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Oral Supplementation of *Bauhinia variegata* affects the Behavior of Albino Mice in a Gender Specific Manner

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FI designed and supervised the study and revised the manuscript. AU and SI performed the lab experiments, analyzed the data and prepared the manuscript.

Key words
***Bauhinia variegata*, Rota rod, Open field, Light-dark box, Elevated plus maze, Novel object, Morris water maze test, Albino mice**

ABSTRACT

Present study was designed to investigate the effects of 300 mg/ml solvent/Kg of body weight of *Bauhinia variegata's* leaf extract on selective aspects of albino mice behavior in a gender specific manner. Seven week old female and male albino mice were used as experimental animals. Mice were orally supplemented either with 300 mg/ml solvent/Kg of body weight *Bauhinia variegata's* leaf extract or with saline solution (0.9% NaCl) for 17 days. Behavioral observations were made by applying a series of neurological tests (Rota rod, Elevated plus maze, Light and dark box, Open field, Novel object and Morris water maze test). It was observed that *Bauhinia variegata's* leaf extract supplementation improved neuromuscular co-ordination in female albino mice during rota rod test ($P = 0.002$), had more rotations ($P = 0.02$) and clockwise rotations ($P = 0.01$) during plus maze test and had more stretch attend reflex ($P = 0.005$) than control group. During the second trial of novel object test, *Bauhinia variegata's* leaf extract treated male albino mice approached old object A ($P = 0.04$) and spend more time with object A ($P = 0.05$) as compared to control group. Open filed, light dark test and probe trial of Morris water maze test performances remain unaffected in both genders ($P > 0.05$) when compared between 300 mg/ml solvent/Kg body weight of *Bauhinia variegata's* leaf extract treated and untreated albino mice of both genders. We concluded that applied dose of *Bauhinia variegata's* leaf extract has the potential to improve neuromuscular co-ordination in female albino and should be explored further as potential treatment for neuromuscular problems.

INTRODUCTION

Memories are ability of an individual to record event, information and retain them over short or long period of time, recall the same whenever needed (Joshi and Parle, 2006) Nootropics agents are known to augment the cognitive dexterity (Whitehouse, 2015). Various drugs are known to have detrimental effect on learning and memory. Nootropic agents are used primarily for improving memory, mood, and behavior. However, the resulting side-effects associated with these agents have limited their use (Whitehouse, 2015). Thus, it is necessary to explore the utility of traditional medicines in the treatment of various cognitive disorders. 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). *Bauhinia variegata* Linn (leguminosae) is known as Kanchanar in Hindi. It is a medium-sized tree abundant in Sub-Himalayan tract extending eastwards to Assam, Eastern, Central and South India. Various parts of the plants *viz*., leaves, flower buds, flower, stem, stem bark, seeds, and roots are known for therapeutic activity and are used as tonic, astringent for fever, diarrhea, dysentery, hemorrhoids, piles, edema, laxative, anthelmintic, antileprotic, in skin diseases, for wound healing, antigoitrogenic, antitumor, in obesity, stomatitis, antidote for snake poisoning, in dyspepsia, flatulence and as carminative (Mali *et al*., 2007).

Recent findings on *Bauhinia variegata* Linn have demonstrated its antioxidant, anti-hyperlipidemic (Rajani and Ashok, 2009) and hepatoprotective potential (Bodakhe and Ram, 2007). The plant is also known to inhibit enzyme acetylcholinesterase (Santos *et al*., 2012). Extract of *Bauhinia variegata* stem bark has shown protective action against milk-induced eosinophilia in mice (Mali and

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Dhake, 2011).

The present work is focused to evaluate effect of 300 mg/ml solvent/Kg of body weight of *Bauhinia variegata's* leaf extract on neuromuscular coordination, exploratory and locomotory behavior in adult albino mice.

MATERIALS AND METHODS

Subjects

Seven weeks old albino mice were used as experimental animals in order to demonstrate the effect of *B. variegate* leaf extract on selected behavioral aspects of albino mice in a gender specific manner. Animals were reared at the animal facility of Bio Park at Bahauddin Zakariya University Multan, Pakistan following the colony maintenance conditions as described by Aftab *et al*. (2018). Animals were kept in locally manufactured small rodent cages filled with wood chips. Standard mouse diet and water were available *ad labium*. 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 about 200 Watt from 8am to 6pm.

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 an herbal grinder and sieved to have particle size of 50-300 mm. The powder was stored in an air tight polythene bag at room temperature before extraction. 300 gm of *B. variegata* leaves powder was used to prepare the leaf extract following the Zahra *et al*. (2015). 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* (300 mg) was dissolved in 1ml of distilled water to prepare the working solution. Albino mice were weighed and orally administered either with 300 mg *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 10 consecutive days and a series of neurological tests including Rota rod, light dark box, open field, elevated plus maze, novel object and Morris water maze test were conducted on next 7 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 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. Test was conducted following Sunyer *et al*. (2007).

