BENI-SUEF UNIVERSITY JOURNAL OF BASIC AND APPLIED SCIENCES 4 (2015) 214–224

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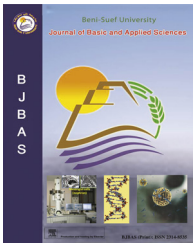

Full Length Article

Behavioral and biochemical characterization of elevated "I-maze" as animal model of anxiety

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ARTICLE INFO

Article history:
Received 5 March 2015
Received in revised form 4 July 2015
Accepted 14 July 2015
Available online 4 September 2015

Keywords:
Anxiety
Animal maze
Mice
Diazepam

ABSTRACT

The elevated I-maze is a modification of the elevated plus-maze model of anxiety in mice. The design of I-maze comprises a straight wooden passage, resembling the English letter "**I**," divided equally into three areas; two enclosed areas (close arms) at both ends of the "maze" and an open area in the center of two enclosed areas. The I-maze completely avoids the central platform of elevated plus-maze, removing any ambiguity in time spent on central platform and allowing uninterrupted animal exploration. In this model, diazepam (1 mg/kg) and gabapentin (10 mg/kg) significantly increased the percentage of time spent in the open areas (%TO) and the number of unprotected head dips (uHDIPS), and reduced the number of protected head dips (pHDIPS) and stretch attend postures (SAP) from close to open arm. Similarly, fluoxetine (5 mg/kg) significantly increased %TO and uHDIPS, and significantly decreased SAP from close to open arm, but it did not have any significant effect on pHDIPS. The 5-HT_3 receptor antagonist, ondansetron (0.1 mg/kg), did not produce any significant change in all the behaviors, observed, as compared to vehicle-treated control mice. On the other hand, the anxiogenic agent, caffeine (15 mg/kg), did produce a significant decrease in %TO and uHDIPS, and significantly increased pHDIPS and SAP from close to open arm. Mice confined in open area of I-maze bring the relevant biochemical changes associated with anxiety behavior, showing significant increase in the levels of plasma nitrate and plasma corticosterone. These data indicate that a combination of novel design of elevated I-maze and a detailed behavioral analysis provides a sensitive model for the measurement of anxiety.

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http://dx.doi.org/10.1016/j.bjbas.2015.07.003

1. Introduction

Human anxiety is defined as a feeling of apprehension, uncertainty and tension stemming from the anticipation of an imagined or unreal threat (Ninan, 2001). Pharmacological evaluation of anti-anxiety agents is done using different exclusively designed behavioral paradigms. A model of anxiety is an experimental system or paradigm to simulate some aspects of anxiety disorders in which the effects of anxiolytic drugs are examined. Animal behavioral profiles such as locomotion, self grooming, defecation and urination have long been used to detect effects on anxiety, and a number of models, based on animal emotional reactivity, have been proven to be sensitive to stressful manipulations. Many of these models have been successfully used to test new anxiolytic drugs by simple, rapid and inexpensive ways of evaluating animals' conditions. In this class of tests environmental stimuli or contexts are used to induce anxiety states in animals. Studies on antianxiety compounds generally involve exposing the animal to a situation that has not been experienced before, or after some time. Most of them involve putting an animal into a novel enclosure such as maze, open field or other test chamber.

Several experimental paradigms have been constructed to test anxiety in the rodent (Belzung, 1999). In particular, the elevated plus-maze has been used and validated as a tool to assess anxiety in rodents, as well as to determine the efficacy of various anxiolytic compounds. In this paradigm, subjects are placed on the central platform of the elevated plus-maze at the start of the test, and the amount and distribution of time spent in the open and closed arms are measured. Increased time spent in the open arms is indicative of a low level of anxiety, whereas increased time spent in the closed arms is indicative of a high level of anxiety. Validation of this procedure has been confirmed by the effects of anxiolytic compounds. Earlier modifications in the design of elevated plus-maze are (a) elevated Zero-maze (EZM) (Shepherd et al., 1992a, 1992b) and (b) elevated T-maze (ETM) (Graeff et al., 1993). These mazes were designed and proposed as animal models of anxiety, which lack the inherent deficiency in the design of elevated plus-maze; there exists an inherent limitation in the plus maze design, of the unavoidable ambiguity, which is associated with time spent on the central square of plus-maze. It is argued by various workers that period spent on central platform compromises interpretation of time spent in open/close arms. Further, mice have been observed to spend 20–30% of the test period on central square (Lee and Rodgers, 1990). The elevated T-maze was designed to offer the provision of testing conditioned and unconditioned fear in the single apparatus, whereas elevated zero-maze was utilized for detailed behavioral analysis including risk assessment by animal, in addition to measuring anxiety. Though, these mazes offer clear advantages over elevated plus-maze *viz.* avoidance of central platform (in case of both ETM and EZM) and uninterrupted exploration of the apparatus (in case of EZM), the I-maze design facilitates (a) expedited exploration of the maze because, as the animal reaches the end of the closed end, it spontaneously turns back and encounters a much clear view (option) of open space, present inherently between two closed arms (choices), as compared to all other mazes like EPM, ETM and EZM. (b) Authors opine that I-maze offers a rather more robust measure of anxiety as compared to the designs of EPM, ETM and EZM, because during the whole period of animal stay (300 sec) in the maze, the design of I-maze facilitates a clear view of all the portions of the maze. Due to such structure of the maze, during its stay in any one arm (open or close), the animal has always a clear vision of other arm, where it can enter as per its own preference. This feature adds to the reliability of the I-maze, because the animal during its stay in open arm still has a clear view of close arms, on both sides of its stay on the straight open platform of the maze. In this situation, still, if the animal does not opt to enter close arm, then it may be taken as a more robust measure of animal preference for open arm i.e. anxiolytic-like behavior of animal or drug action, rather than an artifact or an unexplained stay of animal in any one arm (out of animal's own confusion and retardation of decision making, which is an inherent feature of anxiety or due to lack of a clear view of another arm during its stay in one arm). (c) This further adds to the utility of I-maze to facilitate a significant interpretation of more subtle drug effects. (d) Further, similar to ETM and EZM, I-maze also avoids the central platform in its design.

