



Gender Specific effects of Ethanolic Leaf Extract of *Bauhinia Variegata* on the Behavior of Albino Mice.

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Abstract: The present study was designed to evaluate the effect of 150 mg/ml solvent/Kg of body weight of *Bauhinia variegata*'s leaf extract supplementation on neuromuscular coordination, locomotory and exploratory behavior of adult female and male albino mice. Seven week old female and male albino mice were used as experimental animals. Mice were either orally supplemented with 150 mg/ml/Kg of body weight *Bauhinia variegata* leaf extract or with saline solution for 17 days. Behavioral observations were made by applying a series of neurological tests (rota rod, light dark box, open field, elevated plus maze and Morris water maze). Female mice treated with leaf extract of *Bauhinia variegata* displayed significantly improved rota rod test performance ($P = 0.008$) than control group. *Bauhinia variegata* Linn leaf extract treated male mice had increased dark to light transition frequency than control group during light dark test, leaf extract treated male mice covered more distance ($P = 0.03$) with higher speed ($P = 0.03$), remained mobile for longer time ($P = 0.01$) than control group during elevated plus maze test. Open field, novel object and Morris water maze test performance remained unaffected for both genders when compared between *Bauhinia variegata* leaf extract treated and untreated mice.

Supplementation with leaf extract of *Bauhinia variegata* improved neuromuscular coordination in female while improved the exploratory behavior in male albino mice.

Keywords: *Bauhinia Variegata*; leaf extract; behavioral Testing; Albino Mice

1. INTRODUCTION

Traditional medicines deal with use of plants and plant products for maintenance of good health. This indigenous form of medicinal system uses the active ingredients present in plants for treating various diseases (Nair 1998). Flower buds, flowers, leaves, stem, seed, roots and stem bark of *Bauhinia variegata* (*B. variegata*) are used for pharmacological purposes in various systems such as Homeopathy, Unani and Ayurveda. It is globally distributed in tropical regions (Amrani *et al.*, 2009) and is used to treat multiple diseases (Sahu *et al.*, 2012) including hypoglycemia (Abd *et al.*, 1987) and inflammations (Yadava 2002). 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 (Raj Kapoor *et al.*, 2003) and it is known to be chemo preventive and cytotoxic against human liver, breast and epithelial larynx cancer (Raj Kapoor *et al.*, 2006). Despite the known medicinal importance of *B. variegata*, effects of its leaf extract on mouse behavior have not been explored in much detail. The objective of the present study was to explore the effect of 150 mg/ml solvent/Kg of body weight of *Bauhinia variegata*'s leaf extract on selected aspects of behavior of albino mice in a gender specific manner.

2. MATERIAL AND METHODS

Subjects

Seven weeks old, female albino mice were used as experimental animals in order to demonstrate the effect

of *B. variegata* leaf extract on selected behavioral aspects of adult female and male 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 $22 \pm 1^\circ\text{C}$. The light/dark rhythm was maintained at 14:10. The room was illuminated with artificial light at an intensity of about 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

Dry leaf powder of *Bauhinia variegata* was used to prepare leaf extract following the Zahra *et al.* (2015).

Experimental design

Leaf extract of *B. variegata* (150 mg) was dissolved in 1ml of distilled water to prepare the working solution. Albino mice were weighed and orally administered either with 150mg *B. variegata* leaf extract /ml solvent/Kg body weight ($N = 14$) or with 0.9% saline solution [Otsuka, Pakistan ($N = 14$)].

Assessment of Neurofunction

Dose were applied for 17 consecutive days and a series of neurological tests including Rota rod, light

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dark box, open field, elevated plus maze, novel object and Morris water maze test were conducted on consecutive days to determine the effect of *B. variegata*'s leaf extract on adult female and male albino mice behavior. Doses were administered during neurological testing, at least 30 minutes prior to start of each test.

Rota Rod

Rota Rod test was performed by using a locally manufactured apparatus comprised of rotating drum with acceleration of 40 rpm. Test was conducted following Zahra *et al.* (2016).

