field and Novel object test]. Exploratory and locomotory behavior during Open Field test was improved in leaf extract treated mice as they had more means speed [P = 0.01], rotations [P = 0.001], clockwise rotations [P = 0.004] and anti-clockwise rotations [P = 0.001] than control mice. *Bauhinia variegata's* leaf extract treated mice had higher transition frequency [P = 0.002] and spent more time in light compartment during light dark box test than saline treated controls. *Bauhinia variegata's* leaf extract treated mice covered more distance [P = 0.033] with higher speed [P = 0.028] compared to control mice during Elevated Plus Maze test. *Bauhinia variegata's* leaf extract improves the locomotory and exploratory behavior in adult female albino mice. Leaf extract of *Bauhinia variegate* can be further explored to be used as a therapeutic agent for the treatment of neurological ailments.

Keywords: *Bauhinia variegate* ; open field test ; Rota rod test; novel object test; elevated plus maze; female albino mice.

Introduction

Conditioning is the form of learning that is often studied in entirely behavioral context as experience-dependent long-lasting changes occur in motor activity [1, 2]. Since last decade or so, researches are focused on investigating effective drugs and herbal treatment to improve the learning processes [3, 4]. Medicinal values of many plants are known to cure varieties of diseases but their effects on cognition are rarely studied [5].

Flower buds, flowers, leaves, stem, seed, roots and stem bark of *Bauhinia variegate* [*B. variegate*] are used for pharmacological purposes in various systems such as Homeopathy, Unani, and Ayurveda. It is globally distributed in tropical regions [6] and is used to treat multiple diseases [7] including hypoglycemia [8] and inflammations [9]. Ethanolic stem extract of *B. variegata* is reported to be useful against Dalton's ascetic lymphoma in Swiss albino mice where it increases the count of peritoneal cells [10] and it is known to be chemo preventive and cytotoxic against human liver, breast and epithelial larynx cancer [11]. Despite the known medicinal importance of *B. variegate,* effects of its leaf extract on

mouse behavior has not been explored in much detail. The objective of the present study was to explore the effect of 200 mg/ml solvent/Kg of body weight of *Bauhinia variegata's* leaf extract on neuromuscular coordination, exploratory and locomotory behavior in adult female albino mice.

Methods

Subjects

Six weeks old, female albino mice [BALB/c strain] were used as experimental animals in order to demonstrate the effect of *B. variegate* leaf extract on selected behavioral aspects of adult female albino mice. Animals were reared at the animal facility of Bio Park at Bahauddin Zakariya University Multan, Pakistan. Animals were kept in locally manufactured small rodent cages filled with wood chips. Standard mouse diet and water were available *ad libitum*. Room temperature was maintained at 22±1ºC. The light/dark rhythm was maintained at 14:10. The room was

illuminated with artificial light at an intensity of 200 Watt from 8 a.m. to 6 p.m.

All the experimental protocols and mouse handling procedures were approved by the ethical committee of the Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Pakistan.

Preparation of *Bauhinia variegata* leaves extract

The leaves of *Bauhinia variegata* plant were collected from various localities in Multan and thoroughly washed with distilled water and air dried under shade for 15-20 days. The completely dried plant leaves were ground well in to a fine powder in a herbal grinder and sieved to have particle size of 50-150 mm. The powder was stored in an air tight polythene bag at room temperature before extraction. 200 gm of *B. variegata* leaves powder was used to prepare the leaf extract following the Zahra et al. [5]. The crude extract was stored in dried and airtight container and stored in refrigerator at 4ºC till further use.

Experimental design

Leaf extract of B. variegata [200 mg] was dissolved in 1ml of distilled water to prepare the working solution. Albino mice were weighed and orally administered either with 200mg B. variegata leaf extract /ml solvent/Kg body weight [N = 10] or with 0.9% saline solution [Otsuka, Pakistan [N = 10]].

Assessment of Neurofunction

Dose were applied for 7 consecutive days and a series of neurological tests including Rota rod, light dark box, open field, elevated plus maze and novel object test were conducted on consecutive days to determine the effect of *B. variegata's* leaf extract on adult female albino mice behaviour. Doses were administered during neurological testing, at least 30 minutes prior to start of each test.