Various categories of drugs, like benzodiazepines, barbiturates, alcohol, tri-cyclic antidepressant, have been used for long time to treat anxiety. Apart from these established categories of drugs, there are certain categories, which have been explored for their anxiolytic potential. These include selective serotonin reuptake inhibitors (SSRIs) like paroxetine, citalopram and fluoxetine. These categories have been considered as possible therapeutic replacements for some of traditional anxiolytics because they are found to have comparable anxiolytic effect to diazepam. Further, some anti-epileptics like tiagabin, gabapentin and pregabalin have been explored for anxiety treatment.

The present study was undertaken to examine the possible anti-anxiety activity of selected categories of drugs on a proposed model of anxiety, named as "I-maze." A model is said to be behaviorally validated, if a model facilitates the expression of a significant pharmacological activity of selected drugs in mice, thereby showing a clear behavioral change in mice, as a result of administration of selected drug(s).

In the brain, NOS has been localized in regions involved with anxiety, such as hypothalamus, amygdala and hippocampus (Vincent, 1994). There is evidence suggesting the role of NO/cGMP signaling pathway in effect of NO on anxiety (Eroglu and Caglayan, 1997). Inhibition of nitric oxide–cGMP pathways by inhibition of NOS has been reported to produce antianxiety effect (Spolidório et al., 2007). Stressful events have been found to enhance anxiety in rodents, and plasma corticosterone is reported to be a hormonal marker of anxiety (Mitra and Sapolsky, 2008; Zhao et al., 2014). Therefore, in the present hypothesis, if the present model facilitates the expression of a significant change in plasma nitrite and plasma corticosterone in mice, it will show the biochemical validation of animal model.

2. Materials and methods

2.1. *Animals*

Swiss albino mice (male; 20–25 g) were used in this study. Animals were housed under standard laboratory conditions,

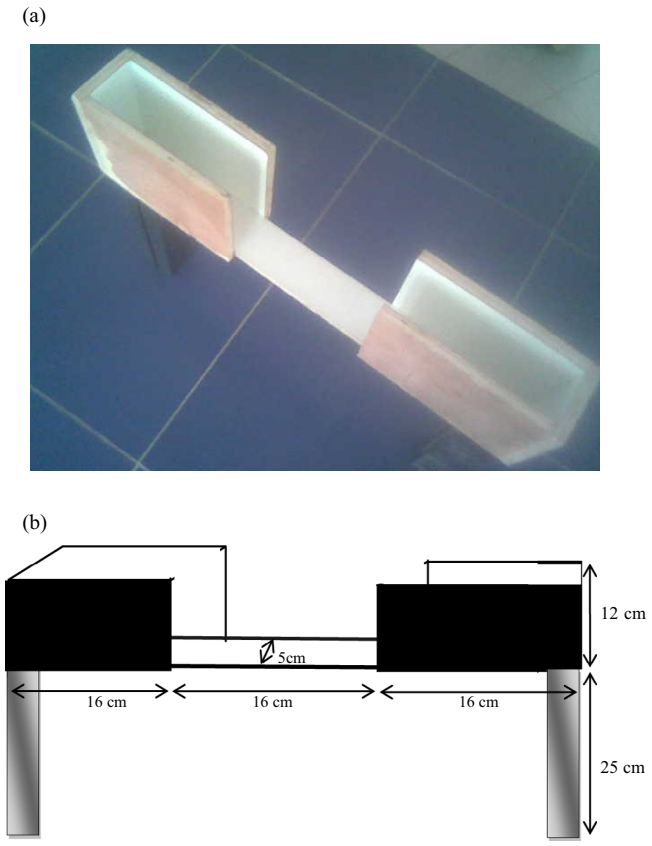

Fig. 1 – (a) Top view of elevated I-Maze. (b) Scheme of I maze, showing proposed dimensions.

maintained on a 12 hour light and dark cycle. Animals had free access to standard food and water. Animals were acclimatized to laboratory conditions before the test.

2.2. *Apparatus*

The apparatus is a straight wooden passage, resembling the English letter "I" (Fig. 1a). It consists of a 48 cm × 5 cm, straight passage, divided equally (16 cm each) into two enclosed areas (close arms) at both ends of the "maze" and an open area in the center of two enclosed ends (arms). Height of the walls of enclosed areas is 12 cm. Final dimensions of "I" maze are proposed to be 48 cm long × 5 cm wide with 12 cm high walls at both ends. The entire maze is elevated to the height of 25 cm (Fig. 1b).

2.3. *Plasma corticosterone estimation*

The quantitative estimation of plasma corticosterone level was performed by the method of Bartos and Pesez (1979).

2.4. *Plasma nitrite estimation*

For nitrite estimation, plasma was collected using cooling centrifuge at 2500 rpm and 4 °C for 10 min. The plasma was stored in a refrigerator for estimation of nitrite content within 24 h. Plasma nitrite was measured by a spectrophotometric assay based on the Griess reaction (Green et al., 1982).

2.5. *Experimental protocol*

Each experimental animal group consisted of ten mice each (n = 10). For behavioral characterization, vehicle (distilled water) (10 ml/kg, ip), diazepam (1 mg/kg, ip), Gabapentin (10 mg/kg, ip), Fluoxetine (5 mg/kg, ip), Ondansetron (0.1 mg/kg, ip), caffeine (15 mg/kg, ip) were administered in separate groups of mice, 30 min before subjecting them to behavioral testing. After 30 min of vehicle or drug treatments, animals were observed for total 5 min duration with the help of a video tracking system, and behaviors were analyzed and documented by a blind observer from these recordings. In all experiments, i.e. before placing each animal on the maze, the test apparatus (maze) was cleaned with 5% ethanol and thoroughly dried between each test period. All the experiments were carried out between 09:00 a.m. and 03:00 p.m. The experimental protocols were approved by institutional animal ethics committee and were conducted as per CPCSEA guidelines on use and care of experimental animals. The following behavior parameters were studied in the mice.

2.5.1. *Percent time spent in open arm (% TO)*

It denotes the time which is spent by animals in open area (open arm). It is calculated as percentage time spent in open arm. Animals were scored as in the open area, when all four paws of animal were in an open arm (Fig. 2). It was determined as follows:

$$\%\ \text{Time spent} = 100 \times \frac{\text{number of seconds spent on open arms}}{300\ \text{seconds (5 min observation time)}}$$

2.5.2. *Unprotected head dips (uHDIPS)*

It denotes scanning by animal over the sides of the maze downward towards the floor from unprotected area i.e. uncovered open arm. uHDIPS are counted as the number of head dips from open arm (Fig. 3).