Light and 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 (Bourin and Hascoet, 2003). The test was conducted as we have previously reported by Zahra *et al*. (2015).

Open field test

Open field test is used to assess locomotory and exploratory behaviour of an animal (Prut and Belzung, 2003). The test was performed following Weitzdoerfer *et al*. (2015).

Elevated plus maze

Elevated plus maze test is used to assess the anxiety related behaviors in rodents (Walf and Frye, 2015). The test was conducted following Zahra *et al*. (2015).

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 (Broadbent *et al*., 2009; Ennanceur and Delacour, 1988). The test was performed following Zhanga *et al*. (2012).

Morris water maze test

The Morris Water Maze has been used extensively to study strain differences in spatial learning in mice (Upchurch and Wehner, 1988). The test was conducted following Ullah *et al*. (2017).

Statistical analysis

All the data was expressed as mean ± standard deviation (SD) statistical package Minitab (Version 17, USA) was used for the analysis of results. Probability (P) values less than 0.05 were considered to be significantly

different. Two 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.

RESULTS

Rota rod test

Statistical analysis of the Rota rod test revealed that female albino mice treated with 300 mg/ml solvent/Kg body weight of *Bauhinia variegata* leaf extract spent significantly more time on rotating rod as compared to their saline treated control group (P = 0.002) (Fig. 1). On the other hand, rota rod reflex did not varied significantly when compared between male mice treated with 300 mg/ml solvent/Kg body weight (P = 0.77).

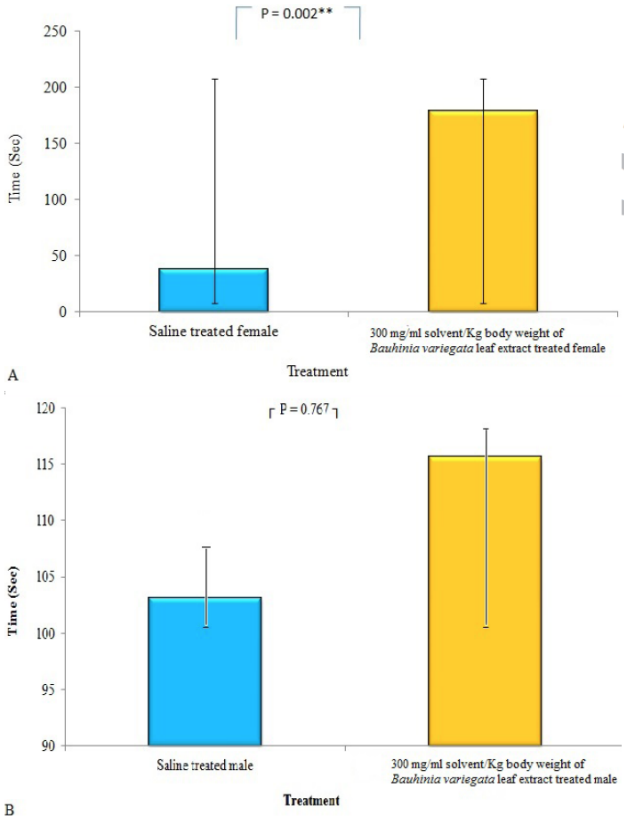

Fig. 1. Comparison of rota rod test performance between (A) female and (B) male albino mice treated with 300 mg/ml solvent/Kg body weight of *B. variegata* leaf extract treated and their respective untreated control group. N: 7 for each treatment. P - value represents the results of 2 sample t – test.

Elevated plus maze test

Analysis of the elevated plus maze data for female albino mice revealed that Female mice supplemented with dose (300 mg/ml solvent/Kg body weight) had more rotations (P = 0.02) and clockwise rotations (P = 0.01) than their saline treated control group (Table I). On the other hand, all the studied parameters varied non significantly (P > 0.05) when compared between saline treated and male mice supplemented for 300 mg/ml solvent/Kg body weight of *Bauhinia variegata* leaf extract (Table I).

Light dark box test

Analysis of the results revealed that all the studied parameters of light and dark test varied non-significantly (P > 0.05) for both genders when compared between plant extract treated and control group (Table II).

Open field test

Analysis of the data revealed that the applied dose of *Bauhinia variegata* leaf extract (300 mg/ml solvent/Kg body weight) had no influence on studied parameters of open field as all open field parameters varied non significantly (P > 0.05) when compared between leaf extract treated and untreated albino mice of both genders; female and male, indicating that *Bauhinia variegata* is not potent to influence the exploratory and locomotory behavior of albino mice (Table III).

Novel object test

Analysis of the data revealed that during novel object test (trial-1) female albino mice treated with 300 mg/ml solvent/Kg body weight had more stretch attend reflex (P = 0.005) as compared to their control group (Table IV). All other studied parameters varied non-significantly (P > 0.05) when compared between plant extract treated and untreated animal of both genders (Table IV).

