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EVALUATION OF ANXIOLYTIC ACTIVITY OF LEAF PARTS OF *BASELLA ALBA LINN*

ASTHANA A AND SHARMA N

Ph.D. Scholar, Department of Pharmacy, Bhagwant University, Rajasthan Sikar Rode, Ajmer-305004, Rajasthan, India

***Corresponding Author: Dr. Amrita Asthana: E Mail: amritaasthanaamh@gmail.com**

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ABSTRACT

Methanol was prepared from dried leaf parts of *Basella alba* by cold maceration method. Anxiolytic activity of the extract of *B. alba* was evaluated by diazepam induced by elevated plus maze on open and close arm and light and dark model by dark arm and light arm test in mice. Methanol extract (100 and 200 mg/kg, oral rout) showed highly significant ($p<0.001$) anxiolytic activity. All the results were compared with reference drug, diazepam. The presence of various phytoconstituents in the extracts was identified by preliminary phytochemical studies. The results of this study suggested that *B. alba* methanol extract possesses anxiolytic activity.

Keywords: *Basella alba*, anxiolytic activity, elevated plus maze model, light and dark model diazepam,etc.

1. INTRODUCTION

Basella alba (Family: Basellaceae), is commonly known as Indian spinach. The paste of root of red *B. alba* along with rice washed water is taken in the morning in empty stomach for one month to cure irregular periods by the rural people of Orissa, India **[1]**. Leaves of B. alba is used for the treatment of hypertension by Nigerians in Lagos **[2]**, and malaria in cameroonian folk medicine **[3]**. A literature survey revealed that the plant has reported its antifungal **[4]**, anticonvulsant, analgesic, anti-inflammatory **[5]** and

androgenic **[6]** activities and for the treatment of anaemia **[7]**. Also the leaves of *B. alba* is traditionally used in ayurveda system of medicine for to bring sound refreshing sleep when it is applied on head about half an hour before bathing **[8]**. Hence, the present investigation was aimed to scientifically evaluate anxiolytic and analgesic activity of the leaf parts of *B. alba*.

2. Plant Material: The leaf parts of *B. alba Linn*. were collected from Azamgarh, Jaunpur, Varanasi and Allahabad. It was authenticated by Prof. N.k Dubey FNASc, FNAAS centre of advanced study in Botany Institute of Science, Banaras Hindu University Varanasi. A voucher specimen was deposited in the departmental herbarium.

2.1 Preparation of Extract: The collected leaf parts of *B. alba* were shade-dried at room temperature. The dried materials were size reduced to coarse powder and macerated with methanol and distilled water separately for seven days. Methanol extract (MEBA) of *B. alba* was collected separately, filtered and concentrated under vacuum using rotary vacuum evaporator **[9]**. The colour, consistency and the % yield of the extracts was noted in **Table 1**. All the extracts was kept in desiccators until further use.

2.2 Preliminary Phytochemical Analysis: Preliminary phytochemical investigation of the extracts for identification of phytoconstituents such as alkaloids, carbohydrates, and flavanoids, proteins, was carried out **[10]**. The results are shown in **Table 2**.

2.3 Experimental Animals: Swiss albino mice of either sex (20 - 30g) were used for this investigation and kept at the Laboratory Animal House. They were kept in well ventilated room for 1 week before and during the experiments. Animals were provided with commercial rodent pellet diet and water ad libitum. All the experiments were performed according to current guidelines for the care of the laboratory animals and the ethical guidelines. The standard orogastric cannula was used for oral drug administration.

2.4 Determination of Acute toxicity studies (LD 50) [10]

It is further planned to study the acute toxicity of solvent extract of *Basella alba* leaves. In albino mice of either sex (20-30gm). Fixed dose method (OECD guideline number 420) of CPCSEA will be adopted for toxicity studies to obtained dose range of extracts of *Basella alba* leaves.