2.5.3. *Head dipping from close arm (pHDIPS)*

It denotes scanning by animal over the sides of the maze downward towards the floor from protected area i.e. covered close

Fig. 2 – Time spent in open arm (% TO).

Fig. 3 – Unprotected Head Dipping (uHDIP).

Fig. 4 – Protected Head Dipping (pHDIP).

arm. pHDIPS are counted as the number of head dips from close arm (Fig. 4).

2.5.4. *Stretch attend posture (SAP)*

It is characterized by a forward elongation of the body exhibited when the animal is either standing still or moving slowly forward (Fig. 5).

All the above mentioned behavioral patterns are helpful to assess anxiety behavior in rodents, which has been shown by mice behavior on elevated I maze.

For biochemical estimation, plasma nitrite and plasma corticosterone were made on samples collected from animals that were confined to open arm of the maze for 20 min by placing a wooden block at both entries of the closed arm and compared with the vehicle treated mice.

Fig. 5 – Stretched Attend Posture (SAP).

2.6. *Statistics*

All the results are expressed as Mean ± S.E.M. The data were analyzed by Student's t-test. $P < 0.05$ was considered as statistically significant.

3. Results

In the present model, a significant increase in time spent by mice on open arm (%TO) and unprotected head dips (uHDIPS) and a significant decrease in protected head dips (pHDIPS) and stretched attend postures (SAP) indicate an antianxiety-like behavior. On the other hand, a significant decrease in time spent by mice on open arm (%TO) and unprotected head dips (uHDIPS) and a significant increase in protected head dips (pHDIPS) and stretched attend postures (SAP) indicate an anxiety-like behavior.

3.1. *Effect of diazepam*

Diazepam (1 mg/kg) produced a significant increase in %TO, an increase in uHDIPS, a decrease in pHDIPS and a decrease in SAP, as compared to that in vehicle-treated mice (Figs. 6–9). All these data suggest that diazepam show an anxiolytic-like activity on I-maze.

3.2. *Effect of gabapentin*

Gabapentin (10 mg/kg) produced a significant increase in %TO, an increase in uHDIPS, a decrease in pHDIPS and a decrease in SAP, as compared to that in vehicle-treated mice (Figs. 10–13). All these data suggest that gabapentin shows an anxiolytic-like activity on I-maze.

3.3. *Effect of fluoxetine*

Fluoxetine (5 mg/kg) produced a significant increase in %TO, an increase in uHDIPS, a decrease in SAP but did not produced

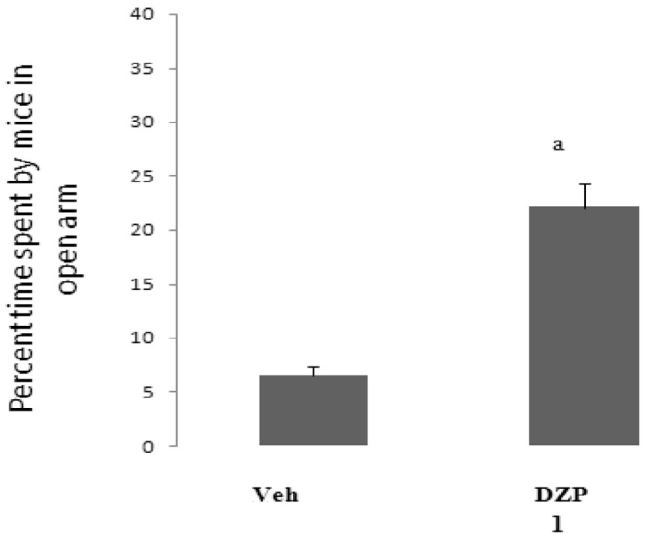

Fig. 6 – Effect of diazepam on percent time spent by mice in open arm (%TO). n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant difference from vehicle-treated control group. Values mentioned are doses in mg/kg.

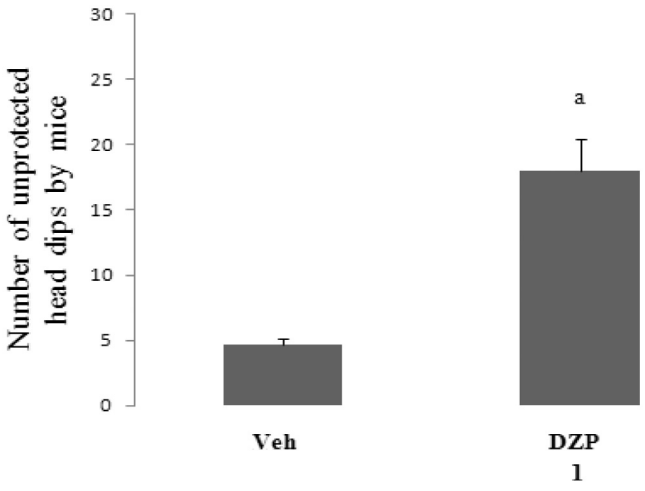

Fig. 7 – Effect of diazepam on number of unprotected head dips (uHDIP) by mice. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant difference from vehicle-treated control group. Values mentioned are doses in mg/kg.

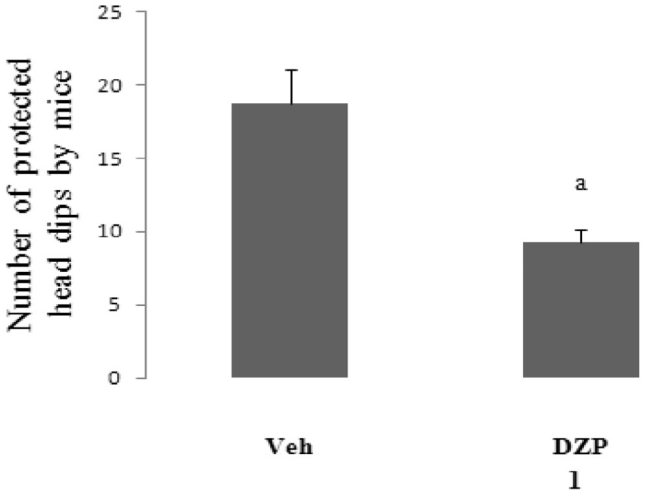

Fig. 8 – Effect of diazepam on number of protected head dips (pHDIP) by mice. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant difference from vehicle-treated control group. Values mentioned are doses in mg/kg.

