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SCREENING OF STEM BARK EXTRACT OF *BAUHINIA VARIEGATA* LINN. FOR PHYTOCHEMICAL CONSTITUENTS AND PHARMACOLOGICAL ACTIVITY

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Abstract
Bauhinia variegata named orchid tree, belongs to the family leguminosae. The methanolic extract of *Bauhinia variegata* (MEBV) revealed the presence of carbohydrates, proteins, tannins, steroids, triterpenoids and flavonoids. Various CNS models were used to find out the antianxiety activity of *Bauhinia variegata*. The study was designed to evaluate the antianxiety activity of stem bark extract of *Bauhinia variegata*. The effect of dried stem bark of *Bauhinia variegata* (200 mg/kg and 400 mg/kg) was evaluated using Elevated Plus Maze, Light and Dark Box, Restrained Stress Model and Novelty Suppressed Feeding Test using a wistar albino rats (n=6) and was statistically analyzed using ONE WAY Annova followed by Dunnett's test. Oral administration of the methanolic extract of *Bauhinia variegata* (200 mg/kg and 400 mg/kg) showed significant increase in %OAE and %TSOA values for EPM, NEL and TSL values in Light and Dark Box, Restrained Stress Model showed significant increase in %OAE and %TSOA and NEL and TSL values. Novelty suppressed feeding behaivior test showed significantly lower values for latency to feed.From the present study it may be concluded that among both the test groups, MEBV at a dose of 400 mg/kg was found to possess significant anti-anxiety activity.
Keywords: EPM- Elevated Plus Maze, NEL- Number of Entries in Light, TSL- Time spent in light.

Introduction

Nature has been source of medicinal agent since time immemorial and man has been dependent on plant products for his needs. The importance of herbs in the cure of human ailment cannot be neglected. Phytomedicines used to cure several diseases are well acknowledged in indigenous traditional system Ayurveda, Unani and Charak Samhita. With growing awareness for the last three decades, it was needed to explore the potential of plant-derived products against several diseases.[1]

Synthetic drugs are known to cause side effects, as a result of which people are more keen on using natural products obtained from plants. Standardization and formulation of the plant product is necessary to describe the isolation, identification and qualification of active ingredients in plant materials. [2]

Plants are an important source of medicinally valuable bioactive secondary metabolites. The subcontinent of India is one of the major biodiversity centers and is a home for around 45000 plant species. Around 15000 medicinal plants have been recorded in India. Different Indian communities use around 7000- 7500 plants for curing various diseases. [3]

There is a growing demand for phyto-pharmaceutical products, derived from Ayurveda, in western countries. This is because the medicinal plant-derived products are more safe and economical than the synthetically derived drugs. Many national and multinational pharmaceutical companies are now concentrating more on production of Ayurvedic products.[4]

The human brain is a complex organ. Its major task is to receive information from rest of the body, interpret it and then modulate the body's response to it. The brain and the spinal cord together comprise the central nervous system (CNS). The CNS is the major control network that influences the body's function and abilities. It helps in automatic operation of vital organs and also enables conscious communication within the body.[5]

Bauhinia variegata, commonly known as orchid tree, belongs to the family Leguminosae, grows 10-12 meter tall with a spreading crown of briefly deciduous leaves which are 10-20 cm across and rounded with lobed ends and heart shaped bases. "Kachnar" is an herbaceous plant, reaching up to 6-12 meters.[6]

The phytochemical screening revealed that *Bauhinia variegata* contains terpenoids, flavonoids, and tannins,

saponins, reducing sugars, steroids and cardiac glycosides.

Pharmacological studies have revealed that *Bauhinia variegata* possesses anticancer, antioxidant, hypolipidemic, antimicrobial, anti-inflammatory, nephroprotective, hepatoprotective, antiulcer, immunomodulating, molluscicidaland wound healing effects.

The present study employs various models to screen the methanolic extract of dried stem bark of *Bauhinia variegata* for its antianxiety activity.

Materials

Test material: Dried methanolic extract (MEBV) of *Bauhinia variegata* Linn. was procured from Amsar Pvt. Ltd respectively.

Control: 2% Tween 80.

Standards: Diazepam (2mg/kg i.p.) was obtained from market.