2.5 Experimental design:

For all experiments, the animals are randomly divided into nine groups of (n = 2) animals each. Group I: Control

Group II: Treated With dizepam

Group III: Treated With *basella alba* extract

Experimental models:

1. Elevated Plus-Maze Test in mice [11-14]

Albino mice of either sex weighing between 20-30gm are selected and divided into Different groups of 6 animals (n=6) each where the control group will receive 2% gum Acacia per oral (p.o.) and the standard group receives drug diazepam at a dose of 2mg/kg.

Procedure: The plus-maze apparatus comprises of two open arms (16×5cm) and two closed arms (16×5×12cm) that extend from a common central platform (5×5cm). The entire maze is elevated to a height of 25 cms above the floor level. Mice are placed individually in the center of the maze facing one of the enclosed arms for recording various parameters in a period of 5 minutes.

2. Light-dark model transition test in mice [15]:

The light/dark transition test is based on the innate aversion of rodents to brightly Illuminated areas and on the spontaneous exploratory behavior of rodent in response to Mild stressor, that is, novel environment and light. A natural conflict situation occurs. When an animal is exposed to an unfamiliar environment or novel objects. The conflict is between the tendency to explore and the initial tendency to avoid the unfamiliar (neophobia). The exploratory activity reflects the combined result of these tendencies in novel situations. Thus, in the light/dark test, drug induced increase in behavior in the white part of a two compartment box, in which a large white compartment is illuminated and a small black compartment is darkened, is suggested as an index of Anxiolytic activity. Albino mice of either sex weighing between 20-30gm are selected and divided into Different groups of 6 animals (n=6) each, where the control group will receive 2% gum Acacia per oral and the standard group receives drug diazepam at a dose of 2mg/kg.

Procedure: The light-dark apparatus consists of two-compartment chamber (40×60×20cm/h) Comprising of a brightly illuminated area (40×40cm) and a dark area (40×20cm) separated by a wall with around hole (7cm diameter) will be used. Mice are placed individually in the illuminated part of the cage and following parameters are recorded during the test Session of 5 minutes:

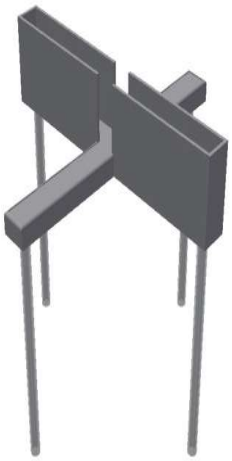
a. Total number of crossings

b. Number crossing between the light and dark area

c. Total time spend in the illuminated part of the cage

Time spend in the dark part of the cage.

Ethanol extract of *Basella alba*



Elevated Plus-Maze model

Light-dark model

Figure 1

Statistical Analysis: All the data are expressed as mean ± SEM. The values obtained were compared with control group by sudents t test. The values of $p < 0.001$ and $p < 0.01$ were considered to indicate a significant difference between the groups.

3. RESULTS AND DISCUSSION

The results of the present work showed that there was significant CNS depressant activity in mice pre-treated with MEBA as compared to control. MEBA exhibited a dose-dependent reduction of the onset time of sleep and increased the duration time of sleep in pentobarbitone-induced hypnosis. A dose-dependent reduction was observed up to 60min in MEBA treated mice and no movement was observed at 120min for locomotor and exploratory activities by open field and hole cross tests respectively. The maximum activity was observed at 120min in MEBA treated animals.

Table 1: Colour, consistency and % yield of various extracts of leaf parts of *B.alba*

Extracts	Colour	Consistency	% yield
MEBA	Dark Greenish colour	semisolid	10.6%

MEBA-Methanol extract

Table 2: Preliminary phytochemical studies of various extracts of aerial parts of B.alba

S. No.	Chemical constituents	MEBA
1	Alkaloids	-
2	Carbohydrates & glycosides	+
3	Flavonoids	+
4	Proteins	+

MEBA-Methanol extract

Table 3 shows effect of various extracts of leaf parts of *B. alba* on Dizepam induced anxiolytic activity by Elvated Plus Maze model and Light and Dark model.

The results showed that the number of open arm entries and time spent in the open arms were increased and number of closed arm entries and time spent in the closed arms were decreased significantly in the extracts treated groups which were comparable with the standard (Diazepam). The test results are dose dependent and decline with time. The responses of the extract of methanol was expressed in the **Table 3, 4,** etc. respectively.

