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ANXIOLYTIC EFFECTS OF BACOPA MONNEIRI L. IN ANIMAL MODELS OF POSTTRAUMATIC STRESS DISORDER

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Abstract: The present study was conducted to explore the role of *Bacopa monneiri* in posttraumatic stress disorder (PTSD) in mice. Swiss albino mice were subjected to a 2-day electric foot-shock stress of 5 min, which included 15 alternating inescapable foot shocks of 0.8 mA intensity with 10-s duration and 10-s intershock interval on the electrified grid floor. It was followed by 3 week re-exposures (on day 2, 7, and 14) in the same context (as situational reminders) for 5 min without delivering any foot-shocks. Trauma and situational reminders results a significant development of behavioral deficits as assessed on the 21st day. A significant development of freezing behavior was also observed in response to situational reminders on 2nd, 7th and 14th days. Repeated administration of hydroalcoholic extract of *Bacopa monneiri* (HEBM) for 21 days of (50 mg/kg, 100 mg/kg and 200 mg/kg), diazepam (DZP) 1 mg/kg and flouxetine (FLX) 15 mg/kg considerably restored the behavioral changes evaluated on elevated plus maze, open field and contextual fear paradigm. *Bacopa monneiri* led to significant reduction in the freezing behavior in response to situational reminders suggesting the inhibition of formation of aversive fear memory. It may be concluded that *Bacopa monneiri* L. may be beneficial in preventing the PTSD symptoms in response to a traumatic event.

Index terms: Behavior, foot-shock trauma, memory, posttraumatic stress disorder, *Bacopa monneiri*

1. Introduction

Post-traumatic stress disorder (PTSD) is among the most psychiatric illnesses that cause the affected individual great stress and other complications. Individuals that are subjected to serious trauma such as rape, wartime combat and motor vehicle accident that threatens physical harm or death are at greater risk of developing post-traumatic stress disorder. Individuals that experience a trauma of this nature may develop symptoms that fall into three distinct clusters: reexperiencing phenomenon; avoidance and numbing; and autonomic hyperarousal [1]**.** Some of the symptoms of PTSD include intrusive flashback memories, persistent anxiety, hyperarousal and cognitive impairments. People who develop PTSD respond to a traumatic experience with intense fear, helplessness or horror and subsequently endure chronic psychological distress by repeatedly reliving their trauma through intrusive, flashback memories [2-7]. These intrusions are frequently precipitated by the presence of cues associated with the traumatic event; therefore, PTSD patients make great efforts to avoid stimuli that remind them of their trauma. Several therapies to avoid and treat patients with PTSD have been suggested. For the treatment of PTSD symptoms, selective serotonin reuptake inhibitors, sertraline and paroxetine, and serotonin-norepinephrine reuptake inhibitor agents such as venlafaxine have been approved [8]. Despite their effectiveness against stressful symptoms caused by PTSD, their use is limited because of the number of adverse effects [which] emphasize the need for more research on therapeutic targets in this area [9].

Bacopa monneiri L. is a small perennial creeping herb of scrophulariaceae family. It has small oblong leaves and light purple flowers. It grows in wet and sandy area. Flowers and fruits appear in summer.

The whole plant is used in traditional medicine. Brahmi is the Sanskrit name for the herb *Bacopa monnieri*. It has been used in ayurvedic medicine, since centuries [10, 11]. The plant extracts has been used for enhancing memory and improving functions of brain [12, 13], Cognitive enhancer [14, 15] antioxidant [16, 17], antiulcerogenic agent [18], anti-inflammatory, anxiolytic [19]. Studies have reported antidepressant activities of *Bacopa monneiri* [20-22].

2. Materials and methods: To execute and achieve the aim and objectives of this study, the following materials and methods were employed

2.1 Experimental Animals

Swiss albino mice of either sex (weighing 20-25g) of 8-10 weeks age were used for the study, which were obtained from the animal house of Department of Pharmacology, Vidyabharati College of Pharmacy, Amravati. All the animals were acclimatized to the animal house prior to use. The animals were housed at temperature (25±1°C) with 50±55% of relative humidity under 12 h day and night cycle and fed standard rodent chow and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee (Reg.No. No.1504/PO/Re/S/11/CPCSEA) and care of the experiment on animal was carried out as per the guidelines of CPCSEA, Ministry of Environmental and Forests, Government of India.

