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PHYTOPHARMACOLOGICAL EVALUATION OF *BUTEA MONOSPERMA* AS AN ANTI ANXIETY AGENT

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ABSTRACT

Butea monosperma plant has been used for the anxiolytic activity. Then the acute toxicity of the extract of higher dose of 2000 mg/kg has been performed on the albino mice for 14 days. The ethanolic extract of *Butea monosperma* was evaluated for anxiolytic activity in mice. In open field test in which mice treated with dose 250 and 500 mg/kg of *Butea monosperma* showed dose dependent increase in open field ambulation, rearing, self grooming and center activity with compared to vehicle treated control mice, producing significant anxiolytic activity of *Butea monosperma* extract at the dose of 500mg/kg. In hole cross test in which a significant increase in the exploratory hole crossing behavior were observed after treatment with 500 mg/kg of *Butea monosperma* but there is no change at the dose of 250 mg/kg. In elevated plus maze model in which *Butea monosperma* treated mice exhibited dose dependent significant increase in time spent in open arm and entries in open arms at the dose of 500 mg/kg in comparison to control mice. In novelty induced suppressed feeding latency test in this test the latency to eat food decreased at the dose of 500 mg/kg in the novel environment as compared to control group animals. Fifth model was social interaction in this mice treated with *Butea monosperma* dose of 250 and 500 mg/kg spent significantly more time in social interaction at dose 500 mg/kg in comparison to control mice and effect of *Butea monosperma* extract was found to be dose dependent.

KEYWORDS: *Butea monosperma*, Anxiety, Elevated plus Maze**,** Open Field Test, γ-Aminobutyric acid.

INTRODUCTION

Anxiety

Anxiety is a psychological and physiological state characterized by somatic, emotional, cognitive, and behavioral components. The root meaning of the word anxiety is to vex or trouble; in either presence or absence of psychological stress, anxiety can create feelings of fear, worry, uneasiness and dread. (National institute of mental health, 2008) Anxiety is considered to be a normal reaction to a stressor. It may help someone to deal with a difficult situation by prompting them to cope with it. When anxiety becomes excessive, it may fall under the classification of an anxiety disorder. (Ohman, 2000).

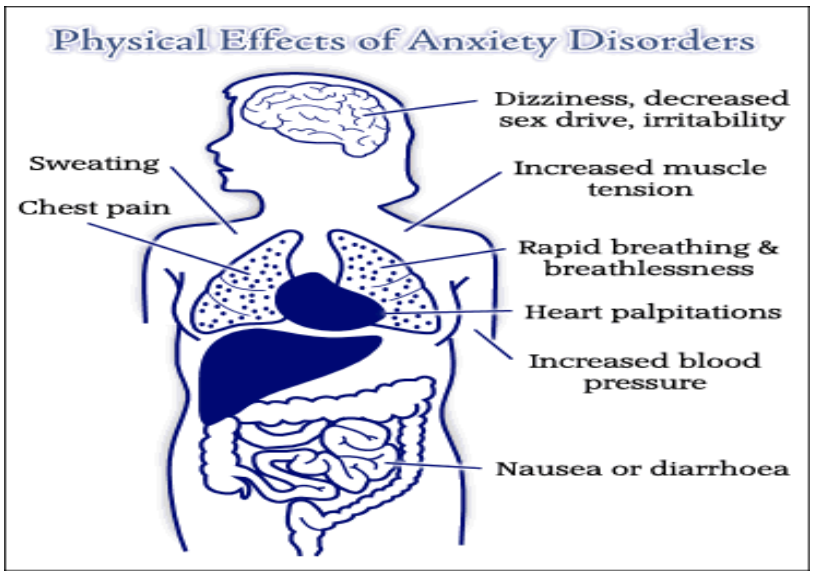


Fig. 1.1: Physical effect of anxiety disorder.

Common Anxiety Disorders

Generalized anxiety disorders (GAD)

Obsessive-Compulsive Disorder (OCD)

Post-Traumatic Stress Disorder (PTSD)

Butea monosperma - dhaak

Plant Profile

Photochemical constituents

Triterpene (Mishra, M., Yogendra, S., 2000), several flavonoids butein, butin, isobutrin, coreopsin, isocoreopsin (butin 7-glucoside), sulphurein, monospermoside (butein 3-e-D-glucoside) and isomonospermoside, chalcones,aurones, isobutyine, palasitrin, 3',4',7-trihydroxyflavone (Gupta, S. R., Ravindranath, B.,1970). Myricyl alcohol, stearic, palmitic, arachidic and lignoceric acids (Murti, P. Bhaskara, R., 1940), glucose, fructose, histidine, aspartic acid, alanine and phenylalanine.

MATERIAL AND METHODS

Plant Materials

Fresh leaves of *Butea monosperma* were collected from local area of Lucknow (India) in the month of November 2011. The Sample was authenticated and a voucher specimen no. NBRI/CIF/258/2011 has been deposited at the National Botanical Research Institute (NBRI), Lucknow. The Sample was finely powdered in a blender, weighed and stored in a dry polythene bags.

Drugs and Chemicals

The following drugs were used: diazepam and *Butea monosperma* preparation, 95% methanol extract of *Butea monosperma* leaves, resulting in a dry powder containing 40% butrin, isobutrin and sodium carboxymethyl cellulose. The animals received either diazepam (2.5 mg/kg), (adjusted to 250 and 500 mg/kg *Butea monosperma* or their respective vehicle 60 min prior testing. All drugs were suspended ultrasonically in vehicle immediately prior to use. The drugs were given orally in a volume of 10 ml/kg.