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 (Table IV). Analysis of the data indicated that during the second trial of novel object test male albino mice treated with 300 mg/ml solvent/Kg body weight of *Bauhinia variegata* leaf extract approached old object A (P = 0.04) and spend more time with object A (P = 0.05) as compared to control group. All other studied parameters varied non-significantly (P > 0.05) when compared between *Bauhinia variegata* leaf extract treated and untreated female albino mice (Table IV).

Table I. Comparison of various studied parameters of elevated plus maze between *Bauhinia variegata* leaf extract (300 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	*Bauhinia variegata* treatment	P-value	Saline treated	*Bauhinia variegata* treatment	P-value
Distance (m)	9.76 ± 5.67	13.15 ± 3.3	0.2	6.6 ± 3.3	9.56 ± 5.79	0.27
Mean Speed (m/s)	0.032 ± 0.02	0.43 ± 0.01	0.2	0.02 ± 0.01	0.03 ± 0.01	0.3
Time mobile (sec)	180.8 ± 97.9	224.3 ± 47.2	0.3	140.9 ± 70.1	184.3 ± 70.1	0.3
Time immobile (sec)	119.2 ± 97.7	75.7 ± 47.2	0.3	159.1 ± 70.1	155.7 ± 70.1	0.3
Mobile episodes	13.17 ± 4.75	15.57 ± 4.65	0.5	18.29 ± 6.9	17.71 ± 8.26	0.9
Immobile episodes	12.71 ± 4.75	15.29 ± 4.64	0.3	17.86 ± 6.54	12.14 ± 8.21	0.2
Rotations	5.86 ± 3.85	10.57 ± 1.99	0.02*	6.57 ± 1.72	5.86 ± 3.18	0.06
Clockwise rotations	2.86 ± 1.95	5.86 ± 1.68	0.01*	3.71 ± 1.60	3.29 ± 1.70	0.6
Anticlockwise rotation	3.0 ± 2.77	4.71 ± 2.21	0.2	2.86 ± 1.77	2.57 ± 2.07	0.8
Urination	0.29 ± 0.49	0.14 ± 0.38	0.6	1.14 ± 1.46	0.14 ± 0.37	0.1
Defecation	1.43 ± 0.53	1.57 ± 1.81	0.9	2.71 ± 1.6	0.71 ± 1.11	0.02*
Head dipping	7.29 ± 7.78	16.3 ± 10.5	0.09	6.29 ± 7.65	11.86 ± 6.82	0.2

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

Table II. Comparison of various studied parameters of light and dark box test between *Bauhinia variegata* leaf extract (300 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	*Bauhinia variegata* treatment	P-value	Saline treated	*Bauhinia variegata* treatment	P-value
Transition frequency	19.43 ± 7.93	19.00 ± 9.40	0.9	8.9 ± 10.2	12.6 ± 8.50	0.5
Rearing frequency	1.000 ± 0.82	4.57 ± 4.47	0.08	3.57 ± 2.07	2.57 ± 1.51	0.3
Stretch attend frequency	4.86 ± 4.85	4.14 ± 2.85	0.7	2.43 ± 1.90	3.86 ± 4.30	0.4
Time in dark (sec)	149.6 ± 54.5	130.4 ± 51.8	0.5	170 ± 107	178.7 ± 52	0.9
Time in light (sec)	150.4 ± 54.5	169.6 ± 51.8	0.5	130 ± 107	121.3 ± 52	0.3
Urination	0 ± 0	0 ± 0	0	0.143 ± 0.4	0.143 ± 0.4	1
Defection	19.43 ± 7.93	19.00 ± 9.40	0.9	8.9 ± 10.2	12.6 ± 8.50	0.5

Morris water maze test

Acquisition phase

Analysis of results revealed that male mice treated with 300 mg/ml solvent/Kg body weight of *Bauhinia variegata* leaf extract reached the platform significantly earlier on training day 3(P = 0.01) than their saline treated control group (Data not shown here). Analysis of results revealed that average swimming speed of *Bauhinia variegata* leaf extract treated female albino mice was significantly less on training day 3 (P = 0,02) and 4 (P = 0.04) when compared with their control group (Data not shown here). The time mobile for male albino mice treated with 300 mg/ml solvent/Kg body weight of *Bauhinia variegata* leaf extract was significantly higher (P = 0.01) on training day 3 when compared with the control group. Analysis of results revealed that time immobile for *Bauhinia variegata* leaf extract treated female albino mice was significantly less on training day 2 (P = 0.009) and 3 (P = 0.02) as compared to their control group. All other studied parameters varied non significantly (P > 0.05 when compared between *Bauhinia variegata* leaf extract treated and untreated mice of both genders.

