

Research Article

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Comparative Role of Paroxetine and Clomipramine on Anxiety in Mice Recovering from Stress

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Abstract

Stress is the body's reaction to any change that requires an adjustment or response. The body reacts to these changes with physical, mental, and emotional responses. It is the body's reaction to harmful situations whether they're real or perceived. When stressed, a chemical reaction occurs in the body that allows it to act in a way to prevent injury. This reaction is known as "fight-or-flight," or the stress response. During stress response, heart rate increases, breathing quickens, muscles tighten, and blood pressure rises. The present study investigated behavioral pattern between stress-induced mice and stressed mice treated with antidepressants (paroxetine and clomipramine) using open field apparatus and elevated-plus maze test. Twenty eight (28) Swiss Mice were divided equally into four groups i.e. Group one (control), Group two (stressed), Group three (stressed mice treated with paroxetine) and Group four (stressed mice treated with clomipramine). Animals in group-one were given normal feed and water only. Group two animals were also given normal rat chew and water and subjected to prolonged stress by social isolation, deprivation, confinement to small cages and exposure to bright light at night for a period of five weeks. Animals in group three were administered with paroxetine at a dose of 10mg/kg body weight orally for two (2) weeks after they were stressed for five-week, while animals in the group four were administered with clomipramine at the same dose orally for two (2) weeks after subjecting them to the same measure and duration of stress like those of group three. The treated groups were also allowed access to water and normal rat feeds throughout the experiment period. The four groups were then subjected to tests in both open field and elevated-plus maze apparatus. The results showed increased anxiety in the stressed mice compared to control. The groups stressed and later treated with paroxetine and clomipramine showed significantly reduced anxiety ($P < 0.05$) compared to stressed mice. The group stressed and later treated with paroxetine showed reduced anxiety significantly compared to the group stressed and later treated with clomipramine ($P < 0.05$). These were determined by grooming frequency and duration, rearing frequency as well as closed arm duration and head dip frequency in the EPM. Paroxetine and clomipramine administration resulted in significant improvement from anxiety after stress. Paroxetine caused a more significant improvement from anxiety than clomipramine in mice recovery from stress when the two agents were compared.

Keywords: Anxiety; Stress; Paroxetine; Clomipramine

Introduction

Stress has become undoubtedly an integral part of human life. Stressful events have a damaging effect on normal physiological functions leading to a variety of diseases. Many of the diseases of the modern life like hypertension, diabetes, behavioral disorders have been suggested as some of the many deteriorating effects of stress [1]. Stress is an important factor of depression that causes the changes in various body systems. Animal health including human has been shown to be affected by the stressful events of life including situation which alters cognition, learning, memory and emotional responses, causing mental disorders like depression and anxiety. Depression is a state of low mood and aversion to activity that can affect a person's thoughts, feelings, behavior and sense of well-

being [2]. However, many of these effects are mediated by stress-induced neurochemical and hormonal abnormalities that are often associated with oxidative stress [3]. People with depressed mood may be notably sad, anxious, or empty; they may also feel hopeless, helpless, dejected or worthless. Other symptoms expressed may include senses of guilt, irritability or anger. These individuals may express a further feeling of shamefulness or an expressed restlessness. They may notably lose interest in activities that they once considered pleasurable to family and friends or otherwise experience either a loss of appetite or overeating. Experiencing problems like concentrating, remembering general facts or details, otherwise making decisions or experiencing relationship difficulties

may also be notable factors in these individuals' depression and may also lead to their attempting or actually committing suicide [4]. In addition to all the aforementioned factors, actions committed by siblings of these individuals may also contribute to the decision-making in individuals experiencing depression or attempting to take their own lives [4]. Expressed insomnia, excessive sleeping, fatigue, pains, digestive problems and a reduced energy may also be present in individuals experiencing depression. A depressed mood is a feature of some psychiatric syndromes such as major depressive disorder and dysthymia, but it may also be a normal temporary reaction to life events such as bereavement, a symptom of some bodily ailments or a side effect of some drugs and medical treatments.

Antidepressants are drugs used for the treatment of major depressive disorder and other conditions, including dysthymia, anxiety disorders, obsessive compulsive disorder, eating disorders, chronic pain, neuropathic pain and, in some cases, dysmenorrhea, snoring, migraine, attention-deficit hyperactivity disorder, addiction, dependence, and sleep disorders [5]. The most important classes of antidepressants are the selective serotonin reuptake inhibitors (SSRIs), serotonin-norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (TeCAs), and noradrenergic and specific serotonergic antidepressant [6]. Antidepressants are recommended by the National Institute for Health and Care Excellence (NICE) as a first-line treatment of several depressions and for the treatment of mild-to-moderate depression that persists after conservative measures such as cognitive therapy. There has been controversy regarding the efficacy of antidepressants in treating depression depending on its severity and duration. The 2008 meta-analysis combined 35 clinical trials submitted to the Food and Drug Administration (FDA) before licensing of four newer antidepressants (including the SSRIs paroxetine and fluoxetine, the non-SSRI antidepressant nefazodone, and the SNRI venlafaxine). The authors attributed the relationship between severity and efficacy to a reduction of the placebo effect in severely depressed patients, rather than an increase in the effect of the medication. SSRIs are recommended by the National Institute for Health and Care Excellence (NICE) for the treatment of generalized anxiety disorder (GAD) that has failed to respond to conservative measures such as education and self-help activities [7]. Among the SSRIs, paroxetine has the highest specificity for serotonin. Paroxetine affects chemicals in the brain that may be unbalanced in people with depression, anxiety, or other disorders. It is effective in depression that has proved resistant to other antidepressants and in depression complicated by anxiety [8].