Preparation of Extract

The powder of *Butea monosperma leaves* was extracted by Soxhlet apparatus using solvent petroleum ether (60-80^{0}C) and followed by chloroform and methanol. Accurately weighed 50 gm of powder was placed in Soxhlet extractor. About 750 ml of solvent was used for extraction. The progress of the extraction was evaluated by applying spot of extract on thin layer chromatography plate. The thin layer chromatography was performed using silica gel plate and the plate was visualized in UV-chamber followed by iodine chamber. The extracts were filtered and concentrated by rotary evaporator and finally dried at very low pressure. The Phytochemical test was performed for extract 14-18 hour.

PHYTOCHEMICAL SCREENING

Test for Alkaloids

To 0.1ml of the extract and fractions in a test tube, 2– 3 drops of Dragendoff's reagent was added. An orange red precipitate with turbidity confirmed the presence of alkaloids (Ciulci, 1994).

Test for Flavanoids

To 4mg/ml of the extracts and fractions a piece of magnesium ribbon was added followed by drop-wise addition of concentrated HCl. A colour change from orange to red indicated the presence of flavones; red to crimson indicated the presence of flavonoids. (Sofowora, 1993).

Test for Glycosides

Ten millilitres of 50% H_2SO_4 was added to 1ml of the filtrate in separate test tubes and the mixtures heated for 15mins followed by addition of 10ml of Fehling'ssolution and boiled. A brick red precipitate indicated presence of glycosides (Sofowora, 1993).

Test for Reducing Sugars

To 1ml of extract and fractions in separate test tubes,2.0mls of distilled water were added followed by addition of Fehling's solution (A + B) and the mixtures were warmed at 40oC. Appearance of brick red precipitate at the bottom of the test tube indicated the presence of reducing sugar (Brain and Turner, 1975).

Test for Saponins

Half gram of the powdered leaf was dispensed in a test-tube and 5.0ml of distilled water was added and shaken vigorously. A persistent froth that lasted for about 15 minutes indicated the presence of saponins (Brain and Turner, 1975)

Test for Steroids

Two millilitres of the extracts were evaporated to dryness in separate test tubes and the residues dissolved in acetic anhydride followed by addition of chloroform. Concentrated sulphuric acid was added by means of a pipette via the side of the test tubes. Formation of brown ring at the interface of the two liquids and violet colour in the supernatant layer denoted the presence of steroids (Ciulci, 1994**).**

Test for Tannins

Two millilitres of the extract/fraction was diluted with distilled water in separate test tubes, 2 – 3 drop of5% ferric chloride ($FeCl_3$) solution was added. A green – black or blue colouration indicated tannin (Ciulci, 1994).

MODELS FOR ANXIOLYTIC ACTIVITY

Elevated plus Maze (EPM)

The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. The apparatus consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 12 cm). The arms extended from a central platform (5 cm × 5 cm) and maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by the mouse to move into any one of the covered arms with all its four legs. TL was recorded on the first day. If the animal did not enter into one of the covered arms within 90 sec., it was gently pushed into one of the two covered arms and the TL was assigned as 90 sec. The mouse was allowed to explore the maze for 10 sec and then re-turned to its home cage. Memory retention was examined 24 h after the first day trial on the second day (Parle M, Singh N, et.al, 2004).

Open Field Test

The effect of the extract and fractions on locomotor activity, exploration and grooming was studied in the open field. Male mice (19 - 30 g) selected at random were divided into groups (n = 5) to receive oral administration of extract of papaya leaf. The control groups received either diazepam (1 mg/kg i.p) or the vehicle (10 ml/kg p.o). Thirty minutes after treatment, each mouse was placed in the centre square of the open field and observed for 5 min with the aid of video camera. Behavioral parameters recorded include line crossing, centre square entries, rearing (in the air and against the wall) and stereotype as shown by frequency and duration of grooming. The floor of the open field was cleaned with 70% ethanol and allowed to dry between tests (Archer J 1973).

Hole Cross Test

A steel partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. The number of passage of a mouse through the hole from one chamber to other was counted for a period of 3

min at 0, 30, 60, 90 and 120 min after the oral treatment with *papaya leaf* extracts (Takagi K, Watanabe M and Saito H, et al 1971).

Social Interaction Test

Male Sprague-Dawley rats (225–275 g body weight) are housed in groups of 5 animals the apparatus used for the detection of changes in social behavior and exploratory behavior consists of a Perspex open-topped box (51 × 51 cm and 20 cm high) with 17 ×17 cm marked areas on the floor. One hour prior to the test, two naive rats from separate housing cages are treated with the test compound orally. They are placed into the box (with 60 W bright illumination 17 cm above) and their behavior is observed over a 10-min period by remote video recording. Two types of behavior can be noted: (Albert's, J.R. 1974)

ACUTE TOXICITY STUDIES

Acute oral toxicity studies revealed the nontoxic nature of *Butea monosperma*. There was no morbidity observed or any profound toxic reactions found at a dose of 2000 mg/Kg p.o. which indirectly pronouns the safety profile of the plant extract.

The acute toxicity study was carried out by guidelines set by OECD 420 guidelines. Albino male and female mice (25-35g) maintained under standard laboratory condition were used. A total number of six animals were used per group which received a single dose (2000 mg/kg, b.wt) of herbal drug.

Principle of the Test

Groups of animals of a single sex are dosed in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg. The initial dose level is selected on the basis of a sighting study as the dose expected to produce some signs of toxicity without causing severe toxic effects or mortality. Clinical signs and conditions associated with pain, suffering, and impending death, are described in detail in a separate OECD Guidance Document. Further groups of animals may be dosed at higher or lower fixed doses, depending on the presence or absence of signs of toxicity or mortality. This procedure continues until the dose causing evident toxicity or no more than one death is identified, or when no effects are seen at the highest dose or when deaths occur at the lowest dose (OECD, 2000).