Analysis of the results revealed that all studied parameters for probe trial varied non significantly (P > 0.05)

Table III. Comparison of various studied parameters of open field test between *Bauhinia variegata* leaf extract (300 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	*Bauhinia variegata* treatment	P-value	Saline treated	*Bauhinia variegata* treatment	P-value
Distance (m)	22.6 ± 7.3	17.98 ± 6.27	0.2	18.67 ± 9.25	17.54 ± 5.40	0.8
Time immobile (sec)	111 ± 109	160 ± 188	0.6	136 ± 164	109.6 ± 89.9	0.7
Mobile episodes	16.43 ± 9.24	14.71 ± 8.7	0.7	20.7 ±12.6	21.14 ± 7.86	0.9
Immobile episodes	15.86 ± 9.51	14.1 ± 8.76	0.7	20.1 ± 12.6	20.29 ± 8.07	1
Rotations	28.6 ± 12.4	21.4 ± 13.6	0.3	20.6 ± 14.8	20.7 ± 11.7	0.8
Clockwise rotations	12.00 ± 5.1	11.71 ± 8.77	0.9	11.71 ± 8.42	11.57 ± 7.72	1
Anticlockwise rotation	16.6 ± 10.4	9.71 ± 6.1	0.2	10.86 ± 7.38	9.14 ± 6.57	0.7

Table IV. Comparison of various studied parameters during first and second trial of novel object test between *Bauhinia variegata* leaf extract (300 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	*Bauhinia variegata* treatment	P-value	Saline treated	*Bauhinia variegata* treatment	P-value
First trial						
Line cross	18.6 ± 14.9	30.29 ± 8.69	0.1	17.14 ± 7.99	12.43 ± 6.19	0.2
Stretch attend reflex	0.14 ± 0.38	1.43 ± 0.787	0.005**	1.29 ± 1.38	1.14 ± 1.35	0.9
Approaches object A	10.43 ± 9.3	14.29 ± 4.54	0.4	6.71 ± 4.46	6.71 ± 4.35	1
Approaches object B	8.86 ± 7.47	13.71 ± 6.34	0.2	9.57 ± 6.53	7.71 ± 4.50	0.6
Time object A (sec)	27.9 ± 28.2	54.1 ± 12.9	0.6	19.6 ± 10.6	34.0 ± 25.6	0.2
Time object B (sec)	36.4 ± 29.0	51.6 ± 27.3	0.3	50.4 ± 41.9	36.9± 24.8	0.5
Second trial						
Line cross	12.86 ± 8.51	10.71 ± 4.11	0.6	4.14 ± 4.38	7.00 ± 4.69	0.3
Stretch attend reflex	1.57 ± 2.15	2.29 ± 1.50	0.4	0.29 ±0.49	1.29 ± 1.80	0.2
Approaches object A	5.29 ± 5.56	6.29± 3.04	0.4	1.00 ± 1.41	3.29 ± 2.21	0.04 *
Approaches Novel object	5.57 ± 6.43	6.43 ± 3.21	0.7	2.29 ± 3.50	4.00 ± 3.32	0.4
Time Old object (sec)	16.7 ± 19.5	61.1 ± 52.9	0.08	6.00 ± 8.49	42.6 ± 39.1	0.05 *
Time Novel object (sec)	15.1 ± 13.0	63.1 ± 78.8	0.2	2.29 ± 3.50	60.7 ± 80.7	0.2

P < 0.05 = Least significant (*).

when compared between *Bauhinia variegata* leaf extract treated and untreated mice of both genders.

DISCUSSION

Medicinal plants, as potential source of therapeutic aids, have attained significance in health system, for both humans and animals, all over the world not only in diseased condition but also for maintaining proper health (Verma and Singh, 2008). 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).The genus *Bauhinia* belongs to the family Caesalpiniaceae (formally Leguminosae) and several members of this genus like *Bauhinia manca*, *Bauhinia divaricata*, *Bauhinia purpurea* and *Bauhinia variegata* are known for their medicinal importance (Vasconcelos *et al*., 2000; Khan *et al*., 2012).

Bauhinia variegata Linn is conventionally used for the treatment of tumors, bronchitis and leprosy and it is an anti hepatotoxic agent (Rajkapoor *et al*., 2003; Kumar *et al*., 2011; Marasani *et al*., 2013; Rajani and Ashok, 2009). This study was conducted to investigate the effect of *Bauhinia variegata* leaf extract (300mg/ ml solvent/ Kg body weight) on behavior of adult albino mice of both genders. As limited information is available in literature regarding the effect of *Bauhinia variegata* leaf extract on the behavior of albino mice, present study was designed to demonstrate the effect of two different doses of ethanolic leaf extract of *Bauhinia variegata* on behavior of albino mice in a gender specific manner.