Rota Rod

Rota Rod apparatus is used to test the balance and neuromuscular coordination of an animal. Rota Rod test was performed by using a locally manufactured apparatus comprised of rotating drum with acceleration of 40 rpm. Prior to experimentation, each mouse received three training trials followed by three experimental trials. Mean of the time spent on rotating drum was qualitized among the control and extract treated groups following Test was conducted following Sunyer et al.[12].

Light/Dark Box

The light/dark test is based on the innate aversion of rodents to illuminated areas and on the natural exploratory behavior of rodents in response to new environment and illumination which is a mild stressor for rodents [13]. The light/dark box test equipment was having an area of [45 x 27 x 27 cm] made up of plywood and consists of one third dark safe chamber [18 x 27 cm] and two third light aversive chamber[27 x 27 cm] with light intensity of 200 Watt, connected by an opening [7.5 x 7.5 cm] located at floor and centre of the dividing wall. The floor was divided into 9 x 9 cm squares and is covered with Plexiglas. A mouse was placed in the centre of light chamber keeping its snout towards opening in the wall. Time spent in each chamber, transition frequency, rearing, stretch attended, defecation and urination were counted over a five minutes test following Zahra et al.[5].

Open Field Test

Open field test is used to assess locomotory and exploratory behaviour of an animal [14]. A computational tracking system, Any-Maze [Stoeling, USA] connected with video camera [XPod-058, China] was employed to detect the behavior of mice in the open field chamber [40 cm x 40 cm x 70 cm]. Each mouse was released in the corner of the open field box for ten minutes of test duration. Maximum speed [m], Means spead [m/s], Time mobile and Time immobile [seconds], Mobile episodes, Immobile episodes, Rotations: Clockwise and anticlockwise were noticed following Weitzdoerfer et al. [15].

Elevated Plus Maze

Elevated plus maze test is used to assess the anxiety related behaviors in rodents [16]. The apparatus had a Plexiglas floor of 5 x 5 cm with central square platform from which two open arms [45 x 5 cm] with 0.25 cm high edges and two closed arms [45 x 5 cm] with 40 cm high walls made of clear Plexiglas were radiated. Each mouse was placed in the central square of the apparatus facing towards open arm and let the area to explore for five minutes. Maximum speed, mean speed, time mobile and time immobile, mobile and immobile episodes and rotations pattern was recorder via Limelight video camera [Addlink, Bercelona] based tracking system [Anymaze, USA] fixed at the top of apparatus. Head dips, urination times and number of defecations were counted manually. The test was conducted following Zahra et al. [5].

Novel Object Test

Novel object recognition is a form of memory task that doesn't rely on spatial cues and is used to judge the recognition memory by measuring its liking for novel object [17, 18]. Two objects were placed in the opposite corners of field [40 x 40 cm] with 70 cm high walls to avoid the escaping of animal. Each mouse received two trials. In the first trial, the individual mouse was placed in the middle

of the chamber and let to explore the area for five minutes. Line cross, stretch attend frequency, approach to object A, approach to object B, time spent near object A and time spent near object B was measured using stop watch. After 5 minutes, the mouse was removed from the field and let it to relax for 20 minutes. In the second trial, the procedure was repeated replacing one of the objects with novel one. All the calculations were manually recorded and analyzed following Zhanga et al. [19].

Statistical Analysis

All the data was expressed as mean ± standard deviation [SD] statistical package Minitab [Version 16, USA] was used for the analysis of results. 2 sample t-test was applied to compare various parameters of Rota rod, open field, elevated plus maze, novel object and light and dark box test between leaf extract treated and untreated female albino mice.

Results

Rota Rod test

Analysis of the results revealed a non-significant [P > 0.05] difference in mean time spent on rotating drum of Rota Rod when compared between *B. variegata's* leaf extract and untreated female albino mice [Figure-1].