Experimental Animals

Male Wistar albino rats weighing about 150-250 g were used in this study and all experimental protocols were reviewed and accepted by the Institutional Animal Ethics Committee (IAEC) prior to commencement of the experiment- GCP/IAEC/17/04.

METHODS

Dose selection

Acute (single-dose) toxicity studies are carried out on laboratory animals by using high doses (sufficient to produce death or morbidity) of the substance in question and/or based on previous reports of its toxicity or toxicity of structurally related compounds. Since no previous reports on the toxicity of *Bauhinia variegata* were available, two dose levels starting at 2,000 and limit dose 4,000 mg/kg were selected for acute toxicity study. No mortality was observed at the selected dose levels, and no changes in general appearance occurred during the observation period, indicating that AE of B. variegata was well tolerated in rats up to 4,000 mg/kg b.w orally[7.] Hence doses of 200 mg/kg and 400 mg/ kg were selected as test doses for the pharmacological screening in this study.

Groups

The rats were divided into 7 groups, each group consisting of 6 rats, and were administered the following:

Group I: 2% Tween 80, which served as control.

Group II: Diazepam (2mg/kg) which served as standard for anti-anxiety activity.

Group III: Methanolic extract of stem bark extract of *Bauhinia variegata* (200 mg/kg) (MEBV).

Group IV: Methanolic extract of stem bark extract of *Bauhinia variegata* (400 mg/kg) (MEBV).

Group V: Methanolic extract of stem bark extract of *Bauhinia variegata* (400 mg/kg) (MEBV) following restraint stress (4 hours a day for 6 days).

Group VI: Diazepam (2 mg/kg), following restraint stress (4 hours a day for 6 days).

Group VII: 2% Tween 80, following restraint stress (4 hours a day for 6 days) which served as control.

Screening Methods

The following methods were used to screen the methanolic extract of Dried stem bark of Bauhinia variegata Linn. for antianxiety activity:

A) Elevated Plus Maze

B) Light and Dark Model

C) Restraint stress Model
D) Novelty Suppressed Feeding Test

A) Elevated Plus Maze [8]

The rats were individually weighed and numbered .One hour after the administration of the respective solutions / suspensions to each group i.e. control (2% Tween 80), standard (Diazepam 2mg/kg, i.p.), methanolic extracts, the rats were individually placed at the centre of the maze, facing one of the open arms. During a 5-minute test period, the following parameters were monitored using the smart video tracking software:

- Number of entries in the open and closed arms
- Time spent in open and closed arms
- Percentage of open arm entries
- Percentage time spent in open arms

From the above mentioned parameters, the percentage of open arm entries and the percentage time spent by the rat in open arm were taken as indices to evaluate the anti-anxiety activity. The control group (Group I), diazepam-treated group (Group II) and methanolic extract-treated groups (Groups III and IV), were administered the respective suspension / solution for a continuous period of 7 days. The experiment was repeated on the 4th and the 7th day and the readings obtained were noted.

B) Light and Dark Model[8]

The rats were individually weighed and numbered.After 30 mins of administration of the control (1% Tween 80), standard (diazepam 2 mg/kg, i.p), methanolic extracts of stem bark extract of *Bauhinia variegata,*the rats were observed for 5 mins after 1hour, 4 hour and 8 hour.Each

animal was placed at the illuminated compartment near the wall separating light and dark compartment.During the five-minute test period, the following parameters were monitored for:

i) Number of entries in the light and dark compartments.

ii) Time spent in the light and dark compartments

C) Restraint Stress Model

i) Elevated Plus Maze

The rats were restrained in a wire mesh restrainer for 4 hours a day, for 6 consecutive days to induce anxiety. Twenty four rats were used in this study and they were divided in four groups of six rats each.

