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Comparative behavioural profile of newer antianxiety drugs on different mazes

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Anxiety is associated with diverse range of psychiatric conditions. In the present study, antianxiety effect of fluoxetine, citalopram (SSRI's), gabapentin (antiepileptic drugs), venlafaxine (SNRI), clozapine and resperidone (atypical antipsychotics) and a herbal preparation ashwagandha on elevated zero maze and elevated plus maze paradigms was examined. Anti-anxiety potentials of these drugs were compared with diazepam. The drugs tested i.e. fluoxetine (10 mg/kg), citalopram (10 mg/kg), clozapine (0.25, 0.5, 1 mg/kg), resperidone (0.5, 1 mg/kg), venlafaxine (4, 8, 16 mg/kg), citalopram (10 mg/kg), fluoxetine (10 mg/kg), gabapentin (10, 20 mg/kg) and ashwagandha (100, 200 mg/kg) significantly increased the number of open arm entries and time spent in open arm. These drugs also decreased the latency to enter in open arm as compared to control in both the paradigms. Present study confirms the antianxiety activity of different newer classes of drugs and found some of them comparable to diazepam in both the elevated zero maze and elevated plus maze paradigm.

Keywords: Antiepileptic, Anxiety, Atypical antipsychotic, Mazes

Human anxiety is defined as feeling of apprehension, uncertainty or tension stemming from the anticipation of an imagined or unreal threat[1]. Benzodiazepine, barbiturates, alcohol and tricyclic antidepressants (TCA's) have been used for long time to treat anxiety disorders[2-3]. The serious side effects associated with these drugs, namely rebound insomnia, sedation, muscle relaxation, withdrawal and tolerance (benzodiazepine, barbiturates and alcohol), sexual dysfunction anticholinergic and antihistaminic effects (TCA's) [2] have limited and/or discouraged their use in patients. Due to the serious side effects associated to these classical anti-anxiety drugs there is need for the development of newer anti-anxiety drugs.

There are several drugs belonging to different therapeutic classes which have been recently studied for their anti-anxiety effects. The Selective Serotonin Reuptake Inhibitors (SSRI's) (fluoxetine, citalopram, and paroxitine) have been considered as the possible therapeutic replacements of some of these traditional anxiolytics. They are found to have significant anxiolytic effects i.e. comparable to benzo-diazepines[4,5] . The new class of drugs studied recently for the treatment of anxiety disorders are antiepileptic (gabapentin, tiagabaine, and pregabalin). Gabapentin is the first potential GABA facilitating medication that was studied in anxious patients[6]. Besides this a typical antipsychotics (resperidone, clozapine)[7], and a typical antidepressants (selective norepinephrine and serotonin reuptake inhibitor (venlafaxine)[8] have also been studied. An important feature of using atypical antipsychotics and atypical anti-depressants in mood and anxiety disorders is that they can be effective at doses that would be considered sub-therapeutic for the management of respective disorders. Research linked to herbal antianxiety drugs is gaining momentum. Several investigations support the use of ashwagandha as a mood stabilizer in clinical conditions of anxiety[9].

Based on this the present study was undertaken to examine the antianxiety effect of Selective Serotonin Reuptake Inhibitors (SSRI's) (fluoxetine, citalopram), gabapentin (antiepileptic drugs), venlafaxine (SNRI), clozapine and resperidone (atypical antipsychotics) and ashwagandha (herbal drug) on elevated zero maze and elevated plus maze paradigms. Their effects were compared with the antianxiety potentials of diazepam (classical antianxiety drug).

Materials and Methods

Animals—Male Laca mice bred at Central Animal House (CAH) Panjab University, Chandigarh weighing in between 25-30 g were used. Animals were housed under standard laboratory conditions, maintained on a 12 hour natural light and dark cycle,

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with free access to standard food and water. Animals were acclimatized to laboratory conditions before the test. All the experiments were carried out between 09.00 to 15.00 hrs. The experimental protocols were approved by the Institutional Animal Ethical Committee and conducted according to the CPCSEA guidelines on the use and care of experimental animals.

Drug treatment and experimental procedure—Mice were treated with different drugs as diazepam (2 mg/kg), fluoxetine (10 mg/kg), citalopram (10 mg/kg), clozapine (0.25, 0.5, 1 mg/kg), resperidone (0.5, 1 mg/kg), venlafaxine (8, 16 mg/kg), gabapentin (10, 20 mg/kg) and ashwagandha (50, 100, 200 mg/kg). Doses were chosen on the basis of previous literature of these drugs for their anti-anxiety effect or their respective disorder[4-9]. All the drugs were dissolved in distilled water and were administered 30 minutes prior to the experimentation. Elevated zero maze and plus maze tests were carried out simultaneously in each of the drug treatment group.