Table 3: Anxiolytic Activity of MEBA in rat by Elevated plus maze model (mean ± SEM)

Group	Dose (mg/kg)	Avg. no. of entry in the open arms				Avg. time spent in the open arms (in minute)			
		I (.30 hr)	II(1.40 hr)	III (2.50 hr)	IV(4.0 hr)	I (.30 hr)	II(1.40 hr)	III (2.50)	IV(4.0hr)
I	50	1.0± 0.0*	1.0± 0.0*	2.0± 0.0*	1.0± 0.0*	0.30± 0.00**	0.30± 0.00**	1.15± 0.15**	0.15± 0.05**
II	100	0.5± 0.5*	0.0± 0.0*	0.5± 0.5*	1.0± 0.0*	0.15± 0.15**	0.00± 0.00**	0.30± 0.30*	0.12± 0.07**
III	150	0.0± 0.0*	0.5± 0.5*	0.0±0.0**	1.0± 0.0*	0.00± 0.00**	0.05± 0.05**	0.00± 0.00**	0.07± 0.02**
IV	200	0.0± 0.0*	0.0± 0.0*	0.5± 0.5*	1.0± 0.0*	0.00± 0.00**	0.00± 0.00**	0.05± 0.05**	0.20± 0.10*
V	250	1.0± 0.0*	1.5± 0.5*	1.0± 0.0*	1.0± 0.0*	0.30± 0.00**	1.00± 0.50**	0.75± 0.25*	0.07± 0.02**
Std.	4	1.0± 0.0	1.5± 0.5	2.0± 0.0	1.5± 0.5	0.07± 0.02	0.20± 0.00	1.10± 0.30	1.10± 0.10
Cont.	_	1.0± 0.0	0.5± 0.5	1.0± 0.0	1.0± 0.0	0.10± 0.00	0.20± 0.20	0.35± 0.05	0.07± 0.02
		V (5.10 hr)	VI (6.20 hr)	VII (7.30 hr)	VIII (24.0 hr)	V (5.10 hr)	VI (6.20 hr)	VII (7.30 hr)	VIII (24.0 hr)
I	50	0.5± 0.5*	1.5± 0.5*	0.5± 0.5*	0.5± 0.5*	0.20± 0.10**	0.23± 0.08**	0.05± 0.05**	0.05± 0.05**
II	100	0.5± 0.0*	1.5± 0.5*	0.5± 0.5*	1.0± 0.0*	0.05± 0.05**	0.18± 0.75*	0.05± 0.05**	0.15± 0.05*
III	150	0.5± 0.0*	1.0± 0.0*	0.5± 0.5*	1.0± 0.0*	0.07± 0.02**	0.10± 0.05**	0.17± 0.02**	0.12± 0.07*
IV	200	1.5± 0.5*	0.5± 0.5*	0.0± 0.0*	1.5± 0.5*	0.25± 0.05**	0.25± 0.05**	0.00± 0.00**	0.30± 0.10*
V	250	0.5± 0.5*	1.0± 0.5*	0.5± 0.5*	0.5± 0.5*	0.15± 0.15**	0.45± 0.25*	0.15± 0.15*	0.25± 0.05*
Std.	4	2.0± 1.0	2.0± 0.0	1.0± 0.0	1.0± 0.0	1.30± 0.10	1.80± 0.20	1.00± 0.00	0.07± 0.02
Cont.	_	1.5± 0.5	0.0± 0.0	0.5± 0.5	1.0± 0.0	0.10± 0.05	0.00± 0.00	0.05± 0.05	0.12± 0.07

All values are expressed in mean ± standard error mean (n=6).

All data were found to be significant at 5% level of significance and non-significance where **p<0.05 and *p>0.05 respectively.