Figure 1: Comparison of Rota Rod test performance between *Bauhinia variegata* leaf extract (200 mg/ml of solvent/ Kg body weight) and saline treated adult female albino mice. All values are expressed as mean ± standard error of mean. P value presents the results of 2 sample t-test.
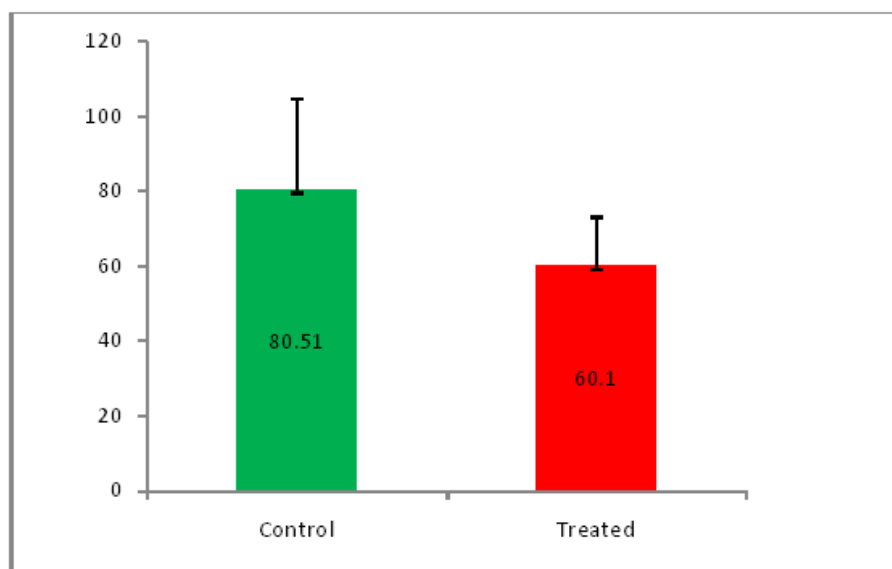
P > 0.05 = Non significant

Open Field test

It was observed that *B. variegata* leaf extract treated mice had higher mean speed [P = 0.01], rotations [P = 0.001], clockwise rotations [P = 0.004] and anti-clockwise rotations [P = 0.001] than control mice indicating their improved exploratory and locomotory behavior [Table 1].

Table 1: Effect of *Bauhinia variegata* leaf extract on studied parameters of open field test, in adult female albino mice. All values are expressed as mean ± standard deviation. N = 10 for each treatment. P value represents the results of 2 sample t-test calculated for each parameter.

Parameters	Saline treated control	*Bauhinia variegata* treated	P- Value
Maximum speed (m)	0.563 ± 0.426	0.22 ± 0.0341	0.147
Mean Speed (m/s)	0.023 ± 0.013	0.05 ± 0.01	0.01**
Time mobile (sec)	362 ± 207	527.8 ± 15.1	0.148
Time immobile (sec)	238 ± 207	72.2 ± 15.1	0.148
Mobile episodes	21.0 ± 19.4	15.25 ± 3.63	0.55
Immobile episodes	20.3 ± 19.2	15 ± 3.61	0.580
Rotations	8.75 ± 8.14	34.25 ± 6.22	0.001***
Clockwise rotations	4.75 ± 4.92	17.25 ± 4.26	0.004*
Anti clockwise rotation	4.00 ± 4.00	17.00 ± 2.55	0.001***

P > 0.05 = Non significant; P < 0.05 = Least significant (*); P < 0.01 = Significant (**); P < 0.001 = Highly significant (***)

Light Dark Box test

Data analysis indicated that *B. variegata* leaf extract treated mice had significantly higher transition frequencies [P=0.002] between light and dark compartments, spent less time in dark [P = 0.003] and more time in light [P = 0.003] as compared to their control group during light dark test indicating anxiolytic effect of *B. variegata's* leaf extract in adult female albino mice [Table 2].

Elevated Plus Maze test

During elevated plus maze test, *B. variegata's* leaf extract treated albino mice covered more distance [P = 0.033] with higher mean speed [P = 0.028] than control mice. All other studied parameters varied not significantly [P > 0.05] between the two treatments [Table 3].