2.2 Drugs and chemicals

The standard drugs used were diazepam (Laborate Pharmaceuticals India Ltd, H.P) and flouxetine (Angel Pharmaceuticals, Nagpur, M.S) was dissolved in saline and was diluted with saline to reach the proper concentrations. HEBM extract was dissolved in saline and was freshly prepared. The extract was administered p.o. and standard drugs or vehicle were injected i.p. once daily from days 2 to 21 during the situational reminder training session and testing session 30 min before each experiment.

2.3 Preparation of plant extract

Each part of the plant was powdered and its extraction was carried out by maceration method of extraction. Fresh samples were air-dried and ground, yielded 1500 g of powder. From which, (500g) powder of dry leaves were separately defatted with petroleum ether (40–60˚C) for 6 h. Then they were filtered and marc dried and extracted with a mixture of ethanol (700 ml): water (300 ml) (70: 30) as solvent for 24 h at room temperature. Mixture was agitated at regular interval in this period. Obtained extract after filtration through muslin cloth followed by using Whatmann No.1 filter paper was concentrated using rotary vacuum evaporator (40˚C), taking precaution that extract does not get powdered. The extraction was repeated two times and the filtered hydroethanolic extracts were mixed and evaporated under reduced pressure. 50 g of hydroalcoholic extracts were dissolved in 200 ml of methanol/water (7:3). The solvent was evaporated in rotary evaporator and dried, dried to constant weight and stored at −10 ◦C until used for experiments [23].

2.4 Induction of electric foot shock trauma

On day 0 and 1, the mice were subjected to a 2-day electric foot-shock stress by introduction of the animal in a plexiglass chamber(300×300×350 mm), with a electrified stainless steel rods grid floor (4 mm diameter, 9 mm interval). After 5 min, A total of 15 intermittent inescapable electric foot shocks (intensity: 0.8 mA, interval: 10 s, duration: 10 s) were delivered through the grid floor by an isolated shock generator. It was followed by 3 week, i.e., day 2nd, day 7th and day 14th re-exposures to the same context (as situational reminders) for 5 min without any foot-shocks. [24]. Animals were randomized to 6 groups: unstressed, Stress control(Stre control), *Bacopa monneiri* (50, 100, and 200 mg/kg)-, diazepam (1 mg/kg) and flouxetine (15 mg/kg) -treated groups (n=6 in each group).

2.5 Behavioral assessment of posttraumatic stress disorder

On 21st and 24th day, at the end of protocol, different behavioral tests including the elevated plus maze and open field test were performed respectively in animals. Every test apparatus was cleaned with alcohol and water after every test. The behaviors in the behavioral test apparatus were recorded and analyzed. The freezing behavior in response to weekly situational reminder was assessed on 2nd, 7th, and 14th day following stress exposure.

2.5.1 Freezing Behavior

All animals were exposed to the reminder situation, that is, the same chamber where the foot-shocks had been delivered, but with no further foot-shocks for 5 min on day 2, 7, and 14 respectively. The duration of freezing behavior of mice were recorded on days 2, 7, and14. Freezing was defined as the absence of movement other than breathing. Total seconds spent freezing during each assessment period was measured and scored [25].

2.5.2 Elevated Plus-Maze

On day 21 post-foot shocks, an elevated plus maze test was conducted. The elevated plus maze apparatus was made of Plexiglas and consisted of two open arms (30×5 cm) and two closed arms (30×5 cm) with 25 cm walls. The arms extended from a central platform (5×5 cm). The maze was elevated 50 cm from the ground. Each animal was placed at the centre of the maze, facing one of the enclosed arms. The number of entries and the time spent in enclosed and open arms were recorded for 5 min. Entry into an arm was defined as the animal placing all four paws onto the arm. Total exploratory activities (number of entries) were registered. After each test, the maze was carefully cleaned up with wet cotton dipped in 10% ethanol solution [26].