Group IV were treated with the dose of extracts showing highest anti-anxiety activity in the EPM test. Group VI was treated with diazepam (2 mg/kg) and Group VII was taken as control (2% Tween 80). At the end of the third hour in the restrainer, the rats were taken out and the respective solution / suspension were administered once a day to them. They were then kept in the restrainer for an additional hour after administration, following which, the screening of anti-anxiety activity using the elevated plus maze was carried out. This was reported over a period of six consecutive days.

ii) Light And Dark Model [9]

The rats were restrained in a wire mesh restrainer for 4 hours a day, for 6 consecutive days to induce anxiety. Twenty-four rats were used in this study and they were divided in four groups of six rats each. Group IV were treated with the dose of extracts showing highest anti-anxiety activity in the EPM test. Group VI was treated with diazepam (2 mg/kg) and Group VII was taken as control (2% Tween 80). At the end of the third hour in the restrainer, the rats were taken out and the respective solution / suspension were administered once a day to them. They were then kept in the restrainer for an additional hour after administration, following which, the screening of anti-anxiety activity using the light and dark box was carried out. This was reported over a period of six consecutive days.

D) Novelty Suppressed Feeding Test [10]

The rats were individually weighed and numbered.Open field apparatus with a dimension of 76cm x 76cm x 46cm was used for testing.24 hours prior to testing, all food was removed from the home cages; however water was available ad libitum.The animals were treated 60 min before the experiment with the control, standard or test drugs.At the time of testing, a food pellet was placed in the center of the open field and the animals were placed individually in an open field area, and were then observed for 05- min after 1 hour of treatment and latencies to approach food and begin eating is recorded.The control, diazepam treated and test solutions treated groups were administered the respective doses for a continuous period of seven days. The experiment was performed 7th day and the readings obtained were noted.

Results and Discussion

I. Antianxiety activity

Table 1: Effect of methanolic extract of dried stem bark of *Bauhinia variegata* Linn. on anxiety using Elevated Plus Maze. (%OAE: Percentage open arm entries, %TSOA: Percentage time spent in open arms)

TREATMENT	TIME (h)	DAY 1		DAY 4		DAY 7	
		%OAE	%TSOA	%OAE	%TSOA	%OAE	%TSOA
CONTROL (GROUP I)	1	6.18±0.91	5.96±1.45	14.75±1.31	5.05±1.85	18.64±4.08	8.08±4.11
	4	5.75±2.63	2.90±1.39	11.90±2.87	9.44±2.11	19.51±1.79	5.13±2.02
	8	13.68±6.70	4.23±1.86	9.261±2.21	6.74±6.16	6.88±2.66	5.7±1.85
DIAZEPAM (GROUP II)	1	24.19±1.68*	13.74±1.57	34.18±6..56	40.13±12.83**	46.12±1.47**	38.28±10.56*
	4	23.93±2.87**	47.48±6.53**	31.62±3.86	38.89±3.34**	45.81±1.77**	33.38±2.72**
	8	38.71±2.36**	44.07±9.29**	31.63±6.43*	36.67±2.36**	27.61±6.33*	34.64±2.78*
MEBV 200 mg/kg (GROUP V)	1	16.63±1.92*	12.57±1.96	26.09±6.22	10.4±4.82	16.035±3.55	14.88±6.12
	4	13.88±1.86	14.09±5.88	10.91±4.90	10.00±5.10	28.97±2.00*	21.19±3.72**
	8	19.88±6.69	28.72±4.67	22.3±6.37	29.03±8.34*	25.41±3.73*	26.30±9.26
MEBV 400mg/kg (GROUP VI)	1	27.83±3.71**	16.34±3.10**	33.67±4.51*	17.59±3.15	29.0±5.148	17.51±2.23
	4	16.19±1.75	28.19±6.92*	16.36±7.13	29.82±6.87*	35.26±2.83**	33.56±4.428**
	8	36.89±4.06**	32.60±6.48*	28.58±4.42*	30.17±4.35*	30.81±5.33**	38.50±10.34*

Values are expressed as mean ± SEM (n=6) *P<0.05,**P<0.01vs. Control

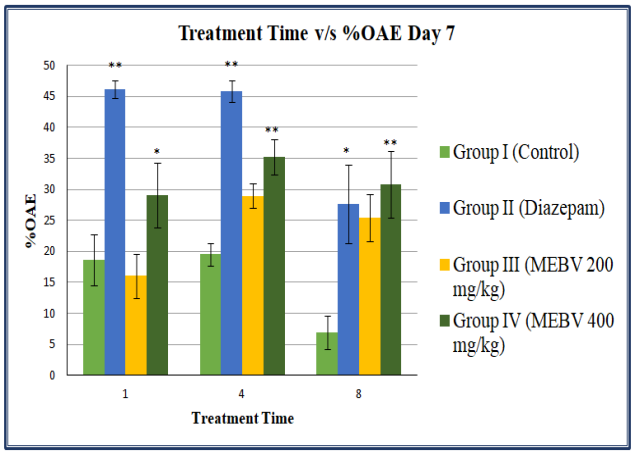

Figure 1: Percentage open arm entries for control, Diazepam, Group III and Group IV, on Elevated plus maze –Day 7