Table 4: Anxiolytic Activity of MEBA in rat by Light-dark model (mean ± SEM)

Group	Dose (mg/kg)	Avg. no. of entry in the open arms				Avg. time spent in the open arms (in minute)			
		I (.30 hr)	II(1.40 hr)	III (2.50 hr)	IV(4.0 hr)	I (.30 hr)	II(1.40 hr)	III (2.50)	IV(4.0hr)
I	50	1.5± 0.5*	1.5± 0.5*	1.0± 1.0*	1.5± 0.5*	1.90± 0.40*	1.57± 0.47*	1.05± 1.05*	1.75± 0.25*
II	100	1.5± 0.5*	0.5± 0.5*	1.0± 0.0*	1.0± 0.0*	2.15± 0.15*	0.60± 0.60*	1.45± 0.05*	1.15± 0.15**
III	150	1.5± 0.5*	1.5± 0.5*	1.0± 0.0*	1.0± 1.0*	1.85± 0.35*	1.65± 0.35*	1.85± 0.45*	1.53± 0.17*
IV	200	1.0± 0.0*	2.0± 0.0*	1.0± 0.5*	1.0± 1.0*	2.35± 0.05*	1.35± 0.05*	2.15± 0.15*	1.90± 0.40*
V	250	2.5± 0.5*	2.0± 0.0*	1.5± 0.5*	2.0± 0.0*	1.75± 0.25*	2.25± 0.25*	2.50± 0.50*	2.50± 0.50*
Std.	4	1.0± 0.0	1.5± 0.5	1.0± 0.0	1.5± 0.5	1.74± 0.26	3.00± 1.00	1.50± 0.50	3.00± 0.50
Cont.	_	1.0± 0.0	1.0± 0.0	1.5± 0.5	0.5± 0.0	0.90± 0.55	1.20± 0.00	0.70± 0.03	1.90± 0.10
		V (5.10 hr)	VI (6.20 hr)	VII (7.30 hr)	VIII (24.0 hr)	V (5.10 hr)	VI (6.20 hr)	VII (7.30 hr)	VIII (24.0 hr)
I	50	0.5± 0.5*	1.0± 0.0*	0.5± 0.5*	1.0± 0.0*	1.50± 1.50*	1.50± 0.00*	1.27± 0.77*	2.50± 0.50*
II	100	1.5± 0.5*	1.5± 0.5*	1.0± 1.0*	1.5± 0.5*	1.70± 0.30*	1.65± 0.35*	0.65± 0.65**	2.00± 0.00*
III	150	1.0± 0.0*	1.0± 1.0*	1.0± 0.0*	1.5± 0.5*	1.65± 0.35*	1.73± 1.73*	2.05± 0.05*	1.25± 0.75*
IV	200	1.0± 0.0*	1.0± 0.5*	1.0± 0.0*	1.5± 0.5*	2.65± 0.35*	2.50± 0.50*	2.00± 1.00*	2.05± 0.05*
V	250	2.0± 0.0*	2.0± 0.0*	1.5± 0.5*	1.5± 0.5*	1.80± 0.30*	2.40± 0.10*	3.00± 0.00*	1.15± 0.85*
Std.	4	2.0± 0.0	2.0± 0.0	1.5± 0.5	1.0± 0.0	3.00± 1.00	2.50± 0.50	3.50± 0.50	3.50± 0.50
Cont.	_	1.0± 0.5	1.0± 0.0	1.0± 0.0	1.5± 0.5	2.10± 1.10	1.00± 0.00	1.20± 0.40	1.10± 0.30

All values are expressed in mean ± standard error mean (n=6).
All data were found to be significant at 5% level of significance and non-significance where **p<0.05 and *p>0.05 respectively