2.5.3 Open field

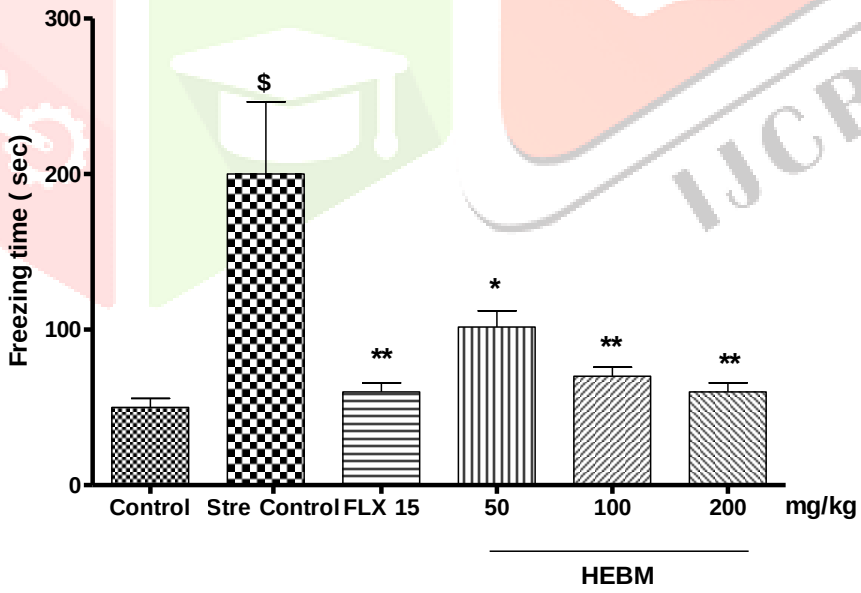
On day 24 post-foot shocks, an open field test was conducted. This method is used to evaluate exploratory activity and emotionality of animals. The open field test apparatus consist of a square arena (60cm×60cm× 60cm) divided into 9 segments. The mice were placed in the centre of arena facing the wall and allowed to explore freely in the apparatus for 5 min with experimenter out of animal sight. Parameters like ambulation (number of partitions crossed with all four paws), rearing (number of times mouse stood on its hind limbs), time spent in central compartment and number of crossings in central compartment were recorded. Mice were carried to the test room in their cages and were handled by the base of their tails at all times. After the 5 min, test mice were returned to their home cages. The open field was cleaned with 70% ethyl alcohol and permitted to dry between tests [27].

3. Results

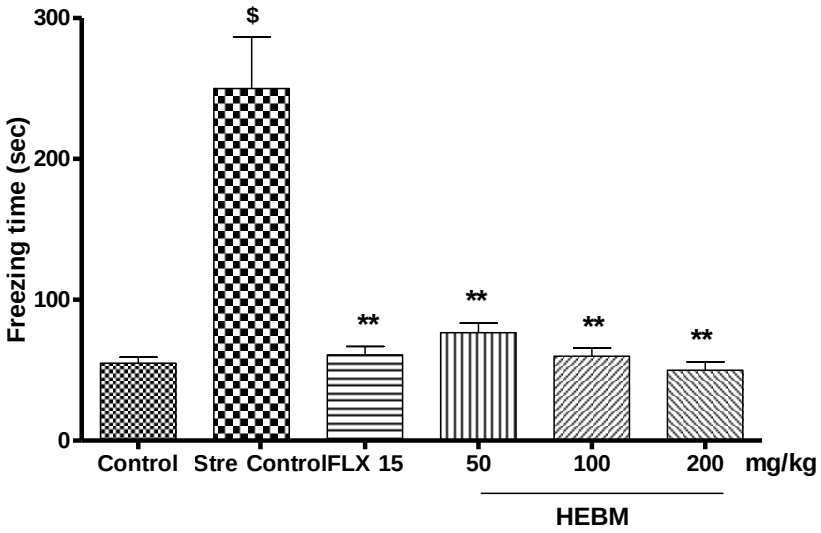
3.1 Freezing Behaviour

On days 2, 7 and 14 after the end of aversive procedure shown in fig. 1, the freezing behaviour was increased significantly, result indicated a persistent fear response of mice to the context as compare to normal control. Repeated administration of HEBM at doses of 50, 100, and 200 mg/kg produced a dose-dependent reduction in freezing behaviour ($P<0.01$), ($P<0.001$). Repeated treatment with a dose of flouxetine 15 mg/kg significantly ($P<0.001$) reduced the freezing behaviour.