Elevated zero maze—All the animals were analyzed for anxiety levels by using elevated zero maze. This test is a pharmacologically validated assay of anxiety in animal models that is based on the natural aversion of mice to elevated zero maze[10,11]. It is composed of a 6 cm wide ring with outer diameter of 45 cm containing 4 equal quadrants of alternating walled (closed) or unwalled (open) sections. The entire ring is elevated to the height of 40 cm (Fig. 1a). Mice were placed in the walled region at the start of 5 minute session and (a) time spent in open arm (b) latency to enter the open arm (c) total number of entries in open arm was analyzed[10,11].

Elevated plus maze—All the animals were also subjected to elevated plus maze assessment of anxiety levels. The plus maze is composed of two open arms (16 × 5 cm) and two enclosed arms (16 × 5 × 12 cm) with an open roof and is elevated to a height of 25 cm (Fig. 1b)[12,13]. Each animal was placed individually in the centre of the maze facing towards the open arm and the following parameters were recorded (a) time spent in open arm (b) latency to enter the open arm and (c) total number of entries in open arm during 5 minutes session[12,13].

Statistical analysis—One specific group of mice was assigned to one specific drug treatment condition and each group comprised six mice (n=6). All the values are expressed as means ±S.E.M. The data were analyzed by student's t-test. $P<0.05$ was considered as statistical significant.

Results

Elevated zero maze—Various drugs such as clozapine (0.25, 0.5, 1 mg/kg), resperidone (0.5, 1 mg/kg), venlafaxine (8, 16 mg/kg), citalopram (10 mg/kg), fluoxetine(10 mg/kg), gabapentin (10, 20 mg/kg) and ashwagandha (50, 100, 200 mg/kg) significantly increased the number of open area entries and time spent in open arm and also decreased the latency to enter in open arm as compared to control. The effect of gabapentin (20 mg/kg), venlafaxine (16 mg/kg) and citalopram (10 mg/kg) was comparable to classical antianxiety drug diazepam (Fig. 2a, b, c).

Elevated plus maze—Various drugs such as clozapine (0.25, 0.5, 1 mg/kg), resperidone (0.5, 1 mg/kg), venlafaxine (4, 8, 16 mg/kg), citalopram (10

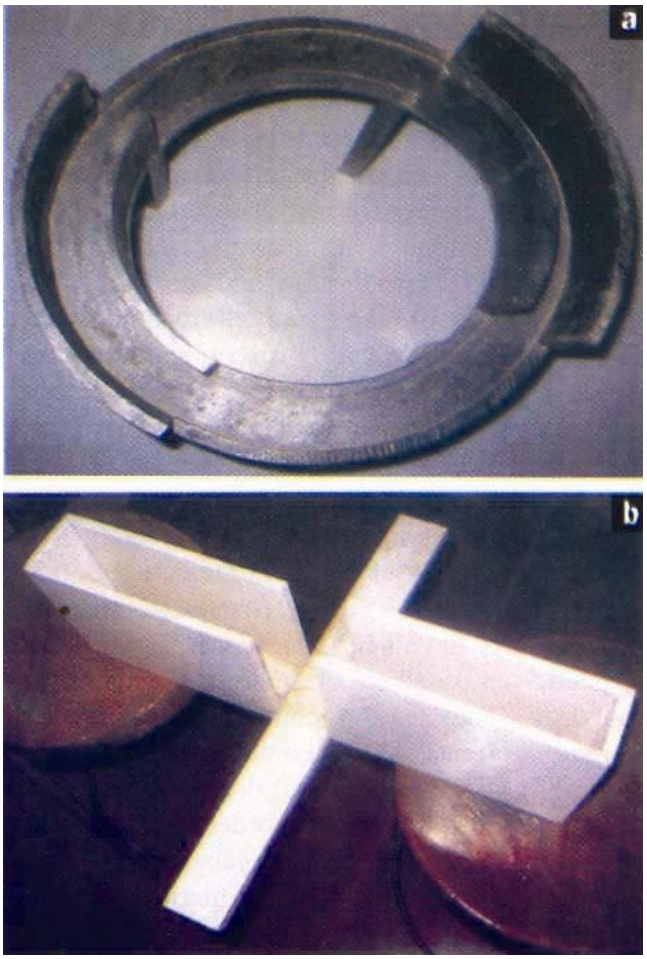

Fig. 1(a)—Elevated Zero Maze (EZM); and (b) - Elevated Plus Maze (EPM)

mg/kg), fluoxetine(10 mg/kg), gabapentin (10, 20 mg/kg) and ashwagandha (100, 200 mg/kg) significantly increased the number of open area entries and time spent in open arm and also decreased the latency to enter in open arm as compared to control. The effect of gabapentin (20 mg/kg), venlafaxine (16 mg/kg) and citalopram (10 mg/kg) was comparable to classical antianxiety drug diazepam (Fig. 3a, b, c).