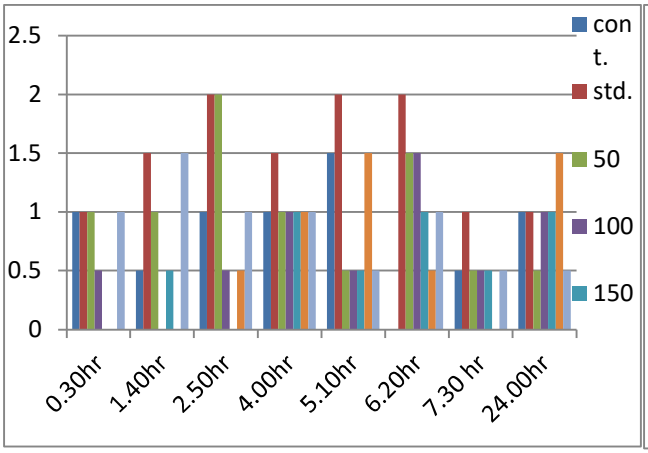


Figure 2: Effect of MEBA on no. of entry in open arm in EPM model

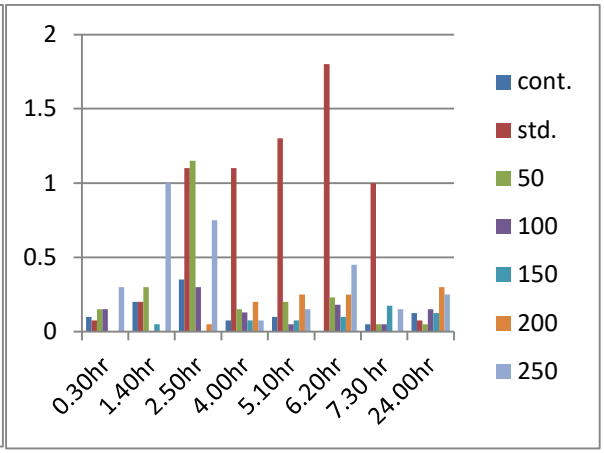


Figure 3: Effect of MEBA on time spent in open arm in EPM model

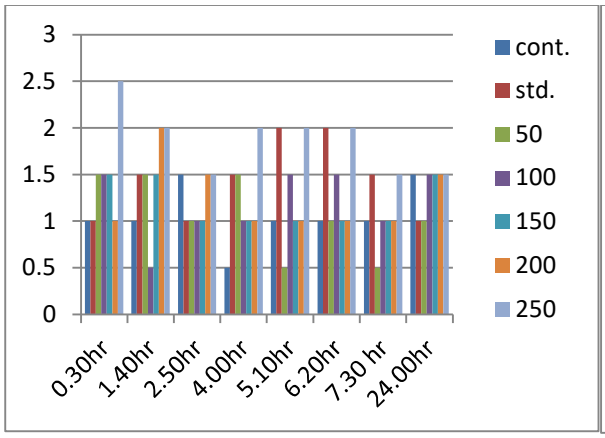


Figure 4: Effect of MEBA on no. of entry in open arm in LD model

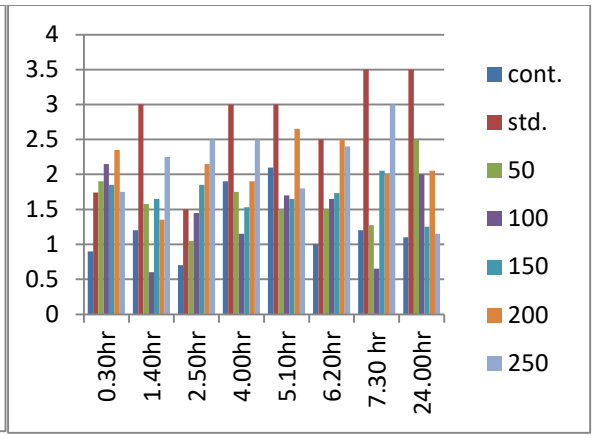


Figure 5: Effect of MEBA on time spent in open arm in LD model

Flavonoids have shown anti-anxiety activity in various studies. Further, the anxiolytic effect of flavonoids has been attributed to its effect on central nervous system and benzodiazepine receptors. Therefore, flavonoids of extracts of *B. alba* may be responsible for the anti-anxiety activity.

4. CONCLUSION

These findings establish the potential of the selected plant as CNS activity and scientifically proved its traditional claim. Hence, the present study concludes that the selected plant directs the importance of further development of some potential anxiolytic drugs as well as their mechanism of action.

5. ACKNOWLEDGEMENT

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