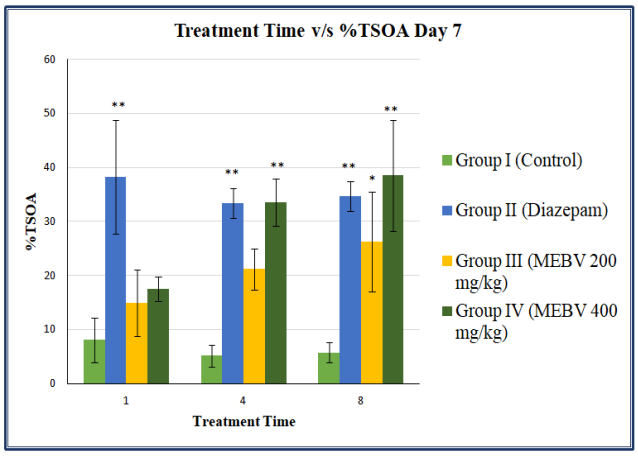

Figure 2: Percentage time spent in open arms for Control, Diazepam, Group III and Group IV on elevated plus maze– Day 7

Table 2: Effect of methanolic extract of dried stem bark of *Bauhinia variegata* Linn. on anxiety using Light and Dark Box. (NEL: Number of Entries in Light, TSL: Time Spent in Light Area)

Treatment	Time	DAY 1		DAY 4		DAY 7	
	(h)	TSL (secs)	NEL	TSL (secs)	NEL	TSL (secs)	NEL
CONTROL	1	31.00±9.32	1.66±0.49	7.16±2.31	1.16±0.40	11.66±5.05	1.00±0.25
GROUP I							
	4	36.66±20.26	2.83±1.32	10.66±4.81	1.00±0.25	13.16±4.54	1.16±0.30
	8	46.83±26.68	1.66±0.55	23.83±8.35	1.33±0.33	18.50±8.75	1.16±0.30
DIAZEPAM	1	31.00±7.21	1.66±0.49	66.55±8.29**	1.83±0.47	90.16±9.95**	2.66±0.42**
GROUP II							
	4	39.00±6.27	4.00±1.03	69.33±9.28**	2.66±0.33*	90.16±9.52**	2.66±0.49**
	8	41.50±5.77	2.50±0.67	62.66±9.20**	2.66±0.33*	102.33±6.76**	3.16±0.40*
MEBV	1	33.83±7.95	1.83±0.54	30.00±3.25	2.66±0.33*	77.05±18.76**	2.1±0.42
(200 mg/kg)							
	4	25.00±2.09	2.33±0.21	31.83±2.60	2.83±0.47*	83.33±26.45**	4.00±0.51**
GROUP III							
	8	30.33±2.47	2.05±0.25	32.55±3.97	3.52±3.93*	93.88±21.59**	3.15±0.34**
MEBV	1	24.50±2.25	1.83±0.65	50.66±10.30**	3.33±0.55*	79.72±5.00*	3.50±0.22**
(400 mg/kg)							
	4	37.83±4.97	3.66±0.76	42.33±5.22**	2.33±0.20*	92.22±8.99**	4.00±0.51**
GROUP IV							
	8	40.66±3.63	2.33±0.42	46.00±5.01	2.83±0.30*	92.22±8.99**	3.66±0.33**