Discussion

The serious side effects associated with classical anti-anxiety drugs, namely rebound insomnia, sedation, muscle relaxation, withdrawal and tolerance (benzodiazepine, barbiturates and alcohol), sexual dysfunction anticholinergic and antihistaminic effects (TCA's)[2,4] have limited and/or discouraged their use in patients. Hence, search for alternative treatment of anxiety disorders have been evolving rapidly. The Selective Serotonin Reuptake Inhibitors (SSRIs) have been reported to be most effective and were considered as the drugs of choice for the anxiolytic therapy[4]. In the present study fluoxetine and citalopram (SSRIs) significantly increased the number of open arm entries and time spent in open arm and also decreased the latency to enter in open arm as compared to control in both the elevated zero maze and elevated plus maze paradigms. Although SSRIs are effective and have replaced the benzodiazepines or they are used as adjuvant therapy with benzodiazepines, clinical studies have shown that they are associated with significant side effects such as sexual dysfunction and weight gain and are effective in approximately 50-60% of patient population[13].

Among anticonvulsants being used in treatment of anxiety disorders are valproate, carbamazepine and the newer anticonvulsant like gabapentin, pregabalin and tiagabaine[14,15]. Although gabapentin is initially synthesized as a GABA analogue and emerging body experience suggest the potential utility of gabapentin for a number of psychiatric disorders including mania, pain and anxiety. Preclinical data suggest the potential anxiolytic effect of gabapentin[6]. It is perhaps the first potential GABA facilitating medication that was studied in anxious patients. It is unclear if gabapentin increases synthesis of GABA or delays its breakdown. Either way mild GABA increases are detected after its administration[16]. Other studies also reported reduction in anxiety symptoms in 18 patients with primary psychotic disorders and in one patient with generalized anxiety disorder. Patients frequently express an interest in using gabapentin rather than mood stabilizing agent due to its limited side effect profile[17]. Gabapentin is also not metabolized in body thus minimizing the possibility of drug-drug interactions in the patients who may require polytherapy[18,19]. In the present study gabapentin significantly increased the number of open area entries and time spent in open arm and also decreased the latency to enter in open arm as compared to control in both the elevated zero maze and elevated plus maze paradigms.

A typical antipsychotics agents have greater affinity for $5HT_{2A}$ binding site than for dopamine D_2 binding sites and possess little or no extrapyramidal side effects. They have also been found to be useful in other mental disorders. Their clinical use in the management of non-psychotic disorder is gradually increasing. The use of atypical antipsychotic (resperidone, clozapine, olanzapine) has recently emerged as an effective strategy in treatment of resistant mood and anxiety disorders[7]. An important feature of using atypical antipsychotics in mood and anxiety disorders is that they can be effective at doses that would be considered sub-therapeutic for the management of psychiatric disorders[20]. Existing evidences strongly suggest both the safety and efficacy of these drugs as augmentation therapy for anxiety. In the present study clozapine and resperidone significantly increased the number of open area entries and time spent in open arm and also decreased the latency to enter in open arm as compared to control in both the elevated zero maze and elevated plus maze paradigm. Further in our study, venlafaxine, a bicyclic antidepressant that produces strong inhibition of norepinephrine and serotonin reuptake significantly increased the number of open area entries and time spent in open arm and also decreased the latency to enter in open arm as compared to control in both the elevated zero maze and elevated plus maze paradigm. It is the first antidepressant that is proved effective in treating patients with generalized anxiety disorder (GAD) with or without depression. Effectiveness of venlafaxine is superior to that of buspirone for the treatment of GAD[8,21].