Preparation of animals

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days prior to the start of dosing to allow for acclimatization to the laboratory conditions (OECD, 1992).

3. RESULTS

Phytochemical Analysis

It comprises of different chemical tests and chemical assays. The isolation, purification and identification of active constituents are chemical methods of evaluation. Quantitative chemical tests such as ash value and extractive values come under these techniques. The qualitative chemical tests are useful in detection of adulteration (Kokate et al., 1999).

The purity of crude drugs is ascertained by quantitative estimation of active chemical constituents present in them.

Qualitative Chemical Tests

The extracts obtained are subjected to qualitative tests for the identification of various plant constituents. The preliminary phytochemical analysis of *Butea monosperma* showed that the plant contains carbohydrates, saponin, alkaloid, tannin, phenols and glycoside are present but steroids are absent. The constituents of which are present or absent are summarized in table 3.1.

Chromatography Techniques

It is necessary to use a two- dimensional chromatography using two different solvent systems. The resolved components of original mixture can be separately eluted from chromatogram by treating the cut out spots with a suitable solvent and then determined qualitatively by some suitable instrumental method of analysis. (Kokate et al., 2005).

Table 3.1 Phytochemical analysis of *Butea monosperma.*

S.NO.	Components	Chemical Tests	*Butea Monosperma* Extract
1.	Alkaloids	Wagner's test	+
2.	Amino acids	Ninhydrin test	-
3.	Carbohydrates	Fehling's test	+
4.	Tannins and Phenolic compounds	Ferric chloride test	+
5.	Terpenoids	Salkowski's test	-

6.	Cardiac glycosides	Kellar kiliani test	+
7.	Fixed oils and fats	Spot test	-
8.	Steroids	Chloroform test	-
9.	Saponins	Foam test	+

Thin Layer Chromatography

Thin layer chromatography (TLC) has been performed and the after spraying the dragandroff's reagent the three yellowish brown spots are obtained and the retention factors (R_f) values has been calculated.

To screen for the presence of known constituents, *Butea monosperma* extract is chromatographed on silica gel. Samples were spotted onto silica gel 60 F254, developed in ethyl acetate: formic acid: acetic acid: water (100:11:11:26) spots with retention factor (R_f) value of 0.22, 0.32, 0.40, 0.44, 0.65, 0.78, 0.90 and 0.96 were collected for further analysis. In this solvent system, R_f values of 0.32 and 0.45 correspond to butrin and isobutrin respectively.

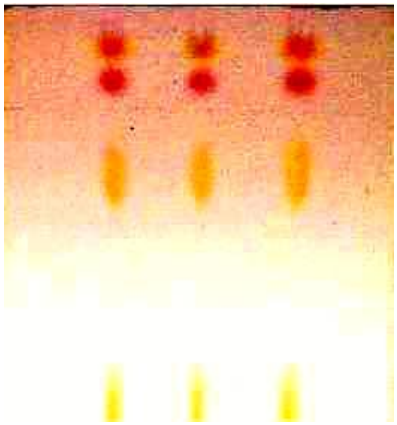


Fig. 3.1: TLC Plate of *Butea monosperma.*

HPTLC ANALYSIS

The chromatographic spectrum of the crude methanol extract of *Butea monosperma* obtained revealed major peaks at the following retention factors: 0.10, 0.40, 0.48, 0.60 and 0.64 (Fig. 3.2). The reference standard, butrin, showed a major peak at the retention factor: 0.64 (Fig. 3.2). Butrin was used to standardize the retention factor. The selected leave extracts were dissolved in methanol and the sample was separated on thin layer Chromatographic plates. Mobile phase was decided based on the separation of compounds on the plates ethyl acetate: formic acid: acetic acid: water (100:11:11:26). Samples were loaded on the thin layer Chromatographic plates and taken for HPTLC processing. *Butea monosperma* methanol

extract showed five compounds having an RF value of 0.10, 0.40, 0.48, 0.60 and 0.64 under λ max at 256 nm and 345nm. The compound 5 appeared to be the major compound with 65.31% area. Results were observed in HPTLC monitor and analyzed using CAMAG software.