Novel Object test

During first trial of novel object test, *B. variegata* leaf extract treated mice had more transition frequencies [P = 0.053] and approached object A more frequently than control mice [P = 0.045] [Table 4A]. During second trial, all the studied parameters varied non-significantly [P > 0.05] between the two treatments indicating no effect of leaf extract treatment on object recognition ability of female albino mice [Table 5].

Table 2: Effect of *Bauhinia variegata* leaf extract on studied parameters of light dark box test in adult female albino mice. All values are expressed as mean ± standard deviation. N = 10 for each treatment. P value represents the results of 2 sample t-test calculated for each parameter.

Parameters	Saline treated control	*Bauhinia variegata* treated	P-value
Transition frequency	8.500 ± 0.866	23.00 ± 4.64	0.002 *
Rearing frequency	1.250 ± 0.829	1.00 ± 1.22	0.717
Stretch attend frequency	1.50 ± 1.66	5.75 ± 4.15	0.087
Time in dark (sec)	202.5 ± 12.9	151.3 ± 19.7	0.003*
Time in light (sec)	97.5 ± 12.9	148.8 ± 19.7	0.003*
Urination	0.750 ± 0.829	1.50 ± 2.06	0.484
Defecation	2.00 ± 0.707	2.25 ± 1.48	0.747

P > 0.05 = Non significant; P < 0.05 = Least significant (*)

Table 3: Effect of *Bauhinia variegata* leaf extract on studied parameters of elevated plus maze test in adult female albino mice. All values are expressed as mean ± standard deviation. N = 10 for each treatment. P value represents the results of 2 sample t-test calculated for each parameter.

Parameters			P – value
	Saline treated control	*Bauhinia variegata* treated	
Distance (m)	8.35±1.88	11.087±0.927	0.033*
Maximum speed (m/s)	0.244±0.0565	0.272±0.081	0.550
Mean Speed (m/s)	0.0280 ± 0.006	0.037±0.003	0.028*
Time mobile (sec)	193.6±25.0	219.9±15.3	0.092
Time immobile (sec)	106.4±25.0	80.1±15.3	0.092
Mobile episodes	22.00±1.22	21.75±4.92	0.917
Immobile episodes	21.50±1.12	21.00±4.64	0.826
Rotations	6.50±1.80	9.250±0.433	0.029
Clockwise rotations	3.75±1.48	4.500 ±0.866	0.366
Anti clockwise Rotation	2.75±1.79	4.750±0.433	0.072
Urination	1.00 ± 1.00	0.250±0.433	0.184
Head dipping	9.0±3.24	10.75±1.48	0.322

P > 0.05 = Non significant; P < 0.05 = Least significant (*)

Table 4A: Effect of *Bauhinia variegata* leaf extract on studied parameters of novel object test (trial 1) in adult female albino mice. All values are expressed as mean ± standard deviation. N = 10 for each treatment. P value represents the results of 2 sample t-test calculated for each parameter.

Parameters	Saline treated control (N = 5)	*Bauhinia variegata* treated (N = 5)	P-value
Line cross	14.20 ± 6.22	23.60 ± 6.54	0.053*
Stretch attend reflex	2.00 ± 1.58	3.80 ± 2.95	0.274
Approaches object A	7.20 ± 3.11	13.60 ± 4.72	0.045*
Approaches object B	10.00 ± 4.24	13.80 ± 2.68	0.141
Time object A (sec)	47.8 ± 19.0	56.0 ± 21.4	0.542
Time object B (sec)	81.8 ± 57.5	68.8 ± 34.6	0.680

P > 0.05 = Non significant; P < 0.05 = Least significant (*)

Table 4B: Effect of *Bauhinia variegata* leaf extract on studied parameters of novel object test (trial II) in adult female albino mice. All values are expressed as mean ± standard deviation. N = 10 for each treatment. P value represents the results of 2 sample t-test calculated for each parameter.