The rota rod test is designed to assess motor coordination, balance and equilibrium and is used to evaluate the pharmacological action of pscychotropic agents on the central and peripheral nervous system (Sunyer *et al*., 2007; Shiotsuki *et al*., 2010; Iqbal *et al*., 2015). Impairment of rota rod performance has been thought to reflect, at least in a part, a behaviorally depressive state. However, it is well known that riding time on rota rod is also decreased by relaxation or weakness of muscle or motor dysfunction (Amos *et al*., 2001). Analysis of our results indicated that applied dose of *Bauhinia variegata* (300 mg/ml solvent/ Kg body weight) had significantly improved the muscular activity in female albino mice as compared to their saline treated controls (Fig. 1). Interesting, the same doses did not affected the time spent by male albino mice on rotating rod indicating a gender specific effect of ethanolic leaf extract of *Bauhinia variegata* on the muscular strength and neuromuscular coordination in adult albino mice. Our results are in agreement with those reported by Jatav *et al*. (2014) as they had demonstrated that flavonoid-rich fraction of *Bauhinia variegata* (200 mg/Kg) significantly decreased the fall of time as compared to Diazepam (1 mg/ Kg, ip) indicating improved muscular activity in Wistar albino rats.

Elevated plus maze is one of the important methods that help to assess state of anxiety in animals (Espejo, 1997). It is also used to determine status of learning and short term memory (Kulkarni and Dhir, 2007). Analysis of our data revealed interesting results. It was observed that ethanolic leaf extract treatment at the dose of 300 mg/ ml solvent /Kg body weight significantly improved the short memory of female albino mice as evident from their elevated plus maze test (Table I).

Interestingly, the same dose did not affect the short term memory of male albino mice indicating a gender specific response of *Bauhinia variegata* leaf extract on elevated plus maze test results of adult albino mice (Table I). The presence of antioxidants like terpenoids, flavonols (Kaempferol, Quercetin), coumarins (Scopoletin and Scopolin), steroids and catchol in *Bauhinia variegata* has probably improved the memory and learning in albino mice.

Our results are in agreement with those of Shah and Goyal (2011) as they have also reported that the animals treated with flavonoid-rich fraction of *Bauhinia variegata* (400 mg/Kg) showed a significant decrease in transfer latency of rats as compared to the control group, indicating cognitive enhancement effect (Jatav *et al*., 2014) has 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. This was found comparable to the standard drug and very significant as compared to the control group.

Pritipadma *et al*. (2015) has recently reported that the methanolic extract of *Bauhinia racemosa* administered orally in mice at two different doses of 150 mg/Kg and 300 mg/Kg were able to increase the time spent and the number of arm entries in the open arms of the elevated plus-maze indicating improved short term memory. Santos *et al*. (2012) has reported that 50 mg/Kg ethanolic extract of *Bauhinia platypetala* has anxiolytic effect and increase the exploration in male Swiss mice where the dose was administered intraperitoneally supporting our findings. 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 neuro inflammation, supportive in efficient learning, increase memory and cognitive role (Rao *et al*., 2008). These flavonoids act together with lipid and protein kinase signaling pathways in the brain and promote neuronal survival and have useful effect on cerebro-vascular blood flow (Vauzour *et al*., 2008). So the improvements in neuofunctioning in adult albino mice can be attributed with the presence of antioxidants like terpenoids, flavonols (Kaempferol, Quercetin), coumarins (Scopoletin and Scopolin), steroids and catchol in *Bauhinia variegata* assumed to improve the memory and learning in mice (Bouwknecht and Paylor, 2002).

The light/dark transition test is one of the most widely used tests to measure anxiety-like behavior in mice. This test is also used to test the exploratory behavior in rodents (Bourin and Hascoet, 2003). Our light and dark box test results again indicated gender specific response as only 300 mg/ml solvent/Kg body weight of *Bauhinia variegata* leaf extract was the dose that significantly improved the parameters associated with exploratory behavior in male albino mice (Table II). The same dose did not affect the exploratory behavior in female albino mice.

Similar results were documented by Shah and Goyal

(2011) as they have reported that exposure to methanolic extract of *Bauhinia racemosa* resulted in increased time spent by mice in the illuminated side of the light–dark test along with significant increase in nose poking (Rearing) in comparison with control animals. Davey *et al*. (2011) had also reported that indicate that methanolic extract of *Bauhinia racemosa*is an effective anxiolytic agent in mice. These results indicate the *Bauhinia racemosa* and *Bauhinia variegata* has similar chemical composition as they have selectively positive impact on rodent behavior.

Open field test is one of the most widely used test in animal psychology experiments. It is used to test the emotionality of rodents, is used to measure general locomotor activity qualitatively and quantitatively and to measure willingness to explore in rodents (Stanford, 2007). Analysis of our results indicated that the applied dose remains unaffected for both the genders indicating the exploratory and locomotory behavior of male and female albino mice is not influenced by *Bauhinia variegata* leaf extract application for 17 days (Table III). Our results are in agreement with those of Santos *et al*. (2012) as they have reported that the exposure to ethanolic leaf extract of *Bauhinia platypetala* did not induce any change in Swiss adult mice behavior during the open field test. Cavalcanti *et al*. (2011) had also reported that the aquous extract (5.0g/Kg) of *Bauhinia forficate* supplementation resulted in decrease in locomotion frequency and increase immobility during open field test. Sathya *et al*. (2011) 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. These results indicate the *Bauhinia platypetala, Bauhinia forficate, Bauhinia tomentosa* and *Bauhinia variegata* leaf extract composition has variation as they are elective different response in rodents during neurological testing.