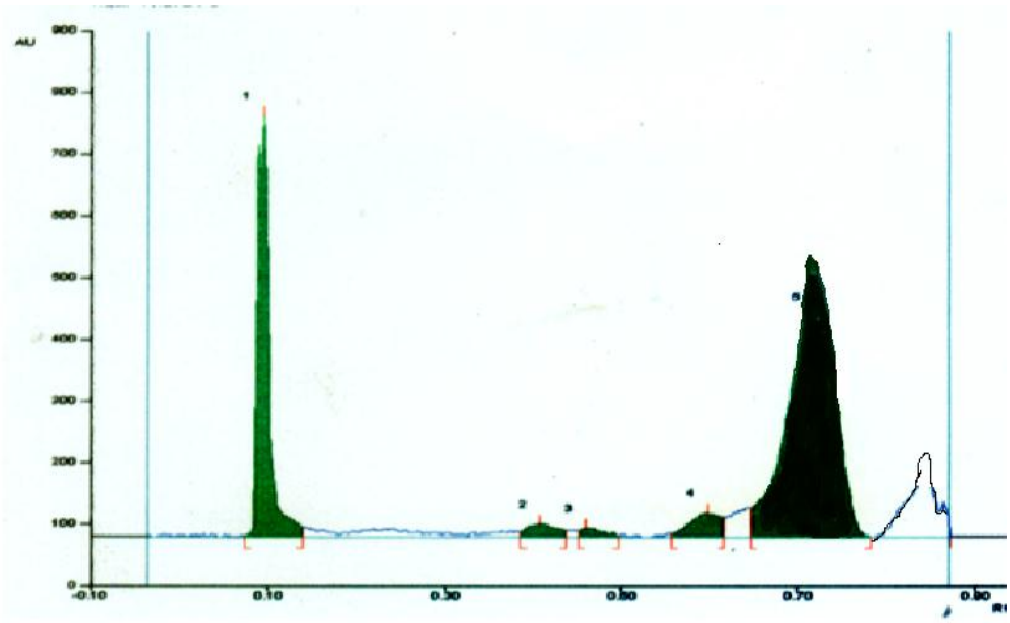


Fig. 3.2: HPTLC analysis of methanolic extract of *Butea monosperma.*

Acute Toxicity Studies

The test animals did not exhibit any visible change and survived beyond recommended duration of observation. Hence, *Butea monosperma* extract was safe up to 2000 mg/kg were selected for further experimentation.

Hematological Analysis

Hematological values measured shows a significant elevation of HGB level and RBC level in treatment group. The value of HCT was significant increased as compared with the control group. Other hematology values WBC, MCV, MCH, MCHC, Lymphocyte and PLT were not significantly different as compare to the control mice and they remained normal limits (control values).

Organ Weight

No toxic symptoms or death were observed in any of the animals and they lived up to 14 days. The end of the experimental period revealed no apparent changes in any organ.

Histopathological Analysis

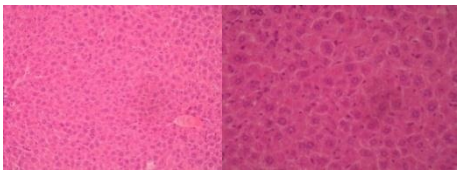
The photomicrographs of liver, kidney, heart, spleen and brain sections from control and experimental rats stained with haematoxylin and eosin are shown below. The tissue sections of the experimental animals were essentially normal when compared with the control sections (Fig.3.2 and Fig.3.3).

Table 3.2: Effect of intake of extract of leaf of *Butea monosperma* on some hematological parameters.

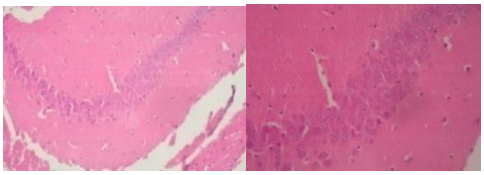
Parameters	Control	2000 mg/kg extract of BM
WBC	7.83±0.236	12.21±1.101
Lym.	57.1±0.266	75.40±0.075
Mon.	3.40±0.141	3.58 ±0.133
Gra.	39.6±0.115	26.10±0.262
RBC	8.83± 0.236	8.240±0.0281
MCV	47.5±0.096	58.30±0.150
Hct	41.9±0.134	62.00±0.065
MCH	14.8± 0.236	14.30±0.080
Mchc	31.2± 0.165	27.90±0.114
Mrbc	0.00±0.00	0.00 ±0.00
Mrbc	0.00±0.00	0.10 ±0.030
RDW	8.70±0.20	8.40 ±0.124
Hgb	13.1±0.266	12.70±0.155
PLT	50.7± 0.20	49.00±0.160
Mpv	5.00±0.115	5.30 ±0.101
PCT	0.30±0.060	0.30 ±0.068

Table 3.3 Effect of extract of *Butea monosperma* on animal organ.

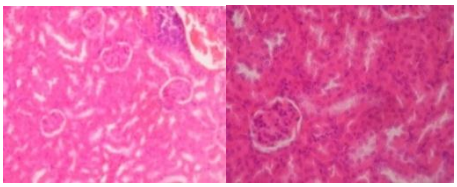
Organ	Control	Extract of BM
Liver	1.550±0.228	1.745 ± 0.211
Spleen	0.205 ± 0.053	0.370 ± 0.010
Brain	0.425 ± 0.038	0.465 ± 0.081
Heart	0.215 ± 0.00002	0.200 ± 0.00001
Kidney	R-0.330 ± 0.039 L-0.315 ± 0.052	R-0.310 ± 0.056 L-0.300 ± 0.057



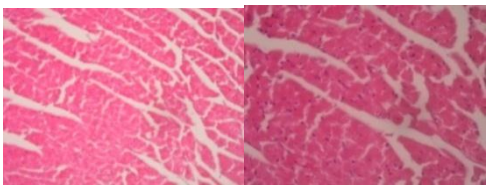
Liver (3.2 a)



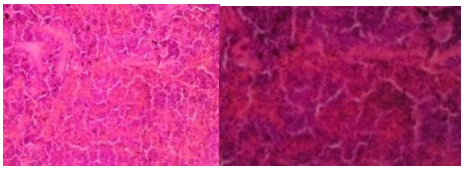
Brain (3.2 b)



Kidney (3.2 c)

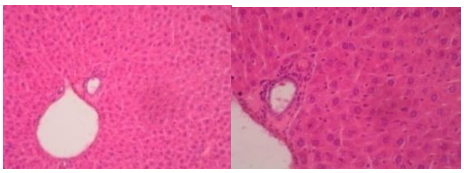


Heart (3.2 d)

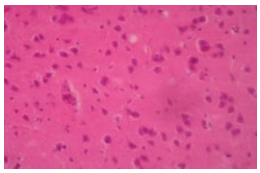


Spleen (3.2 e)

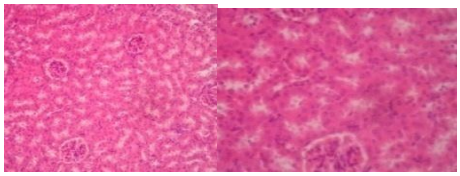
Figure 3.2: Photo 3.2 a, 3.2 b, 3.2 c, 3.2 d and 3.2 e (×20 and ×40) were sections of Liver, brain, Kidney, heart, spleen respectively of animals that received 2000 mg/kg of the extract and stained with H&E.