A) Day 2



B) Day 7



C) Day 14

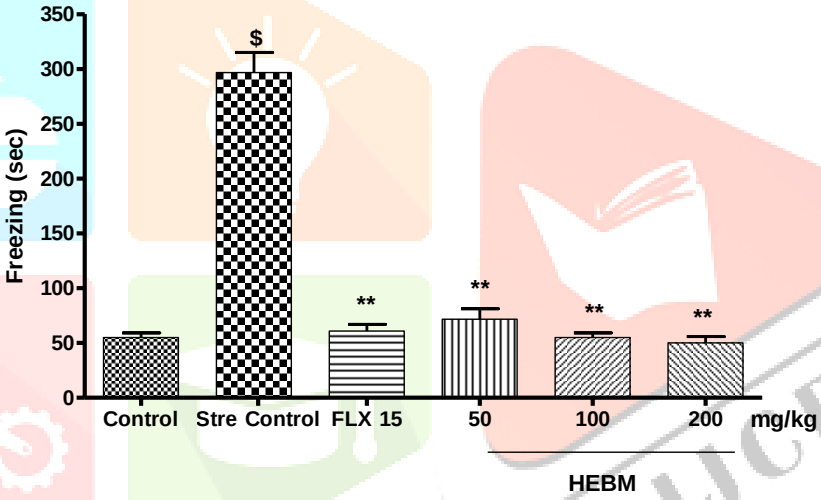
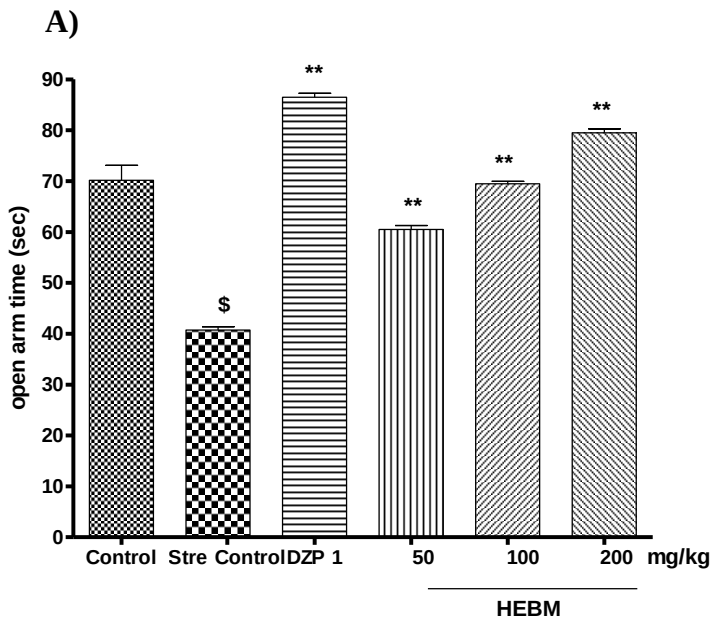


Fig.1. Effects of *Bacopa monneri* and flouxetine on freezing behavior in mice afte re-exposure to aversive procedure. The freezing behavior was determined on days 2 (A), 7 (B), and 14 (C). Daily administrations of *Bacopa monneiri* and flouxetine were started from the first day of training session. The effect on the situational reminder was elucidated 30 min after the treatment. All Data expressed as mean ± SEM (n=6). $^{\$}$P<0.001 compared with vehicle treated control group. *P<0.01, **P<0.001 compared with vehicle treated stress control. One way ANOVA followed by post hoc Tukey's multiple comparisons.

3.2. Elevated Plus-Maze

A daily administration of HEBM (50,100 & 200 mg/kg) for 21 days significantly attenuated foot shock trauma on elevated plus maze as shown in fig. 2. The time spent in the open arms and the number of open arm entries were significantly reduced in the saline stress control (Stre control) animals which had been exposed to the aversive procedure (P<0.001). Repeated administrations of HEBM at doses 50,100 & 200 mg/kg and and diazepam at dose of l mg/kg significantly increased the time spent and the number of entries into the open arm (P < 0.001).