Values are expressed as mean ± SEM (n=6) *P<0.05,**P<0.01vs. Control

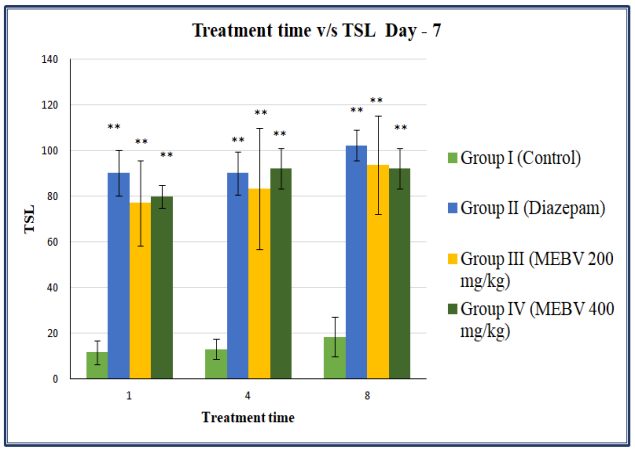

Figure 3: Time spent in Light for Control, Diazepam, Group III and Group IV on light and dark box – Day 7

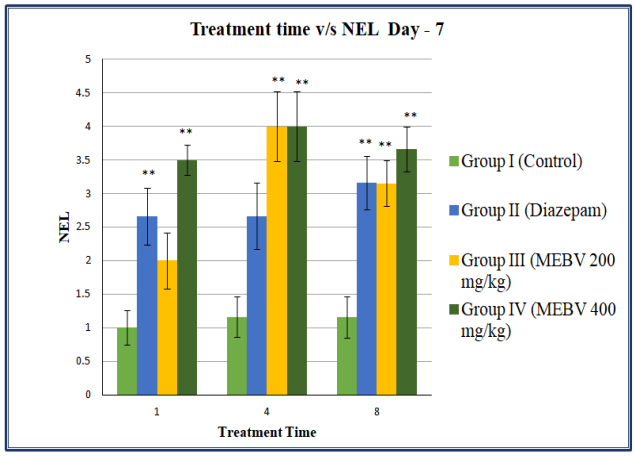

Figure 4: Number of entries in Light for Control, Diazepam, Group III and Group IV on light and dark box – Day 7

Table 3: Effect of methanolic extract of dried stem bark of *Bauhinia variegata* Linn. on anxiety using Restrained Stress Apparatus followed by EPM.(%OAE: Percentage open arm entries, %TSOA: Percentage time spent in open arms)

	TREATMENT					
DAY						
	Control (Group VII)		Diazepam (Group VI)		MEBV (Group V)	
	%OAE	%TSOA	%OAE	%TSOA	%OAE	%TSOA
1	7.998±1.232	18.00±2.840	28.88±4.880**	37.166±2.71**	22.068±4.208*	18.03±4.45
2	15.007±5.326	29.33±5.732	26.306±4.284	81.84±5.52**	15.378±7.716	20.728±5.70
3	17.833±3.525	5.034±2.810	27.55±5.442	60.83±17.08*	33.318±3.907	87.78±6.70**
4	19.648±6.018	16.50±3.836	49.94±8.30*	63.96±16.22**	28.810±4.550	44.94±11.94*
5	16.999±3.479	18.748±4.631	50.61±10.33*	36.73±2.414*	30.445±7.949	91.0±18.40**
6	9.208±4.558	34.218±7.793	42.50±3.294**	84.44±6.80**	32.465±4.85**	87.75±19.91*

Values are expressed as mean ± SEM (n=6) *P<0.05,**P<0.01vs. Control

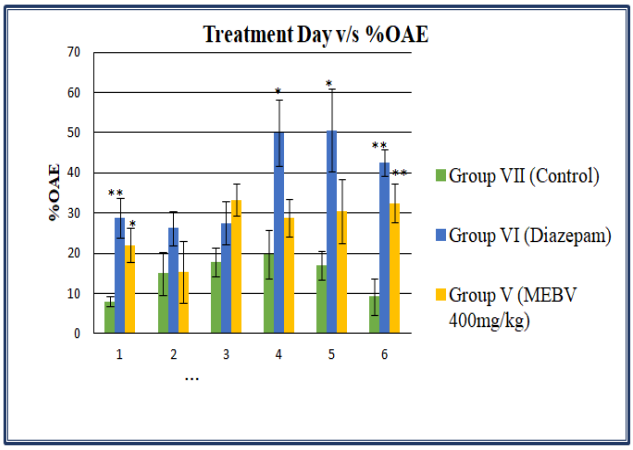

Figure 5: The percentage open arm entries for Control (Group VII), Diazepam (Group VI) and MEBV (Group V) on Elevated plus maze after restrain stress- Day 1 to Day 6