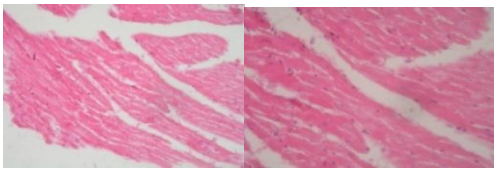
Liver (3.3 a)



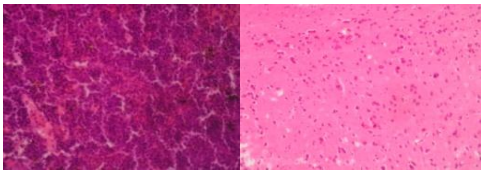
Brain (3.3 b)



Kidney (3.3 c)



Heart (3.3 d)



Spleen (3.3 e)

Figure 3.3: Photo 3.3 a, 3.3 b, 3.3 c, 3.3 d and 3.3 e (×20 and ×40) were sections of Liver, brain, kidney, heart and spleen respectively of control animals.

No alteration were observed in the organs of the control animals as well as treated with 2000 mg/kg of *Butea monosperma.*

PHARMACOLOGICAL ACTIVITY ASSESMENTS

Open Field Test

Mice treated with both the dose 250 and 500 mg/kg of *Butea monosperma* showed dose dependent increase in open field ambulation, rearing, self grooming and activity in center with compared to vehicle treated control mice, evincing significant anxiolytic activity of *Butea monosperma*. However the open-field fecal droppings remain unchanged. Diazepam also induced significant anxiolytic activity and the effects were found to be more than that of *Butea monosperma* (Table 5). In an open field, animals are in a novel environment, they express decreased ambulation, exploration, freezing, rearing and grooming behaviour, and increased defecation due to anxiety and fear which heightened autonomic activity, these behavioral changes are attenuated due to classical anxiolytic and augmented by anxiogenic agents. Observations and results are summarized in table 3.4.

Hole cross test

In the Hole cross model a significant increase in the exploratory hole crossing behavior were observed after treatment with 250 and 500 mg/kg of *Butea monosperma*, thus reinforcing the hypothesis that it has anxiolytic activity. The extract at doses level of 250mg/kg and 500mg/kg body weight showed significant ($p < 0.001$) decrease of movement from its initial value during the period of hole cross experiment as compared to control (Table 3.5).

Elevated plus maze test

In elevated plus maze open closed arm entries and time ratios provide a measure of fear induced inhibition of exploratory activity. These responses are increased by anxiolytic agents.[13] *Butea monosperma* treated mice exhibited dose dependent significant increase in time spent in open arms, entries made on open arms and decrease in time spent enclosed arms and entries on enclosed arms in comparison to control mice. The result obtained by open/closed time and entries ratios also indicated significant anxiolytic in mice by *Butea monosperma*. Diazepam caused more anxiolysis in comparison to *Butea monosperma*. Methanolic extract of *Butea monosperma* significantly increased mean number of entries and mean time spent by mice in open arms of elevated plus maze apparatus at the dose of 250 and 500 mg/kg with respect to control, thereby producing anti-anxiety activity. The results are given in Table 3.6 a and 3.6 b.

Table 3.4: Effect of purified methanol extract of *Butea monosperma* on open field exploratory behavior in mice.

Treated groups	Dose	Ambulation	Rearing	Self grooming	Activity in center square
Control	CMC	22.3±0.33	23±0.55	18.6±2.45	10.6±1.66
Extract BM	250mg/kg	32.3±1.45*	29±1.52#	25.6±1.45*	16±1.85#
Extract BM	500mg/kg	48.6±2.60*	43±2.05*	27.6±1.85*	19.6±1.31**
Diazepam	2mg/kg	53±0.99*	53.6±4.81*	27.6±2.11*	31.6±2.51*

Values are mean ±SEM, (n = 6); ** $p < 0.01$, *$p<0.001$, # $p<0.05$ as compared to control

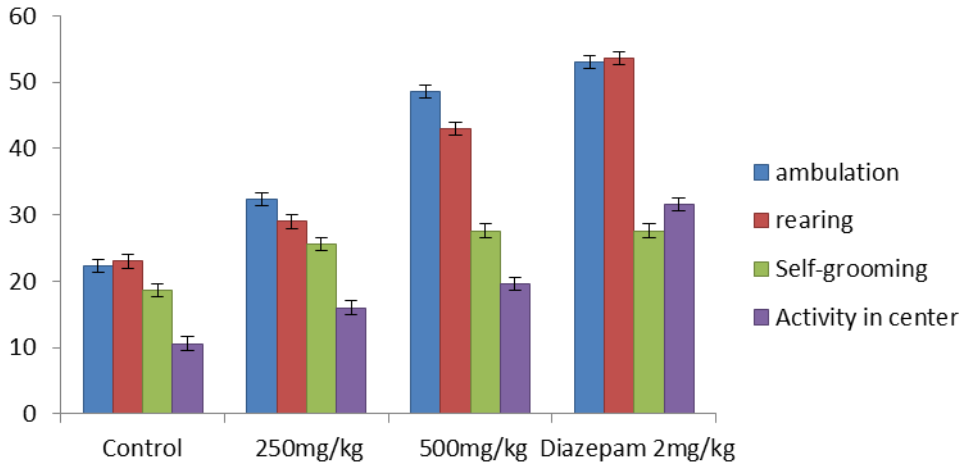


Figure 3.4 Effect of purified methanol extract of *Butea monosperma* on open field exploratory behavior in mice.