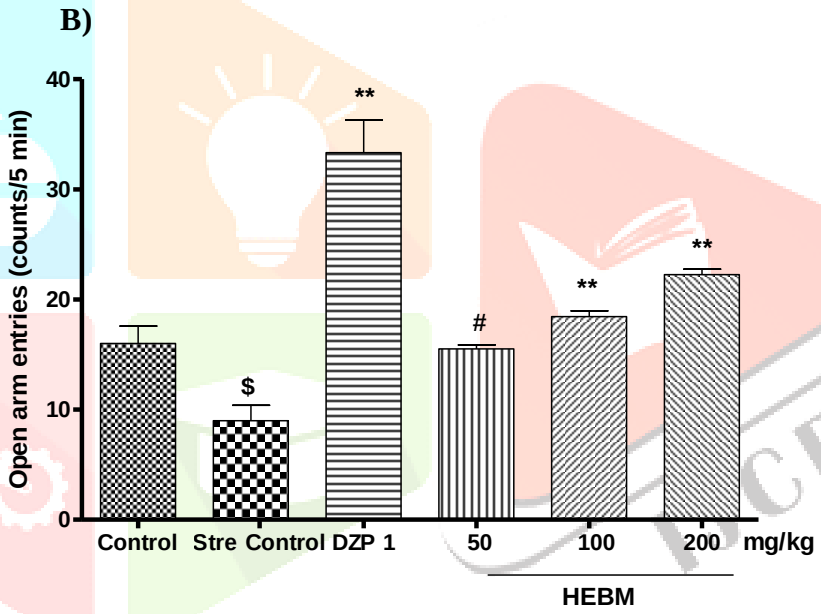
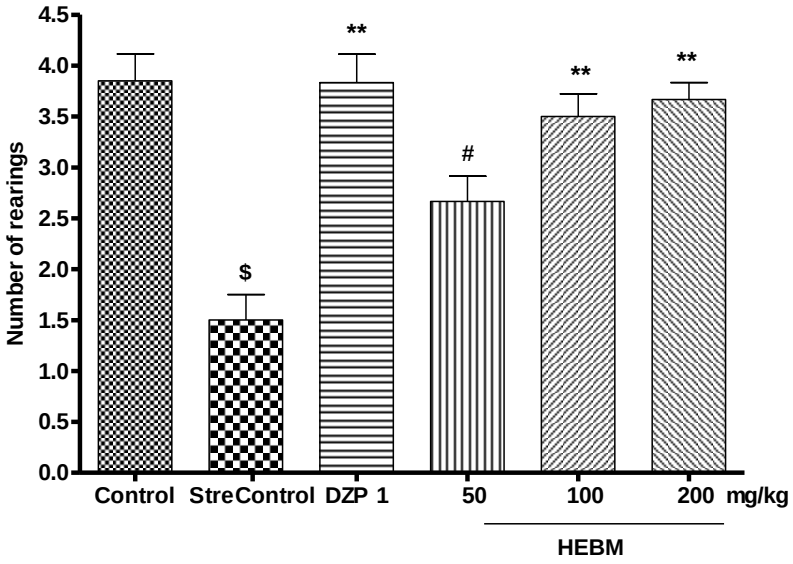


Fig.2. Effects of *Bacopa monneiri* and diazepam on elevated- plus maze test performance of stressed mice. On day 21 after aversive procedure, the animals received the elevated-plus maze test for 5 min. The time spent in open arms (A) and open arm entries (B) were recorded. Daily administration of *Bacopa monneiri* and diazepam were started from the first day of training session. The effects on the plus-maze performance were elucidated 30 min after the treatment. All Data expressed as mean ± SEM (n=6). $^{\$}P<0.001$ compared with vehicle treated control group. $^{\#}P<0.05$, $^{**}P<0.001$ compared with vehicle treated stress control. One way ANOVA followed by post hoc Tukey's multiple comparisons.

3.3 Open field test

A daily administration of HEBM (50,100 & 200 mg/kg) for 21 days significantly attenuated foot shock trauma on open field test. The time spent in the central compartment, number of central squares crossed and rearing were significantly reduced in the saline stress control animals which had been exposed to the aversive procedure ($P<0.001$). Repeated administrations of HEBM at doses 50, 100 & 200 mg/kg significantly increased the time spent in the central compartment, number of central squares crossed and number of rearings ($P<0.05$, $P<0.01$, $P<0.001$). Diazepam at dose of l mg/kg significantly increased time spent in the central compartment, number of central squares crossed and number of rearings ($P < 0.001$) when compared with stress control group.