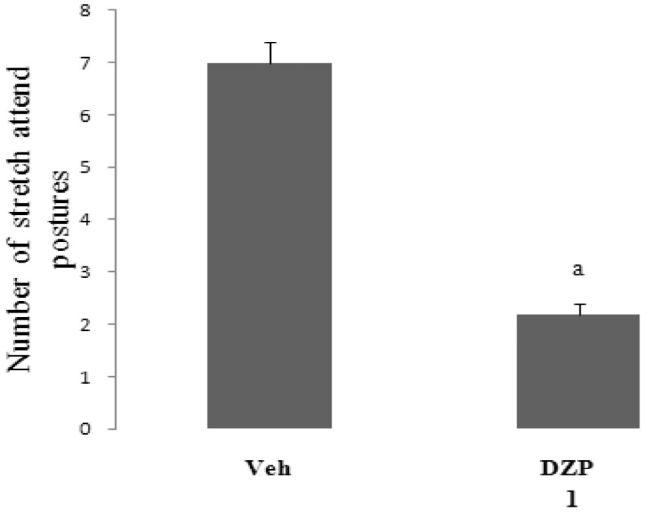

Fig. 9 – Effect of diazepam on number of Stretch Attend Postures (SAP) by mice. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant difference from vehicle-treated control group. Values mentioned are doses in mg/kg.

any change in pHDIPS as compared to that in vehicle-treated control mice (Figs. 14–17). All these data suggest that fluoxetine show an anxiolytic-like activity on I-maze.

3.4. *Effect of ondansetron*

Ondansetron (0.1 mg/kg) did not produce any significant change in all the parameters, as compared to vehicle-treated control group (Figs. 18–21).

3.5. *Effect of caffeine*

Caffeine (15 mg/kg) produced a significant decrease in %TO, a significant decrease in uHDIPS, an increase in pHDIPS and an increase in SAP, as compared to that in vehicle-treated mice (Figs. 22–25). All these data suggest that caffeine show an anxiogenic-like activity on I-maze.

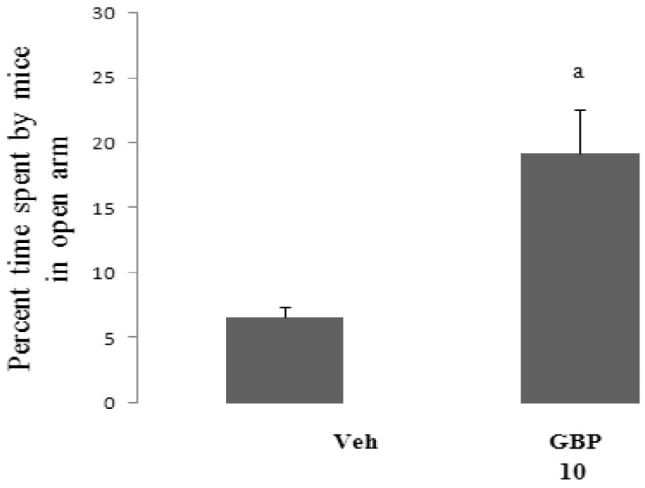

Fig. 10 – Effect of gabapentin on percent time spent by mice in open arm (%TO). n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant difference from vehicle-treated control group. Values mentioned are doses in mg/kg.

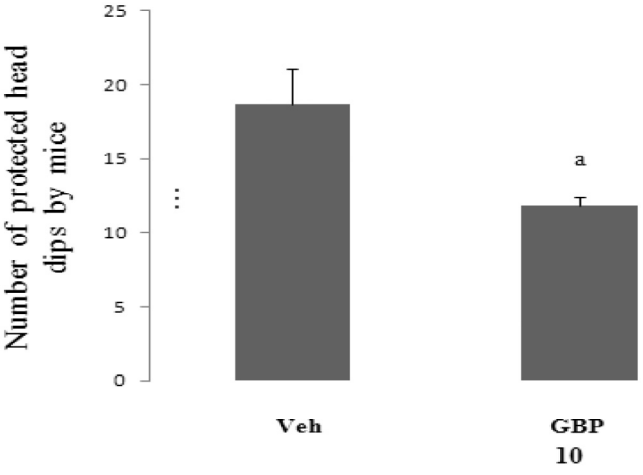

Fig. 12 – Effect of gabapentin on number of protected head dips (pHDIP) by mice. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant difference from vehicle-treated control group. Values mentioned are doses in mg/kg.

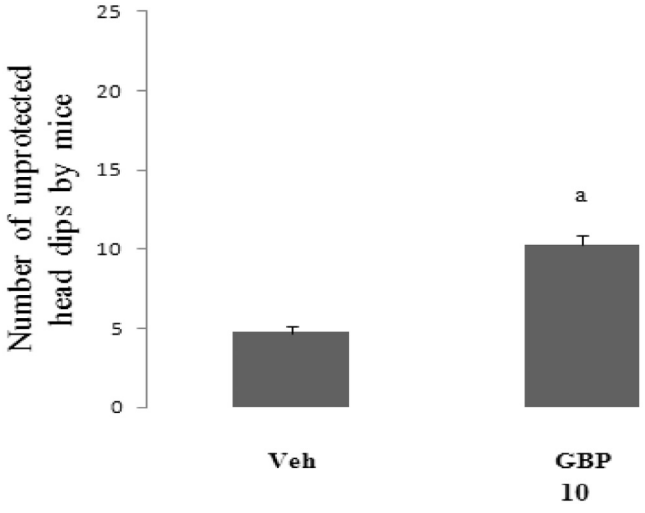

Fig. 11 – Effect of gabapentin on number of unprotected head dips (uHDIP) by mice. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant difference from vehicle-treated control group. Values mentioned are doses in mg/kg.