Light/Dark Box

The light/dark test was performed following Zahra *et al.* (2015).

Open Field Test

Open field test was performed following Iqbal *et al.* (2015)

Elevated Plus Maze

Elevated plus maze test conducted following Zahra *et al.* (2015).

Novel Object Test

Novel object recognition test was performed following Zhanga *et al.* (2012).

Morris water maze

Morris water maze (MWM) test was applied following Gillani *et al.* (2014).

Statistical Analysis

All the data was expressed as mean \pm standard deviation (SD) statistical package Minitab (Version 17, USA) was used for the analysis of results. Significance level was set at $P < 0.05$. 2 sample t - test was applied to compare various parameters of Rota rod, open field, elevated plus maze, novel object, light and dark box and Morris water maze test between leaf extract treated and untreated female and male albino mice.

3. RESULTS

Rota Rod test

Analysis of the rota rod test results revealed that *Bauhinia variegata* leaf extract (150 mg/ml solvent/Kg body weight) resulted in improved balance and coordination in female albino mice as they spent significantly more time on rotating rod ($P = 0.008$) as compared to saline treated female mice. While the same dose did not affected rota rod test performance in male albino mice ($P > 0.05$) (Fig. 1).

Fig. 1 Comparison of Rota rod test performance between 150 mg/ml solvent/Kg body weight of *Bauhinia variegata*'s leaf extract treated and untreated albino mice of both genders. Data is expressed as mean \pm standard deviation. P value represents the results of 2 sample t-test.

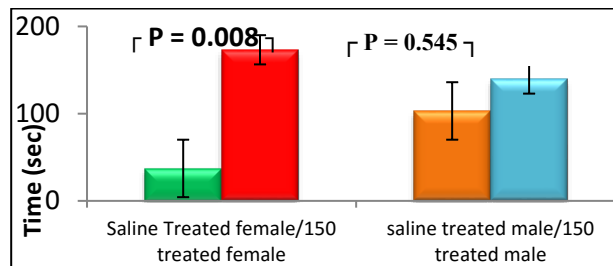


Fig. 1 $P > 0.05$ = Non significant; $P < 0.01$ = Significant

Open Field test

Results of open field test revealed that all the studied parameters varied non-significantly when compared between *Bauhinia variegata* leaf extract treated untreated albino mice of both genders (Table 1).

Light Dark Box test

Analysis of results indicated that female mice treated with 150 mg/ml solvent/Kg body weight of *Bauhinia variegata* leaf extract had significantly higher rearing frequency ($P=0.037$) than control group. All other studied parameters varied non-significantly ($P > 0.05$) when compared between plant extract treated and control animals. Unlike female, male albino mice performed better in light dark box as they showed significant higher transition frequency from dark to light chamber ($P=0.031$) and stretch attend frequency ($P= 0.038$) (Table 2).

Elevated Plus Maze test

It was observed that *Bauhinia variegata* leaf extract treatment had enhanced the short term memory in male albino mice as the extract treated male covered significantly more distance ($P = 0.03$) with higher mean speed ($P = 0.03$), remained mobile for longer time ($P = 0.01$) and immobile for less time ($P = 0.01$) with low rate of defecation ($P = 0.02$) as compared to the saline treated male control (Table 3). On the other hand, mobile episodes was the only parameter that was significantly lower ($P = 0.009$) in 150 mg/ml solvent/Kg body weight *Bauhinia variegata* leaf extract treated female mice than their control group (Table 3).

Novel Object test

Analysis of the data revealed that 150 mg/ml solvent/Kg body weight dose of *Bauhinia variegata* leaf extract did not affected the object recognition capacity of albino mice of both genders. When results of novel object test (Trial 2) were analyzed, it was observed that although leaf extract treated females spent more time with the novel object ($P = 0.16$) but all the studied parameters varied non-significantly when compared between *Bauhinia variegata* leaf extract treated and untreated female albino mice. Analysis of the data indicated that during novel object test (Trial-2) male albino mice treated with 150 mg/ml solvent/Kg body weight showed significant higher tendency to approach old object-A ($P=0.02$) than control group (Table 4, 5).