Values are mean ±SEM, (n = 6); ** $p < 0.01$, *$p<0.001$, # $p<0.05$ as compared to control.

Table 3.5 Effect of purified methanol extract of *Butea monosperma* on Hole cross test in mice.

Group	No. of movements				
	0 min	30 min	60 min	90 min	120 min
A. Control	5.33±0.87	3±0.22	6.33±0.12	3.66±0.5	4.00±0.23
B. Dose 250mg/kg	9±1.52#	8±1.01**	7.66±0.33#	5.66±1.16#	4.66±0.88#
C. Dose 500mg/kg	13.7±1.22**	9±0.578**	14±1.478**	7±0.578**	6.70±0.34**
D. Diazepam 2mg/kg	17.6±2.85*	18.33±2.12*	19.33±3.24*	9±1.12**	8.66±2.31*

Values are mean ±SEM, (n = 5); ** $p < 0.01$, * $p<0.001$, # $p<0.05$ as compared to control.

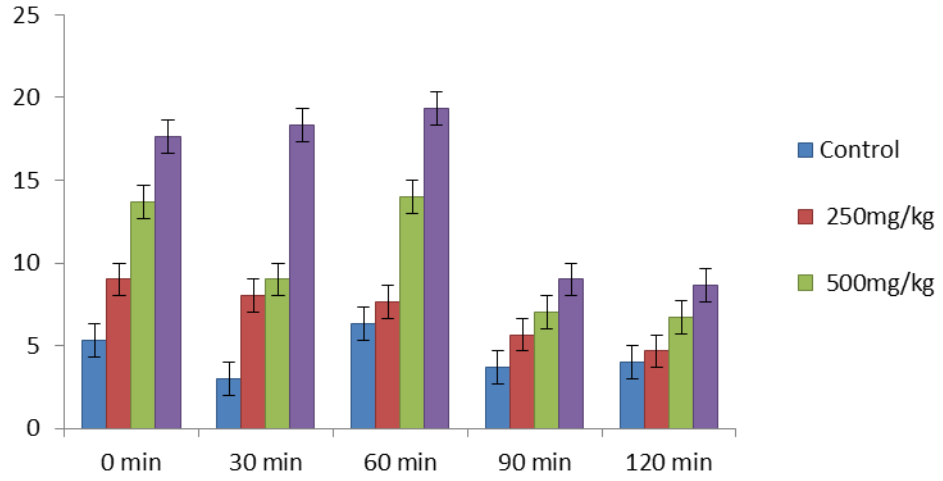


Figure 3.5 Effect of purified methanol extract of *Butea monosperma* on hole cross test in mice.

Values are mean ±SEM, (n = 6); ** $p < 0.01$, *$p<0.001$, # $p<0.05$ as compared to control

Table 3.6: Effect of *Butea monosperma* extract on the elevated plus maze behavior in mice.

Group	Time spent(sec)		no. of enteries	
	Enclosed arms	Open arms	Enclosed arms	Open arms
A. Control	127.33±5.65	17±5.13	10±661.45	3.33±1.23
B. Dose 250mg/kg	102±2.230*	26±1.63$^{\#}$	9.5±0.687$^{\#}$	6.8±1.87**
C. Dose 500mg/kg	98±3.600*	57±2.5*	6.9±0.210*	13.6±0.41*
D. Diazepam 2mg/kg	57±4.368*	87±4.368*	4.0±0.308*	24±0.25*

Values are mean ±SEM, (n = 5); ** $p < 0.01$, * $p<0.001$, # $p<0.05$ as compared to control.

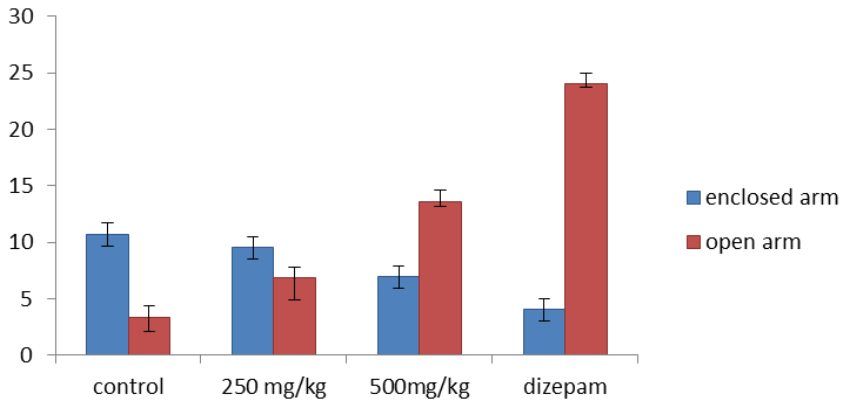


Figure 3.6 a: Effect of purified methanol extract of *Butea monosperma* on the elevated plus maze(no. of entries) behavior in mice.

Values are mean ±SEM, (n = 6); ** $p < 0.01$, *$p<0.001$, # $p<0.05$ as compared to control.