The Novel object recognition test has been introduced by Ennanceur and Delacour (1988) and can be regarded as a spontaneous Delayed-Non-Matching-to-Sample test. The test is based on a spontaneous behavior: the main assumption at the base of this test is that access to novelty (e.g. an object or an environment) can elicit approach behaviors in animals (Gaskin *et al*., 2010). This apparent 'unconditioned preferencefor novelty has been used in the NOR test in order to study memory functions, assessing the ability of animals to recognize a novel object in a familiar environment, because they maintain a representation of those is more familiar stored in memory (Aggleton *et al*., 2010). Analysis of our results indicates that the applied dose (300 mg/ml solvent/Kg body weight) of *Bauhinia variegata* did not affect the object recognition ability of both male and female albino mice (Table IV). Our results are contradictory to De-Laine *et al*. (2011) as they had reported that estrous rats spent a significantly greater percentage of time with the novel object than did diestrous rats. The difference in results is probably due to two reasons. First is the De-Laine *et al*. (2011) used rats as experimental subject while albino mice were used in our study. Two different species may show different behavioral response. Secondly, we did not account the estrous or non-estrous phase of animals during our study. De-Laine *et al*. (2011) had also reported that adolescent rodents have been characterized as showing higher novelty-seeking and risk-taking behavior than adults, potentially due to selection pressures that have favored a willingness to engage with novelty around the time of dispersal.

In conclusion, we have conducted a study to demonstrate the effects of 300 mg/ml solvent/Kg body weight of ethanolic leaf extract of *Bauhinia variegata* on behavior of male and female albino mice. It was observed that applied dose significantly improved the rota rod test performance in females and can be used for them to improve muscular functioning. Female albino mice displayed improved elevated plus maze and light-dark box performance when supplemented with 300 mg/ml solvent/Kg body weight of *Bauhinia variegata* ethanolic leaf extract for 17 days. Open field, Novel object test and Morris water maze results remains unaffected in both the genders following application of 300 mg/ml solvent/Kg body weight of *Bauhinia variegata* leaf extract.

Statement of conflict of interest

Authors declare that they do not have conflict of interest of any sort with any one.

REFERENCES

Aftab, M.N., Ali, A., Asad, M., Fatima, S. and Iqbal, F., 2018. Effect of AlCl3 mediated toxicity on the hemato-biochemical profile of adult male albino mice. *Pakistan J. Zool.,* **50**: 1199-1600. http://dx.doi.org/10.17582/journal.pjz/2018.50.4.sc5

Aggleton, J.P., Albasser, M.M., Aggleton, D.J., Poirier, G.L. and Pearce, J.M., 2010. Lesions of the rat perirhinal cortex spare the acquisition of a complex configural visual discrimination yet impair object recognition. *Behav. Neurosci*., **124**: 55-68. https://doi.org/10.1037/a0018320

Al-Snafi, A. E., 2013. The pharmacological importance of *Bauhinia variegata*: A review. *Int. J. Pharma. Sci. Res*., **4**: 160-164.

Amos, S., Adzu, B., Binda, L., Wambebe, C. and Gamaniel, K., 2001. Neuropharmacological effect of the aqueous extract of *Sphaeranthus senegalensis* in mice. *J. Ethnopharmacol*., **78**: 33-37. https://doi.

org/10.1016/S0378-8741(01)00316-6

Bodakhe, S.H., Ram, A., 2007. Hepatoprotective properties of Bauhinia variegata bark extract. *Yakugaku Zasshi*, **127**: 3003-3007. https://doi.org/10.1248/yakushi.127.1503

Bourin, M. and Hascoet, M., 2003. The mouse light/dark box test. *Eur. J. Pharmacol.,* **463**: 55-65. https://doi.org/10.1016/S0014-2999(03)01274-3

Bouwknecht, J.A. and Paylor, R., 2002. Behavioral and physiological mouse assays for anxiety: a survey in nine mouse strains. *Behav. Brain Res*., **136**: 489-501. https://doi.org/10.1016/S0166-4328(02)00200-0

Broadbent, N.J., Gaskin, S., Squire, L.R. and Clark, R.E., 2009. Object recognition memory and the rodent hippocampus. *Learn. Mem*., **17**: 5-11. https://doi.org/10.1101/lm.1650110

Cavalcanti, E.M., Bonafé, C., Silva, M.G. and Gerenutti, M., 2011. Properties of *Bauhinia forficata* LIK in rats: Behavioral evaluations. *Pharmacol*. *Online,* **2**: 205-211.