A)


Number of rearings
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4.0
3.5
3.0
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1.5
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50
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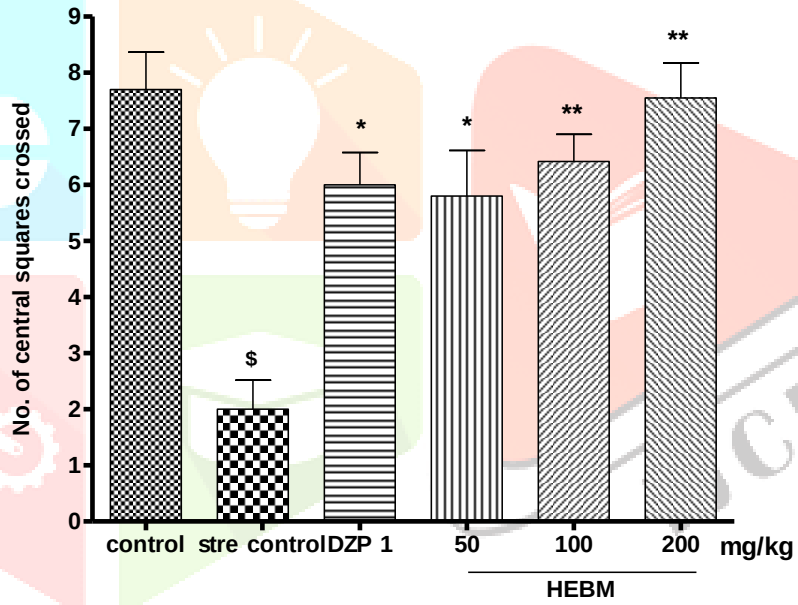
HEBM

B)


No. of central squares crossed
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8
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control
stre control
DZP 1
50
100
200
mg/kg
HEBM

C)

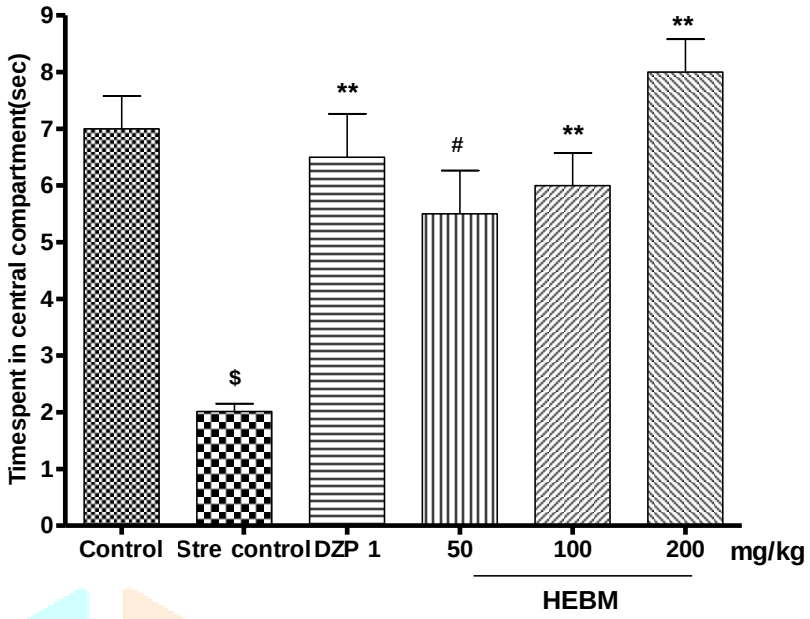

Fig.3. Effects of *Bacopa monneiri* and diazepam on open field test performance of stressed mice. On day 24 after aversive procedure, the animals received the open field test for 5 min. The number of rearings (A), number of central squares crossed (B) and time spent in central compartment (C) were recorded. Daily administration of *Bacopa monneiri* and diazepam were started from the first day of training session. The effects on the open field test were elucidated 30 min after the treatment. All Data expressed as mean ± SEM (n=6). $^{\$}$P<0.001 compared with vehicle treated control group. $^{\#}$P<0.05, *P<0.01, ** P<0.001 compared with vehicle treated stress control. One way ANOVA followed by post hoc Tukey's multiple comparisons.