Table 1 Comparison of various studied parameters of open field test between *Bauhinia variegata* leaf extract (150 mg / ml solvent / Kg body weight) and saline treated adult albino mice. N = 7 for each treatment. P- value represents the results for two sample t – test calculated for each parameter.

Studied parameters	Female mice			Male mice		
	Saline treated	<i>Bauhinia variegata</i> treatment	P-value	Saline treated	<i>Bauhinia variegata</i> treatment	P-value
Distance (m)	18.67 ± 9.25	21.37 ± 6.51	0.54	22.66 ± 7.28	22.42 ± 7.22	0.06
Mean Speed (m / s)	0.03 ± 0.01	0.04 ± 0.01	0.56	0.04 ± 0.01	0.04 ± 0.01	0.96
Time mobile (sec)	464 ± 164	519.9 ± 44.8	0.42	489 ± 109	493 ± 101	0.9
Time immobile (sec)	136 ± 164	80.1 ± 44.0	0.42	111 ± 109	107 ± 101	0.9
Mobile episodes	20.7 ± 12.6	21.57 ± 9.93	0.9	16.43 ± 9.24	22.4 ± 13.2	0.3
Immobile episodes	20.1 ± 12.6	21.1 ± 10.1	0.86	15.9 ± 9.5	21.6 ± 13.1	0.3
Rotations	22.6 ± 14.8	24.3 ± 12.5	0.81	28.6 ± 12.4	29.4 ± 10.1	0.9
Clockwise rotations	11.71 ± 8.42	11.86 ± 7.20	0.97	12.0 ± 5.1	11.7 ± 4.11	0.9
Anticlockwise rotation	10.86 ± 7.38	12.43 ± 7.00	0.69	16.6 ± 10.4	17.7 ± 8.2	0.8

P > 0.05 = Non significant

Table 2 Comparison of various studied parameters of light and dark box test between *Bauhinia variegata* leaf extract (150 mg / ml solvent / Kg body weight) and saline treated adult albino mice. N = 7 for each treatment. P- value represents the results for two sample t – test calculated for each parameter.

Studied parameters	Female mice			Male mice		
	Saline treated	<i>Bauhinia variegata</i> treatment	P-value	Saline treated	<i>Bauhinia variegata</i> treatment	P-value
Transition frequency	19.43 ± 7.93	20.43 ± 7.81	0.82	8.9 ± 10.2	19.9 ± 3.67	0.03*
Rearing frequency	1.000 ± 0.82	3.29 ± 2.21	0.037*	3.57 ± 2.07	3.29 ± 2.69	0.83
Stretch attend frequency	4.86 ± 4.85	4.86 ± 3.24	1.0	2.43 ± 1.90	8.00 ± 5.45	0.04*
Time in dark (sec)	149.6 ± 54.5	183.7 ± 68.8	0.33	170 ± 107	152 ± 40.4	0.68
Time in light (sec)	150.4 ± 54.5	116.3 ± 68.8	0.33	130 ± 107	148 ± 40.4	0.68
Urination	0 ± 0	0 ± 0	0	0.14 ± 0.38	0.14 ± 0.38	0.08
Defecation	1.14 ± 1.35	1.924 ± 0.79	0.49	1.00 ± 0.58	0.43 ± 0.53	0.08

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

Table 3 Comparison of various studied parameters of elevated plus maze between *Bauhinia variegata* leaf extract (150 mg / ml solvent / Kg body weight) and saline treated adult albino mice. N = 7 for each treatment. P - value represents the results of two sample t-test calculated for each parameter.