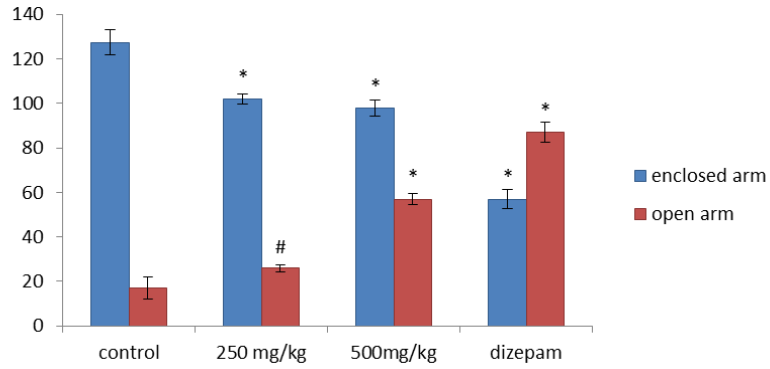


Figure 3.6 b: Effect of purified methanol extract of *Butea monosperma* on the elevated plus maze(time spent) behavior in mice.

Values are mean ±SEM, (n = 6); ** $p < 0.01$, *$p<0.001$, # $p<0.05$ as compared to control

Social Interaction Test

The mice treated with *Butea monosperma* dose of 250 and 500 mg/kg spent significantly more time in social interaction in comparison to control mice and effect of *Butea monosperma* extract was found to be dose dependent. Diazepam also caused significant increase in social interaction (table 8). Diazepam also induced similar effects, however, it was observed to be more than that of *Butea monosperma* extract.

Table 3.7: Effect of *Butea monosperma* extract on the social interaction in mice.

Treated groups	Dose	Crawling	Sniffing	Following	Grooming
Control	CMC	12.33±1.45	15+1.52	14.33+0.882	16+1.52
Extract BM	250mg/kg	12.10±1.0#	21±0.40**	30±0.91*	18±0.57#
Extract BM	500mg/kg	13.33±1.52#	23±0.30**	34±2.084*	25±2.347**
Diazepam	1mg/kg	27.33±1.76*	33±3.21*	25.33±2.6*	28+1.52*

(n = 5); ** $p < 0.01$, * $p<0.001$, # $p<0.05$ as compared to control.

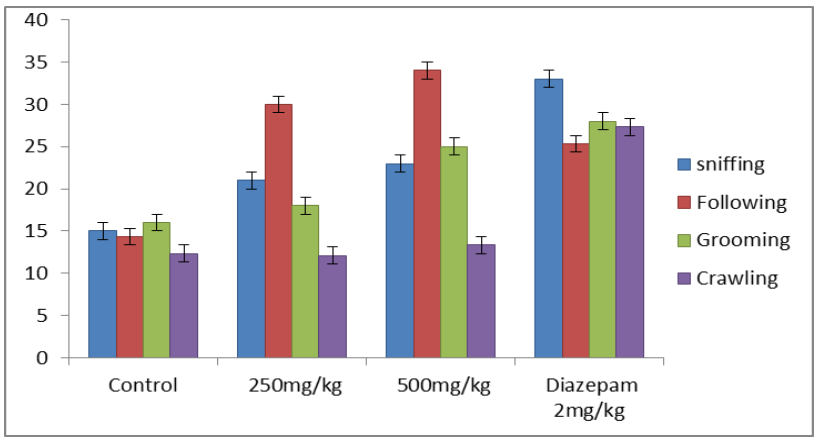


Figure 3.7: Effect of purified methanol extract of *Butea monosperma* on social interaction behavior in mice.

(n = 5); ** $p < 0.01$, * $p<0.001$, # $p<0.05$ as compared to control.

Novelty Induced Suppressed Feeding Latency Test

The *Butea monosperma* of dose 250 and 500 mg/kg caused dose dependent significant decrease of novelty induced feeding latency in mice in comparision to control. There is a decrease in feeding latency in novel environment. Diazepam also caused the decrease in feeding latency more than the control and extracts.

Table 3.8: Effect of *Butea monosperma* extract on latency to feed in mice.

Treated groups	Dose	Latency to eat in novel cages (sec)
Control	CMC	82+4.13
Extract BM	250mg/kg	22±3.53*
Extract BM	500mg/kg	36±3.53*
Diazepam	1mg/kg	51+4.58*

(n = 5); ** $p < 0.01$, * $p<0.001$, # $p<0.05$ as compared to control.

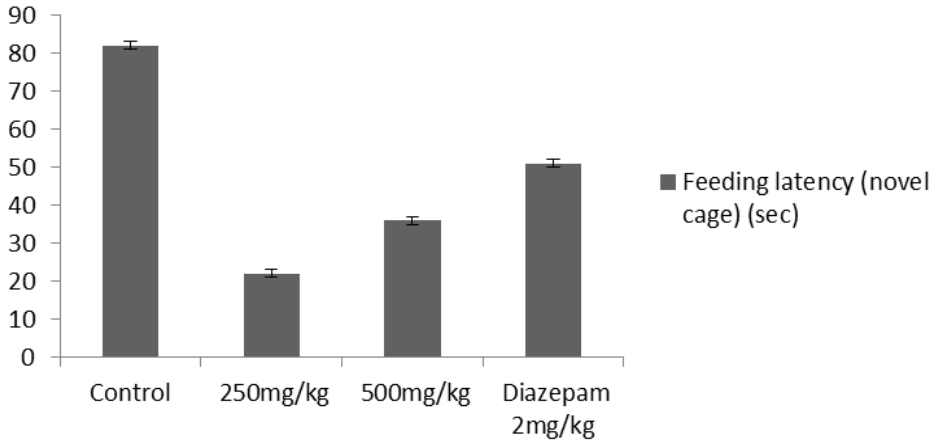


Figure 3.8: Effect of purified methanol extract of *Butea monosperma* on novelity Induced Suppressed Feeding Latency Test in mice.