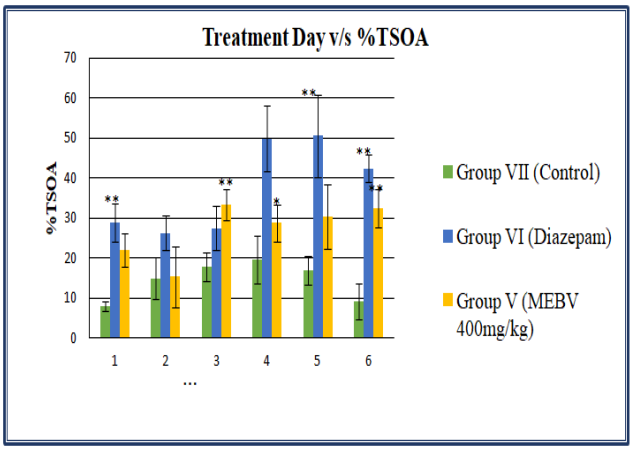

Figure 6: The percentage time spent in open arms for Control (Group VII), Diazepam (Group VI) MEBV (Group V) on Elevated plus maze after restrain stress – Day 1 to Day 6

Table 4: Effect of methanolic extract of dried stem bark of *Bauhinia variegata* Linn. on anxiety using Restrained Stress Apparatus followed by Light and Dark Test. (NEL: Number of Entries in Light , TSL: Time Spent in Light Area)

	TREATMENT					
DAY						
	Control (Group VII)		Diazepam (Group VI)		MEBV (Group V)	
	NEL	TSL (secs)	NEL	TSL (secs)	NEL	TSL (secs)
1	2.16±0.410	32.28±4.41	6.94±0.79**	136.11±20.53*	3.888±0.70*	51.11±15.64
2	1.72±0.133	37.35±7.00	7.77±0.75**	160.55±18.38*	4.722±0.51**	81.94±16.20*
3	3.05±0.51	72.52±19.47	6.38±0.51*	161.66±28.91	5.833± 0.83*	75.55±13.28
4	3.33± 0.43	60.84±19.85	5.55±0.55*	149.44±13.17**	6.388±0.63*	121.38±20.98*
5	3.61±0.51	86.26±13.68	5.27±0.51	148.88±13.40*	5.833± 0.71*	135.83±23.31*
6	2.77±0.55	98.85±5.56	5.83±0.71**	178.33±17.53**	5.833±0.49*	153.05±26.95*

Values are expressed as mean ± SEM (n=6) *P<0.05, **P<0.01vs. Control

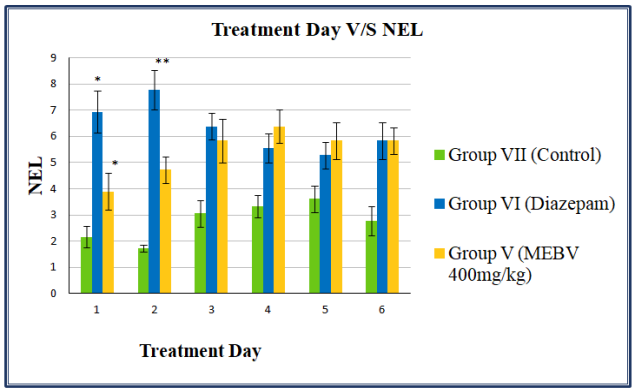

Figure 7: The number of entries in light for Control (Group VII), Diazepam (Group VI) and MEBV (Group V) on Elevated plus maze after restrain stress- Day 1 to Day 6

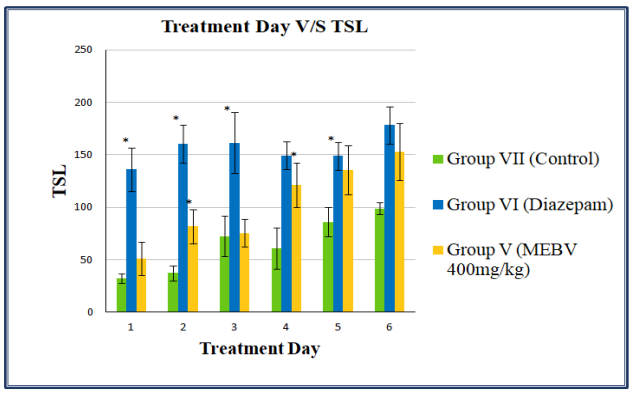

Figure 8: The time spent in light area for Control (Group VII), Diazepam (Group VI) MEBV (Group V) on Elevated plus maze after restrain stress – Day 1 to Day 6