Studied parameters	Female mice			Male mice		
	Saline treated	<i>Bauhinia variegata</i> treatment	P-value	Saline treated	<i>Bauhinia variegata</i> treatment	P-value
Distance (m)	9.76 ± 5.67	21.2 ± 18.5	0.16	6.6 ± 3.3	13.77 ± 6.57	0.03*
Mean Speed (m/s)	0.032 ± 0.02	0.07 ± 0.06	0.16	0.02 ± 0.01	0.04 ± 0.02	0.03*
Time mobile (sec)	180.8 ± 97.9	239.1 ± 87.9	0.27	140.9 ± 70.1	237.2 ± 44.8	0.01**
Time immobile (sec)	119.2 ± 97.7	60.9 ± 87.9	0.27	159.1 ± 70.1	62.8 ± 44.8	0.01**
Mobile episodes	13.17 ± 4.75	6.57 ± 3.74	0.009**	18.29 ± 6.9	12.57 ± 8.38	0.19
Immobile episodes	12.71 ± 4.75	6.0 ± 3.92	0.02*	17.86 ± 6.54	12.14 ± 8.21	0.18
Rotations	5.86 ± 3.85	3.29 ± 3.82	0.24	6.57 ± 1.72	3.29 ± 3.82	0.07
Clockwise rotations	2.86 ± 1.95	1.86 ± 1.77	0.34	3.71 ± 1.60	1.86 ± 1.77	0.06
Anticlockwise rotation	3.0 ± 2.77	1.43 ± 2.15	0.26	2.86 ± 1.77	1.43 ± 2.15	0.2
Urination	0.29 ± 0.49	0.14 ± 0.38	0.55	1.14 ± 1.46	0.14 ± 0.37	0.13
Defecation	1.43 ± 0.53	1.29 ± 0.95	0.74	2.71 ± 1.6	0.71 ± 1.11	0.02*
Head dipping	7.29 ± 7.78	9.14 ± 4.88	0.6	6.29 ± 7.65	11.86 ± 6.82	0.18

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

Table 4 Comparison of various studied parameters during first trial of novel object test between *Bauhinia variegata* leaf extract (150 mg / ml solvent / Kg body weight) and saline treated adult albino mice. N = 7 for each treatment. P- value represents the results for two sample t – test calculated for each parameter.

Parameters	Female mice			Male mice		
	Saline treated	<i>Bauhinia variegata</i> treatment	P-value	Saline treated	<i>Bauhinia variegata</i> treatment	P-value
Line cross	18.6 ± 14.9	23.14 ± 9.34	0.51	17.14 ± 7.99	19.29 ± 7.48	0.41
Stretch attend reflex	0.14 ± 0.38	0.29 ± 0.76	0.67	1.29 ± 1.38	3.14 ± 4.18	0.30
Approaches object A	10.43 ± 9.3	13.86 ± 4.49	0.41	6.71 ± 4.46	8.14 ± 3.13	0.50
Approaches object B	8.86 ± 7.47	11.57 ± 6.21	0.48	9.57 ± 6.53	9.57 ± 3.82	1.0
Time object A (sec)	27.9 ± 28.2	49.3 ± 26.0	0.17	19.6 ± 10.6	45.9 ± 13.2	0.002*
Time object B (sec)	36.4 ± 29.0	52.7 ± 25.1	0.23	50.4 ± 41.9	35.4 ± 16.1	0.41

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

Table 5 Comparison of various studied parameters during second trial of novel object test between *Bauhinia variegata* leaf extract (150 mg / ml solvent / Kg body weight) and saline treated adult albino mice. N = 7 for each treatment. P- value represents the results for two sample t – test calculated for each parameter.

Studied parameters	Female mice			Male mice		
	Saline treated	<i>Bauhinia variegata</i> treatment	P-value	Saline treated	<i>Bauhinia variegata</i> treatment	P-value
Line cross	12.86 ± 8.51	10.9 ± 10.4	0.70	4.14 ± 4.38	8.86 ± 4.14	0.063
Stretch attend reflex	1.57 ± 2.15	0.43 ± 0.79	0.23	0.27 ± 0.49	3.43 ± 5.59	0.19
Approaches object A	5.29 ± 5.56	2.86 ± 4.26	0.38	1.00 ± 1.41	5.00 ± 3.42	0.02 *
Approaches Novel object	5.57 ± 6.43	4.86 ± 5.64	0.83	2.29 ± 3.50	6.71 ± 7.85	0.21
Time Old object (sec)	16.7 ± 19.5	45.0 ± 67.0	0.32	6.00 ± 8.49	28.0 ± 24.1	0.06
Time Novel object (sec)	15.1 ± 13.0	41.1 ± 42.3	0.16	2.29 ± 3.50	6.71 ± 7.85	0.21