(n = 5); ** $p < 0.01$, * $p<0.001$, # $p<0.05$ as compared to control.

4. DISCUSSION

The anti-anxiety activity of methanolic extract of *Butea monosperma* leaves (MEBM) was evaluated by five screening methods widely used for testing BZDs namely Elevated plus Maze (EPM), Open Field Test, Hole Cross Test, Social Interaction Test, and Novelty Induced Suppressed Feeding Latency Test. These are animal models of preliminary pharmacological test of activities on central nervous system, which provide information about action upon

psychomotor performance, motor behavior and anxiolysis. For anxiety most of the animal models were developed for benzodiazepines (BDZ) and, since these compounds also exhibit significant muscle relaxant and anticonvulsant effects, evaluation of anxiolytic activity, even with non-BDZ compounds, invariably now includes tests for these neuropharmacological actions (Wada et al, 1989).

The effect of BME treatments on the behaviour of albino mice in the open-field, elevated plus maze, hole cross test, feeding latency and social interaction tests are respectively shown in Tables 4.1- 4.5. BME dose 250mg/kg and 500mg/kg treatments caused significant reduction in ambulation and activity in the centre squares, and increase in self-grooming and immobility in the open field, in comparison to the control treatment. In the elevated plus maze test, BME -treated albino mice spent significantly more time, and made more number of entries to the enclosed arms, with concomitant less time and fewer number of entries to the open arms, as compared to control rats. The results of the ratio between open arm and enclosed arm time and entries also indicated that both BME dose 250mg/kg and 500mg/kg caused significant anxiogenic behaviour in albino mice.

Similarly, in the elevated zero maze test, BME dose 250mg/kg and 500mg/kg treated albino mice made significantly fewer entries, spent less time on the open arms and showed a significant decrease in the number of head dips and stretched attend postures in comparison to control rats. The drug treatments also had significant effect on novelty induced suppressed feeding (Table 4.5). BME treatments caused significantly enhanced feeding latencies in comparison to control treatment in the novel environment. They also reduced the social interaction time in paired rats in comparison to the control group, but the results are not statistically significant (Table 4.4).

Anxiety is a psychological, physiological, and behavioral state induced in animals and humans by a threat to wellbeing or survival, either actual or potential. It is characterized by increased arousal, expectancy, autonomic and neuroendocrine activation, and specific behavior patterns. The function of these changes is to facilitate coping with an adverse or unexpected situation.

γ-Aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter in the brain. The GABA A benzodiazepine receptor is an important target for several anxiolytic drugs and may therefore play an important role in anxiety-related disorders (Nutt DJ, Malizia AL,

2001). Several GABAA receptor subtypes have been described. (Mohler H, Crestani F, 2002; Mohler H, Fritschy JM, 2002). The diazepam-sensitive α2-GABAA subtype appears to be specifically involved in anxiolysis (Mohler H, Crestani F, 2002). This subtype is largely expressed in the hippocampus, the amygdala, and the striatum (Rudolph U, 2002).

The findings of the present study clearly indicate that the methanolic extracts at a dose of 250 mg/kg and 500 mg/kg significantly improve the condition of anxiety of the learned task as was seen in the increase in the percent ARs, thus demonstrating anti-anxiety activity. The anxiolytic represent a class of agents that facilitate the integrative functions of the CNS, particularly the psychological, physiological, and behavioral state.

In our studies, alcoholic extracts produce significant change in the exploratory activity of the rats in the "Elevated plus Maze (EPM), Open Field Test, Hole Cross Test, Social Interaction Test, and Novelty Induced Suppressed Feeding Latency Test". This indicates the presence of anxiolytic activity in Butea monosperma.

Earlier studies conducted by various workers have revealed that several medicinal plants possess anti-anxiety activity, but only a few plants like W. somnifera possess both antistress (Singh et al., 1982) as well as nootropic activity (Bhattacharya et al., 1995). Similarly, our studies demonstrate that the plant Butea monosperma possesses a combination of activities. As discussed earlier, these properties are complementary to each other and hence the plant could be a valuable contribution to the existing armamentarium of anxiolytic agent having antistress activity.

In elevated plus maze, mice will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces. Drugs that increase open arm exploration are considered as Anxiolytics and the reverse holds true for anxiogenics (Hellion et.al, 2006). Extract shows the similar effects of the anxiolytics as shown by the diazepam. So it can be predict that the mechanism of action is as similar as diazepam by acting on the GABA and benzodiazepine receptors.

Likewise, anxiolytics increase the social interaction and decrease the feeding latency respectively in the social interaction and novelty induced suppressed feeding latency tests in a novel environment (Bhattacharya et al, 1997). Novel environment gives a fear response, a

state of anxiety in experimental animals which decreases their normal social behaviour and this can be overcome with the treatment of anxiolytics. In social interaction test the extract shows the positive response for the anxiolytic activity as compared to the control. In novelty induced suppressed feeding latency test the feeding latency decreases in both diazepam and extract as compared to the control.

Overall, the results of the present study conclude that methanolic extract of Butea monosperma leaves (MEBM) treatment caused significant anxiolysis in rats tested on all the behavioral paradigms. However the anxiolytic activity of Butea monosperma leaves (MEBM) extracts were found to be less marked than that of the common BDZ anxiolytic agent Diazepam.

CONCLUSION

In conclusion, results of the present study on methanolic extract of B. monosperma leaves showed a significant anti-anxiety activity, when given at 250 and 500 mg/kg dose, therefore it can be said that MEBM may reduce the risk of anxiety related disease.

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