Besides the use of synthetic drugs, several herbal antianxiety drugs are also used in the management of anxiety related disorders. The roots of Withania somnifera (WS) are used extensively in Ayurveda, the

classical Indian system of medicine, and WS is categorized as a rasayana, which are used to promote

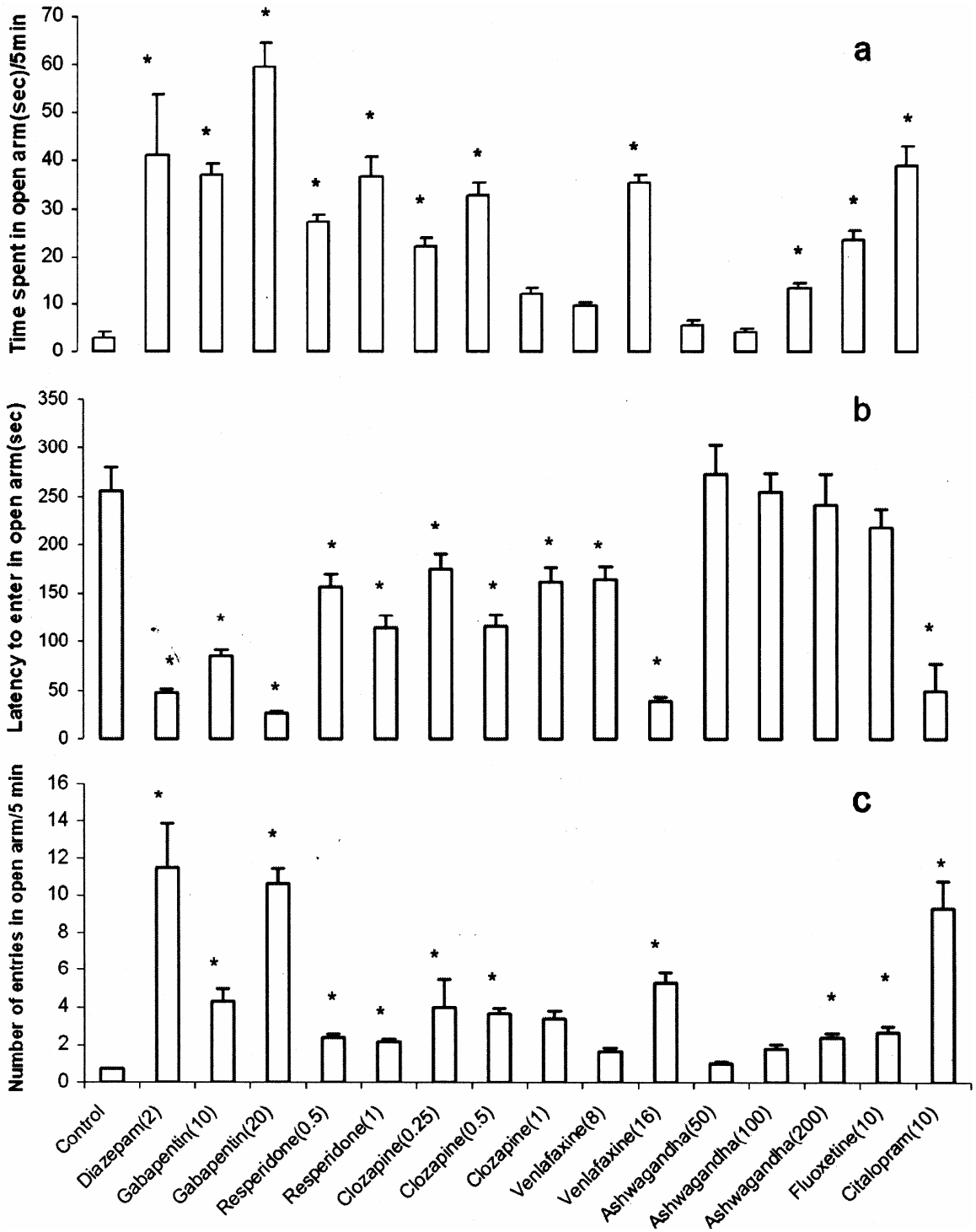

Fig. 2—Effect of different newer anxiolytic drugs on- (a) time spent in open arm; (b) latency to enter in open arm; (c) number of entries in open arm at elevated zero mazes [Values are expressed as mean ± SEM. *$P \leq 0.05$ as compared to control group]