Parameters	Saline treated control	*Bauhinia variegata* treated	P-value
Line cross	11.60 ± 5.13	14.60 ± 3.21	0.310
Stretch attend reflex	4.20 ± 3.03	6.20 ± 3.03	0.328
Approaches Old object	8.20 ± 4.66	11.20 ± 5.31	0.374
Approaches Novel object	6.60 ± 4.56	9.00 ± 1.58	0.329
Time Old object (sec)	33.8 ± 19.7	60.0 ± 43.1	0.271
Time Novel object (sec)	65.2 ± 59.9	46.6 ± 25.2	0.551

P > 0.05 = Non significant

Discussion

Plants are the most precious resource for an extensive range of derivative metabolites utilized as medicine, agrochemicals, biopesticides, food additives and flavors [20]. *Bauhinia variegata* Linn is conventionally used for the treatment of tumors, bronchitis and leprosy and it is an anti hepatotoxic agent [10, 21-24]. The present study was conducted to investigate the effect of *B. variegata* leaf extract [200 mg/ml solvent/Kg of body weight] on behavior of adult female albino mice as little information is available on this topic.

Our results indicated that *B. variegata's* leaf extract treatment did not affect the neuromuscular coordination in adult female albino mice [Figure-1]. Our results are in agreement with Sathya et al. [25] who had reported that the ethanolic extract of the *B. tomentosa* leaves [200 and 400 mg/Kg] had no effect on neuromuscular coordination in mice as both treated and untreated mice spent more than 180 seconds on rotating rod suggesting that extract of *B. tomentosa's* leaves is not neurotoxic.

During open field test, we observed that the *B. variegata's* leaf extract [200 mg/ml/Kg of body weight] improved the locomotory and exploratory behavior as the treated mice remained mobile for longer time with higher speed and having less mobile episodes compared to control group [Table 1]. Our results are contradictory to Sathya et al. [25] and Cavalcanti et al. [26] who had reported reduced mobility in mice and rats upon treatment with alcoholic leaf extracts of *B. tomentosa* [200 mg/Kg and 400 mg/Kg] and *B. forficata* [5 g/Kg] respectively. The differences in performance can be attributed with the differences in leaf extract composition from three *Bauhinia* species.

Results of our light dark test revealed that *B. variegata's* leaf extract mice spent more time in light box and were not in anxiety [Table 2]. These observations are in line withDavey et al. [27] who had reported that oral supplementation with methanolic extract of *B. racemosa* stem bark [150 and 300 mg/Kg] increased the time spent by Swiss albino mice in illuminated areas that recommends its anxiolytic potentials.

Elevated Plus Maze test results revealed that supplementation with leaf extract of *B. variegata's* improves the exploratory and locomotory behavior in female albino mice [Table 3]. Borges dos Santos et al. [28] has reported that 50 mg/Kg ethanolic extract of *B. platypetala* has anxiolytic effect and increase the exploration in male Swiss mice where the dose was administered intraperitonially supporting our findings. Our results are also in agreement with Davey et al. [27] and Sathya et al. [25] who had reported anxiolytic potentials of methanolic leaf extract of *B. racemosa* [150 and 300 mg/Kg] and *B. tomentosa* [200 mg and 400 mg/Kg] in mice during Elevated Plus Maze test.

It has been reported that the aerial non woody parts of *B. variegata* contain flavonoids that have neuro-protective effect and have the ability to protect neurotoxins induced damage and also have the capability to repress neuroinflammation, supportive in efficient learning, increase memory and cognitive role [29]. These flavonoids act together with lipid and protein kinase signalling pathways in the brain and promote neuronal survival and have useful effect on cerebro-vascular blood flow [30]. So the improvements in neuro-functioning in female albino mice during present studies can be attributed with the presence of flavonoids in *B. variegata's* leaf extract.

It may be concluded from the present study that the *B. variegata's* leaf extract has a potential to anxiety and to improve the exploratory and locomotory behavior in female albino mice and can be further explored for the treatment of various neurological ailments.

Authors Contributions

FI has designed the study and revised the manuscript, MJ, SI and AU has performed the lab experiments and MNKK has analyzed the data and written the manuscript.

Conflict of interest

Authors declare that they have no conflict of interest of any sort with anyone.

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on animal experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The authors also assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals

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