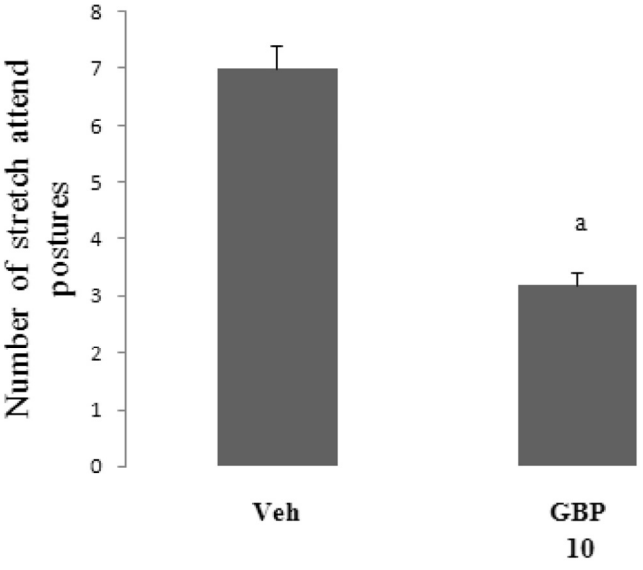

Fig. 13 – Effect of gabapentin on number of Stretch Attend Postures (SAP) by mice. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant difference from vehicle-treated control group. Values mentioned are doses in mg/kg.

3.6. *Plasma nitrite estimation*

Plasma nitrite level was significantly increased in mice confined in open arm as compared to vehicle treated mice (Fig. 26).

3.7. *Plasma corticosterone estimation*

Plasma corticosterone level was significantly increased in mice confined in open arm as compared to vehicle treated mice (Fig. 27).

4. Discussion

An experimental model of anxiety should be analogous to the human disorder in symptoms. A model is required to produce a behavioral change that (a) can be monitored, (b) should respond to standard clinical treatments and (c) should exhibit reproducibility in animal behavior (McKinney and Bunney, 1969). An animal model should at least display three kinds of validity

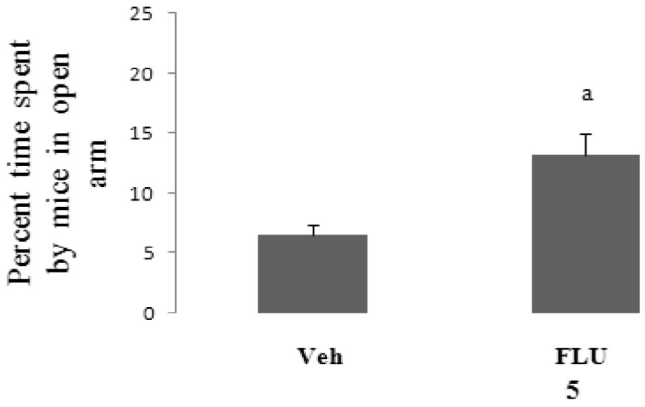

Fig. 14 – Effect of fluoxetine on percent time spent (%TO) by mice in open arm. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant difference from vehicle-treated control group. Values mentioned are doses in mg/kg.

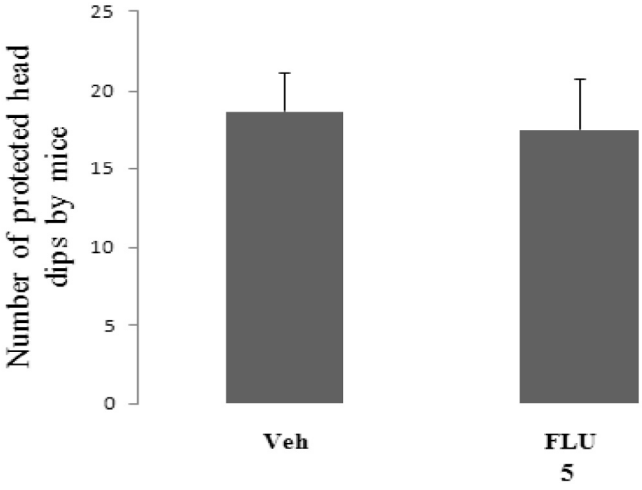

Fig. 16 – Effect of fluoxetine on protected head dips by mice. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. Values mentioned are doses in mg/kg.

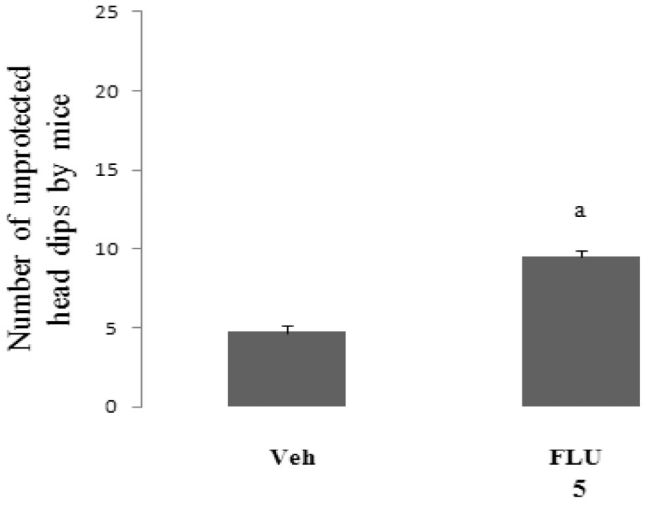

Fig. 15 – Effect of fluoxetine on unprotected head dips by mice. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant difference from vehicle-treated control group. Values mentioned are doses in mg/kg.