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

Morris Water Maze Test

Results of probe trial revealed that mean speed of female albino mice treated with 150 mg/ml solvent/Kg body weight was significantly (P = 0.013) higher than their control group. While all other studied parameters varied non significantly (P > 0.05) when compared between 150 mg/ml solvent/Kg body weight treated albino mice of both genders.

4. DISCUSSION

Plants are the most precious resource for an extensive range of derivative metabolites utilized as medicine, agrochemicals, biopesticides, food additives and flavours (Al-Snafi 2013). *Bauhinia variegata* Linn is conventionally used for the treatment of tumors, bronchitis and leprosy and it is an anti hepatotoxic agent (Marasani et al. 2013, Raj Kapoor et al. 2003). The present study was conducted to investigate the effect of *B. variegata* leaf extract (150 mg/ml solvent/Kg of body weight) on selected aspects of behavior of adult female and male albino mice as little information is available on this topic.

Our rota rod test results indicated that the application of 150 mg/Kg dose improved the balance and neuromuscular coordination in female albino mice. Our results are in agreement with Jatav et al. (2014) who had reported that mice treated with flavonoid-rich fraction of *Bauhinia variegata* (200 mg/Kg) stayed on rotating rod for significantly more time as compared to those treated with Diazepam (1 mg/Kg, ip). Our results indicated that *B. variegata*'s leaf extract treatment did not affect the neuromuscular coordination in adult male albino mice (Fig. 1). Our results are in agreement with those of Sathya et al. (2011) 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.

Our open field results demonstrated that 150 mg *B. variegata*'s leaf extract / ml solvent/Kg body weight supplementation did not affected the locomotory and exploratory behavior of albino mice (Table 1). Santos et al. (2012) had also reported that the exposure to ethanolic leaf extract of *Bauhinia platyptala* did not

induce any change in Swiss adult mice behavior during the open field test.

Results of our light dark test revealed that male albino mice treated with *B. variegata*'s leaf extract spent more time in light box than their control group. These observations are in line with those of Davey *et al.* (2011) who had reported that oral supplementation with methanolic extract of *B. racemosa* stem bark (150 and 300 mg/Kg) increased the time spent in illuminated areas by Swiss albino mice.

It was observed during present study that male mice treated with 150 mg of *Bauhinia variegata* leaf extract performed better during elevated plus maze test (Table 3). Shah and Goyal (2011) had also reported that the animals treated with flavonoid-rich fraction of *Bauhinia variegata* (400 mg/Kg) showed a significant decrease in transfer latency as compared to the control group, which indicates cognitive enhancement effect of flavonoid-rich fraction of *Bauhinia variegata*. Similarly Jatav *et al.* (2014) had also reported that the rats treated with flavonoid rich fraction of *Bauhinia variegata* showed nootropic effect in terms of significant increase in the time spent in enclosed arm than open arm after their training sessions. Pritipadma *et al.* (2015) reported that the mice administered with two different doses (150 and 300 mg/Kg) of methanolic extract of *Bauhinia racemosa* were able to increase the time spent and the number of entries in the open arms of the elevated plus-maze. *Bauhinia variegata* and *Bauhinia racemosa* supplementation has shown improved short term memory in mice indicating that leaf extract composition in two species is pretty much similar.

In conclusion, we are reporting that 150 mg *B. variegata*'s leaf extract/ ml solvent/Kg body weight supplementation has improved the selective aspects of albino mice in a gender specific manner. The applied dose has improved the rota rod test performance in female while improved light dark test and elevated plus maze test performance was observed in male albino mice. We recommend that *B. variegata*'s leaf extract should be investigated for the treatment of neurological ailments.

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