Table No 5: Novelty Suppressed Feeding - Latency to feed (secs)

TREATMENT	Latency to feed (secs)
Group I (Control)	10.166±1.327
Group II (Diazepam)	6.833±0.278*
Group III (MEBV 200 mg/kg)	5±1.183**
Group IV (MEBV 400 mg/kg)	2.83±0.347**

Values are expressed as mean ± SEM (n=6) *P<0.05, **P<0.01 vs. Control

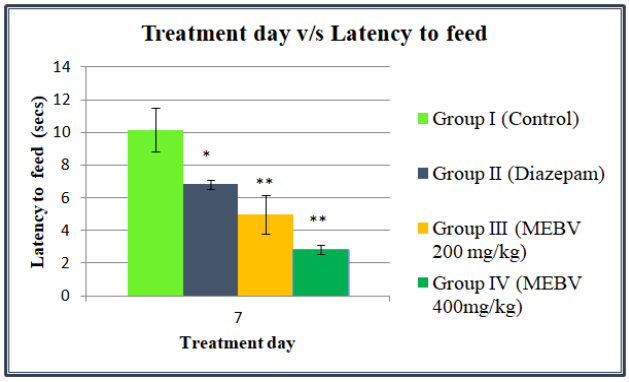

Figure 9: Latency to feed on day 7 for Group I, Group II (Diazepam), Group III (MEBV 200mg/kg), and Group IV (MEBV 400 mg/kg) for novelty suppressed feeding test.

Discussion

Pharmacological screening using Elevated Plus Maze model, after continuous administration of the extracts, demonstrated significant increase in %OAE and %TSOA values for both the doses (200mg/kg and 400mg/kg) of methanolic extract of *B. variegata* out of which dose of 400 mg/kg of MEBV showed most significant increase. Hence it can be said that methanolic extract of *B. variegata* at a dose of 400 mg/kg possesses highest anti-anxiety activity among the two test groups.

Screening using Light and Dark model, after continuous administration of the extracts, demonstrated significant increase in TSL and NEL values for both the doses (200mg/kg and 400mg/kg) of methanolic extract of *B. variegata* out of which dose of 400 mg/kg of MEBV showed most significant increase. Hence it can be said that methanolic extract of *B. variegata* at a dose of 400 mg/kg possesses highest anti-anxiety activity among the two test groups.

Using Restrained Stress model after continuous administration of the extracts, both the doses were subjected to Elevated Plus Maze which demonstrated significant increase in %OAE and %TSOA values for both the doses (200mg/kg and 400mg/kg) of methanolic extract of *B. variegata* out of which dose of 400 mg/kg of MEBV showed most significant increase. Hence it was further confirmed that methanolic extract of *B. variegata* at a dose of 400 mg/kg possesses highest anti-anxiety activity among the two test groups.

The rats were also subjected to Light and Dark model after restrained stress which demonstrated significant increase in TSL and NEL values for both the doses (200mg/kg and 400mg/kg) of methanolic extract of *B. variegata* out of which dose of 400 mg/kg of MEBV showed most significant increase. Hence it was further confirmed that methanolic extract of *B. variegata* at a dose of 400 mg/kg possesses highest anti-anxiety activity among the two test groups.

Novelty suppressed feeding behavior test showed significantly lower values for latency to feed. There was dose dependent decrease in latency to feed which confirmed that that methanolic extract of *B. variegata* at a dose of 400 mg/kg possesses highest anti-anxiety activity among the two test groups.

From the present study it may be concluded that among both the test groups, MEBV at a dose of 400 mg/kg was found to possess significant anti-anxiety and also possess significant CNS depressant activity. Thus the anti-anxiety activity of methanolic extract of *B. variegata* may be attributed to the presence of certain phenolic compounds and other phytoconstituents. Further investigation is required to establish the

complete phytochemical profile and related pharmacological activities of the plant extract.

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