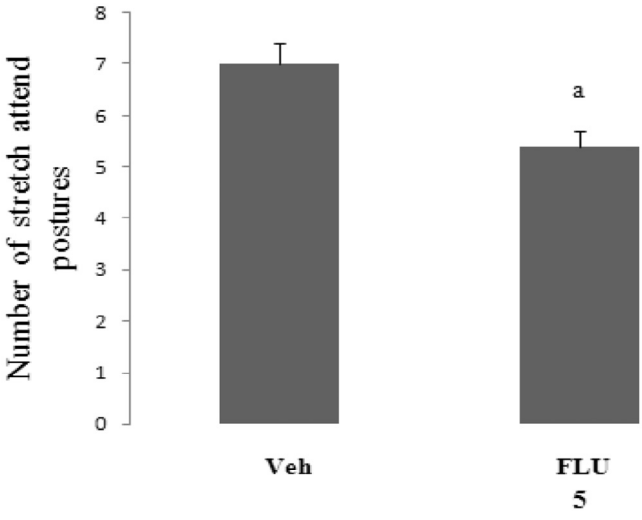

Fig. 17 – Effect of fluoxetine on stretch attend postures (SAP) by mice. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant different from vehicle-treated control group. Values mentioned are doses in mg/kg.

namely (a) face validity i.e. it should produce anxiety-like symptoms in animal, (b) construct validity i.e. its physical design should produce similar biochemical changes as observed in clinical anxiety and (c) predictive validity i.e. animal behavior on the maze should respond to standard therapeutic treatments (Geyer and Markou, 2000). The present maze studies in the present paper bears a physical design that is helpful in studying detailed anxiety-like behavioral changes. Further, "I-maze" provides an environment, which helps to increase the sensitivity to, and facilitating interpretation of drug action. The novel apparatus does not possess central platform, as mice have been observed to spent 20–30% time on central square of elevated plus maze (Lee and Rodgers, 1990). Removal of the central area further helps to detect drug effects on time spent in the open area (Shepherd et al., 1992a, 1992b). Design of I-maze covers maximum relevant features of anxiety – (a) open and close arms for exploration; (b) no central platform; (c) clear demarcation between open and closed areas, that (i) contrasts the stretched attend postures (SAP) and (ii) provides clear differentiation between protected (close) and unprotected (open) environment to observe unprotected and protected head dips. Therefore, I-maze offers feasibility of detailed behavioral analysis by employing *viz.* (a) unprotected head dip, (b) protected head dip and (c) stretch attend posture; the relevant features of human anxiety. The behaviors that are considered as experimentally reliable behaviors for studying anxiety in rodents are (a) time spent in open space, (b) number of head dips from

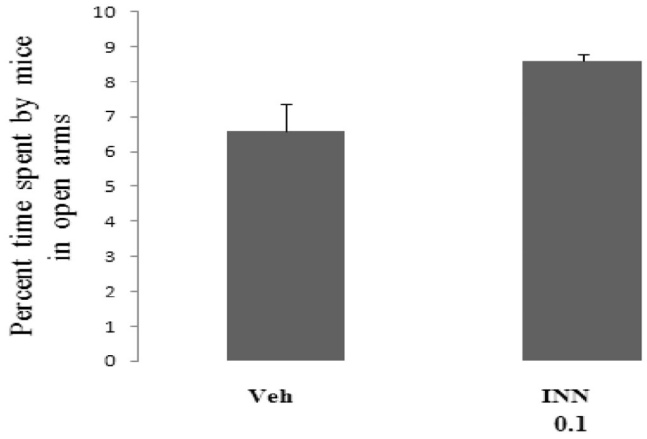

Fig. 18 – Effect of ondansetron on percent time spent (%TO) by mice in open arm. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. Values mentioned are doses in mg/kg.

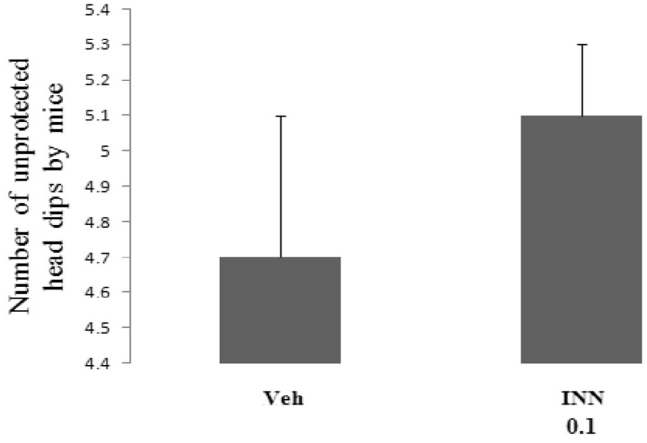

Fig. 19 – Effect of ondansetron on unprotected head dips by mice. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. Values mentioned are doses in mg/kg.

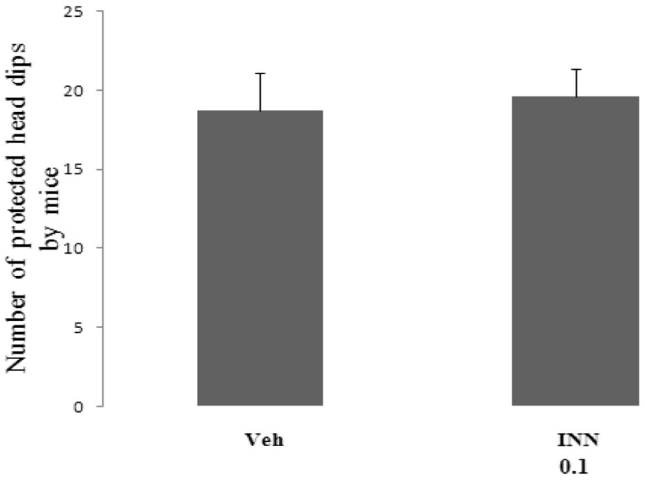

Fig. 20 – Effect of ondansetron on protected head dips by mice. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. Values mentioned are doses in mg/kg.

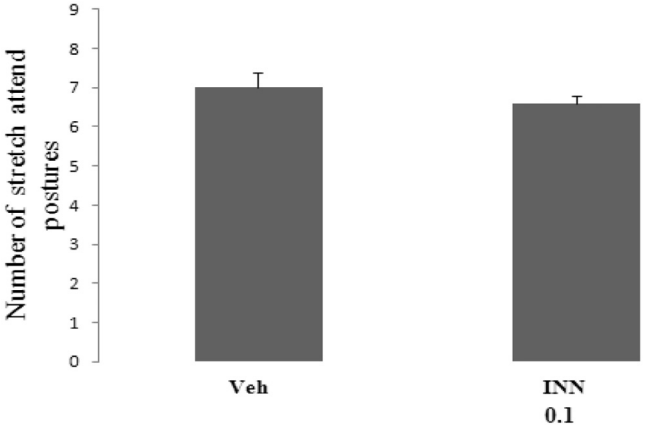

Fig. 21 – Effect of ondansetron on stretch attend postures by mice. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. Values mentioned are doses in mg/kg.

open area of the maze, (c) number of head dips from closed area of the maze and (d) number of stretched attend postures by animal on the maze. These behaviors can be experimentally recorded reliably in a similar manner as adopted and reported by several investigators in anxiety research (Pellow et al., 1985; Shepherd et al., 1994).