Davey, M.S., Atlee, W.C., Bharathi, A. and Farook, M., 2011. Antianxiety effect of methanolic extract of *Bauhinia racemosa* (lamk) stem bark in mice. *Int. J. Pharma. biol. Sci*., **2**: 217-224.

De-Laine, M., Cyrenne and Gillian, R., 2011. Effects of suppressing gonadal hormones on response to novel objects in adolescent rats. *Brown Horm. Behav*., **60**: 625–631. https://doi.org/10.1016/j.yhbeh.2011.08.015

Ennanceur, A. and Delacour, J.A., 1988. New one-trial test for neurobiological studies of memory in rats. Behavioral data. *Behav. Brain Res*., **31**: 47-59. https://doi.org/10.1016/0166-4328(88)90157-X

Espejo, E.F., 1997. Effects of weekly or daily exposure to the elevated plus-maze in male mice. *Behav. Brain Res.,* **87**: 233 – 238. https://doi.org/10.1016/S0166-4328(97)02286-9

Gaskin, S., Tardif, M., Cole, E., Piterkin, P., Kayello, L., Mumby, D.G., 2010. Object familiarization and novel-object preference in rats. *Behav. Proc*., **83**: 61–71. https://doi.org/10.1016/j.beproc.2009.10.003

Iqbal, S., Ali, M. and Iqbal, F., 2015. Long term creatine monohydrate supplementation, following neonatal hypoxic ischemic insult, improves neuromuscular coordination and spatial learning in male albino mouse. *Brain Res*., **1603**: 76-83. https://doi.org/10.1016/j.brainres.2014.10.006

Jatav, N., Ganeshpurkar, A., Gupta, N., Ayachi, C., Ramhariya, R. and Bansal, D., 2014. Nootropic potential of Bauhinia variegata: A systematic study on murine model. *Arch Med. Hlth. Sci*., **2**: 29-35. https://doi.org/10.4103/2321-4848.133792

Joshi, H. and Parle, M., 2006. Brahmi rasayana improves learning and memory in mice. *Evidence-Based Complem. Alternat. Med*., **3**: 79-85. https://doi.org/10.1093/ecam/nek014

Khan, V., Najmi, A.K., Akhtar, M., Aqil, M., Mujeeb, M. and Pillai, K.K., 2012. A pharmacological appraisal of medicinal plants with antidiabetic potential. *J. Pharma. biol. Sci*., **4**: 27-42. https://doi.org/10.4103/0975-7406.92727

Klapdor, K. and Van der stay, F.J., 1996. The Morris water-escape task in mice: strain differences and effects of intra-maze contrast and brightness. *Physiol. Behav*., **60**: 1247-1254. https://doi.org/10.1016/S0031-9384(96)00224-7

Kumar, Yamini, R. and Rajani, G.P., 2011. Extracts of Root of Bauhinia variegata Linn. *Int. J. Pharmacol*., **7**: 616-622. https://doi.org/10.3923/ijp.2011.616.622

Kulkarni, S.K. and Dhir, A., 2007. Effect of various classes of antidepressants in behavioral paradigms of despair. *Progr. Neuro-Psychopharmacol. Biol. Psy*., **31**: 1248-1254. https://doi.org/10.1016/j.pnpbp.2007.05.002

Mali, R.G., Mahajan, S.G., Mehta, A.A. and Rakta, K., 2007. *Bauhinia variegata*: Chemistry, traditional and medicinal uses-a review. *Pharmacog. Rev*., **1**:314-319.

Mali, R.G. and Dhake, A.S., 2011. Evaluation of effects of Bauhinia variegata stem bark extracts against milk-induced eosinophilia in mice. *J. Adv. Pharm. Technol. Res*., **2**: 132-134. https://doi.org/10.4103/2231-4040.82949

Marasani, A., Kavitha, N. and Manohar, S., 2013. Antistress/adaptogenic activity of Bauhinia variegate against different stress paradigms. *Int. J. Pharma. Biol. Arch*., **4**: 956 – 964.

Nair, C.K., 1998. *Medicinal plants of India*. Nag Publications. Delhi, India

Pritipadma, Ghosh, G., Panda, P., Rath, M., Pal, A., Sharma, T. and Das, D., 2015. GC-MS analysis of bioactive compounds in the methanol extract of *Clerodendrum viscosum* leaves. *Pharma. Res*., **7**: 110. https://doi.org/10.4103/0974-8490.147223

Prut, L. and Belzung, C., 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. *Eur. J. Pharmacol*., **463**: 3-33. https://doi.org/10.1016/S0014-2999(03)01272-X

Rajani, P.G. and Ashok, P., 2009. *In vitro* antioxidant and antihyperlipidemic activities of *Bauhinia variegata* Linn. *Ind. J. Pharmacol*., **41**: 227-232. https://doi.org/10.4103/0253-7613.58513