4. Discussion

The current research was carried out to examine the efficacy of *Bacopa monneiri* leaves hydroalcoholic extract in posttraumatic stress disorder in mice. In order to achieve this goal, the study used a model of electrical foot shock stress, accompanied by 3 weekly situational reminders that significantly cause PTSD symptoms [28]. The different behavioral test including freezing behavior, elevated plus maze test and open field test were conducted to assess the development of PTSD.

In the present study, pharmacological profile of HEBM was evaluated in an animal model of PTSD. Data demonstrated that the PTSD-like behavioral deficits were produced in mice after exposure to foot shock aversive procedure. The data here indicated that foot shock procedure induced PTSD-like behavior in mice which mimics the symptoms of PTSD, including avoidance and anxiety state in animal's hyperarousal, aggression and re-experiencing flashbacks, as well as sleep alteration. This causes long-lasting behavioural effects that last up to 3 or 4 weeks and can worsen even further over time. These were in line with previous findings that the PTSD-associated freezing and anxiogenic activity was induced by foot shock which was evidenced by increased freezing time in the contextual freezing paradigm, decreased open arm exploration in elevated plus maze and decreased number of rearings, number of central squares crossed and time spent in central compartment. Accumulating evidence indicates that the mice exposed to foot shock regimen exhibit symptoms of increased arousal, such as exaggerated fear responses to trauma-unrelated and trauma-related stimuli which is similar to the observations in patients with PTSD who show enhanced fear and anxiety in response to stimuli related to trauma. The foot shock procedure has been found to produce contextual conditioning fear, and freezing behavior may serve as a good assessment for the severity of anxiety due to the dysfunction of brain regions (e.g prefrontal cortex, hippocampus and amygdale). Although the foot shock exhibited the PTSD-associated freezing and anxiogenic-like behavior, the locomotor activity was not significantly

affected. These were evidenced by the data of open field and elevated plus maze. The finding is agreed with reports that PTSD-associated multiple foot shocks did not affect the locomotor activity suggesting that the freezing behavior to the context associated with an aversive foot shock and PTSD-associated anxiogenic-like behavior which was not generated by affecting the locomotor activity.

A large number of plants which were used traditionally exhibit pharmacological properties with great potential for therapeutic applications in the treatment of central nervous system disorders [29]. *Bacopa monnieri* L. family Scrophulariaceae have been selected based on their traditional use and their rich flavonoid and saponin content nature [30, 31] as saponins and flavonoids were reported to have potential CNS effects [32].

To date, the exact etiology of PTSD is unclear. The researches into the underlying neurobiology have implicated that the pathology of PTSD is associated with the alterations of a myriad of neurotransmitter and neuroendocrine systems, including serotonin (5-HT), norepi-nephrine (NE), and gamma amino butyric acid type A receptor (GABA-A), as well as dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. PTSD-like behavior deficits elicited by foot shock trauma were attenuated by HEBM. The data showed that decreased freezing time in contextual freezing paradigm, increase in open arm exploration in elevated plus maze and increased time spent in the central compartment, number of central squares crossed and number of rearing in open field test was shown by HEBM, indicated that the PTSD-like contextual fear and anxiogenic behavior was significantly ameliorated by HEBM at certain doses. Based on the results, it may be proposed that *Bacopa monnieri* may be enhancing the level of GABA and 5HT and flavonoids and saponins present in the plant must be having important role in results obtained in present investigation. Since the effects of HEBM which we observed in this study are obtained by using hydroalcoholic extract and not in isolated compound, it is important to understand the effects of active constituents in combination and in isolation and their interactions with other neurochemicals.

5. Conclusion: In conclusion, the plant *Bacopa monnieri* L. have potential anti PTSD activity and serotonin and GABA may play a role in the anti PTSD effects of *Bacopa monnieri* L. The plant may be beneficial in preventing the PTSD symptoms following exposure to a traumatic event. In future studies are required to ascertain mechanism of action for the said activity.

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