Present data indicate that the standard clinical therapeutic option for anxiety, i.e. diazepam, has produced a clear and consistent behavioral change in test animals in the I-maze. The proportion of time spent on the open area (one of the behavioral change recorded in the elevated plus-maze procedure or Zero maze) was increased by diazepam. A detailed behavioral analysis after administration of diazepam also indicated the similar significant increases in exploratory head dipping of animals from open space of the I-maze and decrease in stretched attend postures from the closed to the open arm. There is an evidence to support the view that a decrease in SAP is consistent with reduced anxiety (Blanchard et al., 1990, 1991; Kaesermann, 1986; Shepherd et al., 1992a, 1992b). This finding is in line with observations with the mouse elevated plus-maze, in that diazepam increased percent time spent by mice in open arms. Further, I-maze procedure does not involve measurement of the number of entries in open or closed arm of maze, as this parameter is associated with conflicting opinions that it may not reflect a measure of anxiety, but more of locomotor activity. The dual nature of the design of I maze facilitates not only detailed behavioral analysis but also served to clearly indicate anxiogenic or anxiolytic behavior in mice, because it also facilitates the measurement of stretched attend postures in addition to percent time spent in arms.

Though, it is a generally accepted requirement to validate animal models of anxiety with benzodiazepines. But it is equally needful to search for novel classes of anxiolytics which are free from the numerous problems associated with chronic benzodiazepines. The effect of the 5-HT anxiolyic; ondansetron in

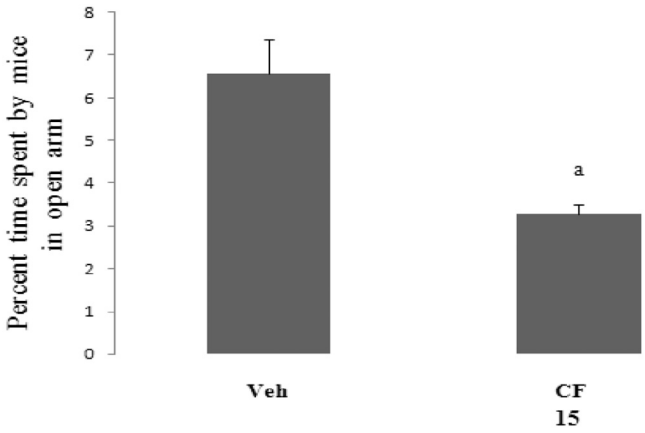

Fig. 22 – Effect of caffeine on percent time spent (%TO) by mice in open arm. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant different from vehicle-treated control group. Values mentioned are doses in mg/kg.

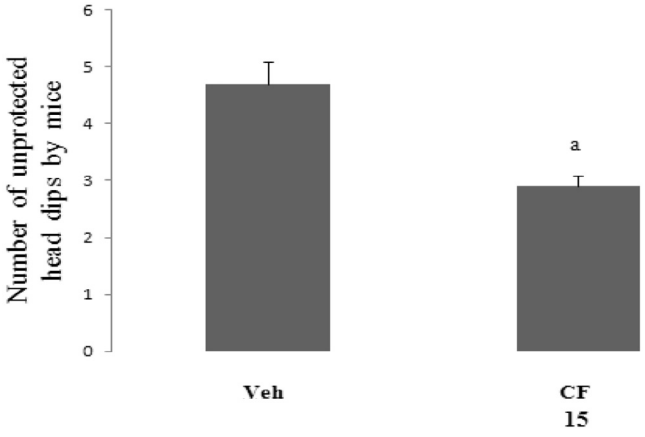

Fig. 23 – Effect of caffeine on number of unprotected head dips (uHDIPS) by mice. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant different from vehicle-treated control group. Values mentioned are doses in mg/kg.

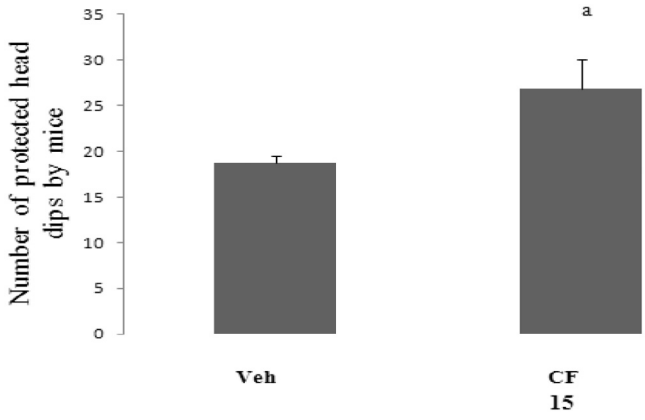

Fig. 24 – Effect of caffeine on number of protected head dips (pHDIPS) by mice. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant different from vehicle-treated control group. Values mentioned are doses in mg/kg.

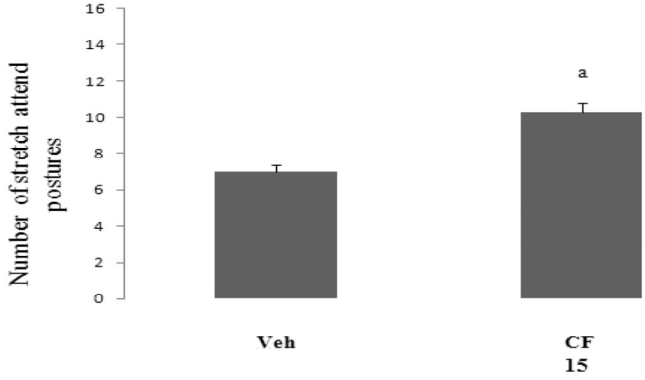

Fig. 25 – Effect of caffeine on stretch attend postures by mice. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant different from vehicle-treated control group. Values mentioned are doses in mg/kg.

the I-maze is important in view of the variable reports in the literature with this drug. In this category, ondansetron has given inconsistent results in the past literature including anxiolytic (Costall et al., 1989; Tomkins et al., 1990) and no effect (File and Johnston, 1989; Wright et al., 1992). In the present study too, data failed to indicate any significant effects of ondansetron. It is understood that an objective of a novel animal model is the detection of anxiolytics which produce their effects in a different manner to those of the benzodiazepines, probably acting via different underlying neurochemical mechanisms. Therefore, it would be acceptable to record behavioral profile that is achieved using non-benzodiazepines.