RajKapoor, B., Jayakar, B. and Murugesh, N., 2003. Anti tumor activity of *Bauhinia variegata* on Dalton's ascitic lymphoma. *J. Ethnopharmacol*., **89**: 107-109 https://doi.org/10.1016/S0378-8741(03)00264-2

Rao, Y.K., Fang, S.H. and Tzeng, Y.M., 2008. Anti inflammatory activities of flavonoids and a triterpene caffeate isolated from *Bauhinia variegata*. *Phytother. Res*., **22**: 957-962. https://doi.org/10.1002/ptr.2448

Santos, F.J., Sidney, G.L., Gilberto, S.C., Antonia, M.G., Ana, A.C., Thiago, H.C. and Rivelilson, M.F., 2012. Chemical composition and anxiolytic-like effects of the Bauhinia platypetala. *Braz. J. Pharmacognos*., **22**: 507-516. https://doi.org/10.1590/S0102-695X2012005000018

Sathya, B., Ariharasiva, K.G., Vimalson, D.C., Subramani, M. and Magesh, M., 2011. Psychopharmacological evaluation of ethanolic extract of leaves of *Bauhinia tomentosa* L in mice. *Int. J. Pharma. Technol*., **3**: 3693-3709.

Shah, J. and Goyal, R., 2011. Investigation of neuropsychopharmacological effects of a polyherbal formulation on the learning and memory process in rats. *J. Young Pharm*., **3**: 119-124. https://doi.org/10.4103/0975-1483.80296

Shiotsuki, Hiromi, Kenji, Y., Yasushi, S., Manabu, F., Yukio, T., Kazutaka, I., Ryosuke, T., Shigeru, K. and Nobutaka, H., 2010. A rotarod test for evaluation of motor skill learning. *J. Neurosci. Meth*., **189**: 180-185. https://doi.org/10.1016/j.jneumeth.2010.03.026

Sunyer, B., Patil, S., Frischer, C., Hoger, H., Selcher, J., Brannath, W. and Lubec, G., 2007. Stain dependent effects of SGS742 in the mouse. *Behav. Brain Res*., **181**: 64-75. https://doi.org/10.1016/j.bbr.2007.03.025

Stanford, S.C., 2007. The open field test: Reinventing the wheel. *J. Psychopharma*., **21**: 134-140. https://doi.org/10.1177/0269881107073199

Ullah, A., Jahangir, M., Nasir, M.K.K., Iqbal, I. and Iqbal, F., 2017. Gender specific effects of ethanolic leaf extract of Bauhinia variegata on the behavior of albino mice. *Sindh Univ. Res. J. (Sci. Ser.)*, **49**: 541-546. https://doi.org/10.26692/surj/2017.09.13

Upchurch, M. and Wehner, J.M., 1988. Differences between inbred strains of mice in Morris water maze performance. *Behav. Genet*., **18**: 55-68. https://doi.org/10.1007/BF01067075

Vasconcelos, F., Sampaio, S. and Arantes, E.C. 2000. Efeitos de extrato de *Bauhinia forficata*. In: *XI Reunia˜oAnual das federac¸o˜es de Sociedades de Biologia Experimental.* Caxambu, MG, Brazil, 216.

Vauzour, D., Vafeiadou, K., Rodriguez-Mateos, A., Rendeiro, C. and Spencer, J.P., 2008. The neuroprotective potential of flavonoids: A multiplicity of effects. *Gen. Nutrit*., **3**: 115-126. https://doi.org/10.1007/s12263-008-0091-4

Verma, S.1. and Singh, S.P., 2008. Current and future status of herbal medicines. *Vet. World*, **1**: 347-350. https://doi.org/10.5455/vetworld.2008.347-350

Walf, A.A. and Frye, C.A., 2015. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocol*, **2**: 322–328. https://doi.org/10.1038/nprot.2007.44

Weitzdoerfer, R., Hoegar, H., Engidawork, E., Engelmann, M., Singewald, N. and Lubec, G., 2015. Neuronal nitric oxide synthase knock - out mice show impaired cognitive performance. *Nitric Oxide*, **10**: 130-140. https://doi.org/10.1016/j.niox.2004.03.007

Whitehouse, O.J., 2015. Neuronal loss and neurotransmitter receptor alterations in Alzheimer's disease. In: *Alzheimer's and Parkinsons disease: Strategies for research and development*. Pelnum, New York. pp. 85-94.

Zhanga, R., Xuea, G., Wanga, S., Zhanga, L., Shia, C., Xie, X., 2012. A novel object recognition as a facile behavior test for evaluating drug effects in aPP/PS1 Alzheimer's disease mouse model. *J. Alzheimer's Dis*., **31**: 801–812. https://doi.org/10.3233/JAD-2012-120151

Zahra, K., Khan, M.A. and Iqbal, F., 2015. Oral supplementation of *Ocimum basilicum* enhances the locomotory, exploratory and learning behavior of adult male albino mice. *Neurol. Sci*., **36**: 73-78. https://doi.org/10.1007/s10072-014-1913-3