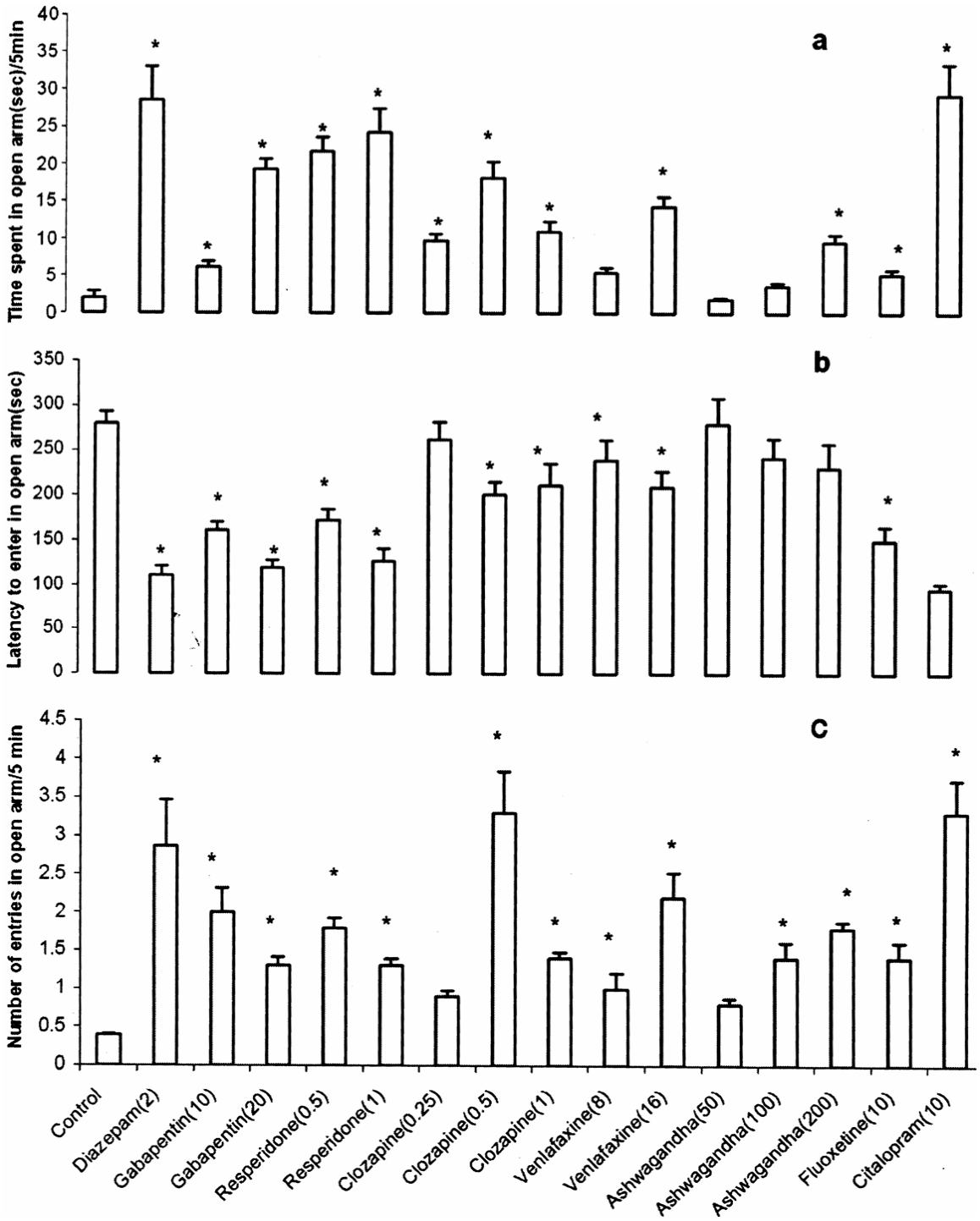

Fig. 3—Effect of different newer anxiolytic drugs on- (a) time spent in open arm; (b) latency to enter in open arm; (c) number of entries in open in open arm at elevated plus maze [Values are expressed as mean ± SEM. *$P \leq 0.05$ as compared to control group]

physical and mental health[9]. The investigations support the use of WS as a mood stabilizer in clinical conditions of anxiety and depression in Ayurveda[9]. In our study it increased the number of open area entries and time spent in open arm and also decreased the latency to enter in open arm as compared to control in both the elevated zero maze and elevated plus maze paradigms at higher doses.

Critically analyzing the results it was found out that among the synthetic drugs, effect of gabapentin (20 mg/kg), citalopram (10 mg/kg) and venlafaxine (16 mg/kg) was comparable to diazepam (2 mg/kg). Further *in vivo* and *in vitro* tests should be carried out to confirm the antianxiety effect these drugs. In conclusion the present study showed the antianxiety activity of different novel class of drugs and found few of them comparable to diazepam (classical anxiolytic) in both the elevated zero maze and elevated plus maze paradigms.

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