Among anticonvulsants, used in the treatment of anxiety are carbamazepine, valproate and gabapentin, pregabalin and tiagabin. Gabapentin has been found to increase GABA content after its administration (Pollack et al., 1998). It has also been used to validate elevated plus maze and zero maze, two effective models of anxiety (Kulkarni et al., 2008). In the present study too, gabapentin has significantly exerted antianxiety-like profile as it has significantly increased time spent by mice in open arm of I-maze as well as increased the unprotected head dips and significantly decreased the protected head dips as well as stretch attend postures by mice.

SSRIs have been observed to be most effective for anxiety treatment (Zohar and Westenberg, 2000). Fluoxetine has also shown a significant antianxiety-like activity as it significantly increased time spent by mice in open arm of I-maze as well as increased the unprotected head dips and significantly stretch attend postures by mice. However, it failed to produce any significant change in the number of protected head dips in mice as compared to vehicle-treated control mice.

Observations with caffeine as an anxiogenic agent (Baldwin et al., 1989), further illustrate the utility of I-maze for detailed behavioral analysis of mice. Results with caffeine on I-maze indicate the anxiogenic-like activity, as evident by a

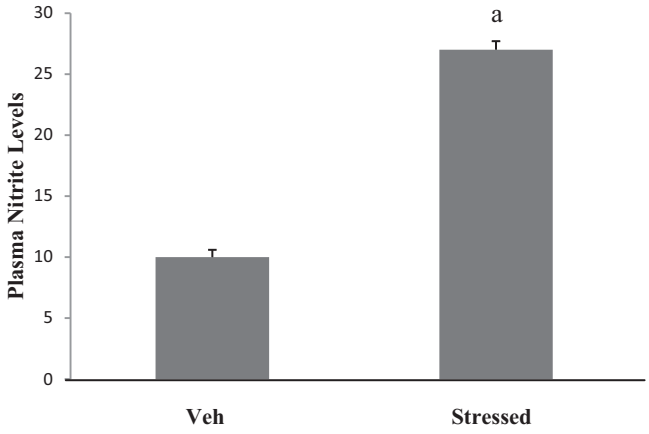

Fig. 26 – Effect of forced open arm exposure on plasma nitrite levels of mice on I-maze. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant different from vehicle-treated control group.

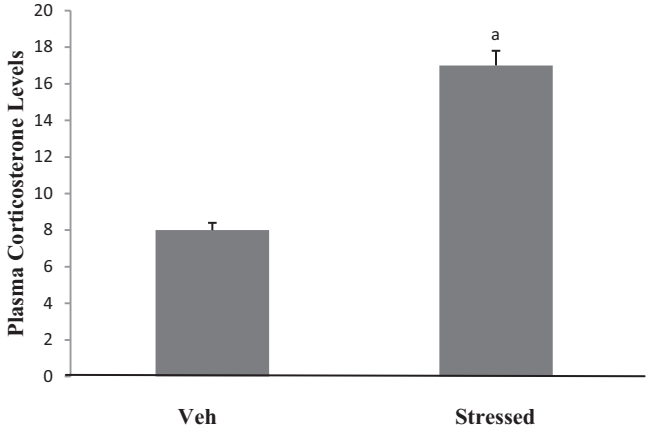

Fig. 27 – Effect of forced open arm exposure on corticosterone levels of mice on I-maze. n = 10 in each group. Values are expressed as mean ± S.E. Data were analyzed by Student t-test. a = p < 0.05 significant different from vehicle-treated control group.

significant decrease in behaviors, indicative of increase in anxiety like (a) time spent by mice in open area of the maze and (b) unprotected head dips; and a significant increase in behaviors, indicative of anxiety like (a) protected head dips and (b) stretch attend postures.

In the present study, significant increases in the levels of plasma nitrate and plasma corticosterone suggest the possible usefulness of the feasible design of the present maze to bring the relevant biochemical changes associated with anxiety behavior in mice.

Finally, resultant observed advantages of "elevated I-maze" comprise (a) I-maze avoids central platform of plus maze, (b) in plus maze, observations are based on animal's preference for any one arm, which is chosen after its movement from the arm in which the animal is present in that very time frame. On the other hand, I-maze offers an ever-ready vision of closed arms in front of its eye. This ever-ready vision helps animals to make a quick choice without spending even a single second of observation period (300 s). Straight vision and choice inherent in I-maze serve to avoid a time lapse spent by animals in any of the arm and a situation, where animals can voluntarily choose or decline a clear visible option, (c) design of I–maze facilitate expedited exploration of maze because, as the animal reaches at the end of closed end, it spontaneously turns back and encounters a much clear and wider view (option) of open space, present inherently between two closed arms (choices). This situation presented by I-maze further helps the animal to clearly make its choice between open or closed arm without being fascinated or intrigued by longer animal stay in closed as well as open arms of plus maze. (d) I-maze provides a visible clue to animals, besides providing a relatively "frank" open space, if, at all, they are lost in its complex thoughts, arising out of integral confusion of anxiety disorder, as compared to the elevated plus maze or Zero maze, where other arms are not visible, when an animal is in any one arm (open or closed). Further, authors opine that I-maze provides a robust measure of animal's preference for any of the two environments because I-maze tests the animal preference in relatively "frank" options.

5. Conclusion

Preliminary evidence suggests the utility of I-maze to explore detailed behavior of mice for testing of mice behaviors for anxiogenic-like as well as anxiolytic-like activity. In conclusion, the results suggest that the design of elevated I maze facilitates expression of anxiety-like behavior of mice and may prove to be a reliable model of anxiety.

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