Paroxetine is used to treat depression, obsessive-compulsive disorder, anxiety disorders, post-traumatic stress disorder (PTSD), and premenstrual dysphoric disorder (PMDD). Common side effects of paroxetine may include: vision changes; weakness, drowsiness, dizziness; sweating, anxiety, shaking; sleep problems (insomnia); loss of appetite, constipation; dry mouth, yawning; or decreased sex drive, impotence, or difficulty having an orgasm. Other

antidepressants used in the treatment of obsessive-compulsive disorder, major depressive disorder, panic disorder, are the tricyclic antidepressants (TCAs). Clomipramine belongs to this group and is a highly selective inhibitor of serotonin reuptake [9]. It is also an antagonist/inverse agonist at the histamine H1 receptor, the muscarinic acetylcholine receptors and the α 1 adrenergic receptor. These last three actions likely contribute to its adverse effects [9].

The nervous system is a coordinating system that controls all the activities of the body. It receives millions of bits of information from the different sensory organs and then integrates all these to determine the responses to be made by the body. It is a communication network that allows an individual to interact appropriately with the environment. The nervous system consists of sensory and motor divisions. The sensory division initiates most of its activities emanating from sensory receptors whether visual, auditory, tactile on the surface of the body or other kinds of receptors. The motor division controls the various body activities like contraction of skeletal muscles, smooth muscles in the internal organs and secretion of both endocrine and exocrine organs [10]. These are collectively called the motor functions of the nervous system. Primarily, the nervous system is divided into two parts namely, the central nervous system and the peripheral nervous system. The central nervous system includes the brain and spinal cord. It is formed by neurons and the supporting cells called neuroglia. The functions of the central nervous system among others include organizing reflexes and other behavioural responses responsible for cognition, learning and memory and plans and executes voluntary movements. Memory refers to the storage mechanisms for what is learned, learning and memory are special forms of information processing that permit behaviour to change appropriately in response to environmental challenges based on past experiences. The peripheral nervous system is formed by the neurons and their processes present in all regions of the body. These consist of cranial nerves from the brain and spinal nerves from the spinal cord. It is further subdivided into the somatic nervous system and autonomic nervous system. The somatic nervous system controls the movements of the body by acting on the skeletal muscles while the autonomic nervous system is concerned with regulation of visceral or vegetative functions [10]. Behaviour consists of the totality of the organism's responses to its environment.

Aim of the study

The aim of this study is to compare the effect of two antidepressants (Paroxetine and Clomipramine) on anxiety in stressed mice.

Materials and Methods

Materials

The following materials were used for the various experiments carried out: methylated spirit, weighing balance, plastic bowl, syringe and needle, distilled water, cotton wool, Toilet paper, open field maze, Elevated plus maze, Marker pen, cages, feeding troughs, milk, spatula, masking tape, stop watches, sawdust beddings.

Experimental animals and drugs

The experimental animals used in this study were 28 Swiss mice, obtained from Department of Pharmacology, University of Calabar, Calabar, Nigeria with body weight between 180g to 250g specifically bred for experimental purposes. The mice were divided into four groups of seven each.

- Group 1: The control (non-stressed).
- Group 2: Stressed group (stressed but not treated).
- Group 3: Stressed + paroxetine group.
- Group 4: Stressed + clomipramine group.

Group 1 was not exposed to any form of stress. Group 2 were stressed but not treated. Group 3 were stressed but treated with oral paroxetine (10mg/kg body weight) daily for 2 weeks. Group 4 were stressed but treated with oral clomipramine (10mg/kg body weight) daily for 2 weeks. All animals were allowed access to feed and water.

Drug administration protocol

The doses of drugs, the duration of the administration were done according to previous studies as reported by [11]. All drugs were dissolved in distilled water.

Administration of paroxetine and clomipramine

A 20mg and a 10mg capsule of paroxetine and clomipramine was dissolved in 20ml and 10ml of distilled water (using a stirrer) to form a stock solution of 0.02mg/20ml and 0.01/10ml respectively. Each of the solution was administered at the dose of 10mg/kg body weight orally, once daily for two weeks.

Stress regimen

This procedure was done through social isolation (prolonged social deprivation), confinement to small cages and exposure to bright light at night for the period of five weeks [12] (Matsumoto et al, 2005).

Statistical Analysis

Values for the result are expressed as mean \pm SEM. The statistical analysis was done using the analysis of variance (ANOVA) and the post/hoc Newmann Keul's test. The computer software was Microsoft excel and SPSS for window.

Differences between means were tested at 0.05 level of significance.

Apparatus and experimental protocol

The open field maze: The large open field (72 \times 72 cm, with 36cm high walls) constructed of plywood was used following the protocol of [13]. Each trial was recorded using a video cassette recorder (VCR) connected to a camcorder. This was for rescoring of behaviour.

Experimental procedure in the open field maze: Mice were carried to the neurobehaviour laboratory in their home cages from

the animal house and were handled by the base of their tails at all times. Each mouse of the four groups was exposed to the open field maze by placing it in the centre square of the maze and allowed to explore the apparatus for 5 minutes. The mouse behavior was scored within this period and the mouse returned to its home cage while the open field was cleaned with 70% ethyl alcohol and then allowed to dry between tests. This was to eliminate olfactory cue.

Behaviour scores in the open field

The behaviours scored included;

1. Line crossing: Number of times the mouse crossed a line drawn on the floor with all its four paws.
2. Rearing: Frequency with which the animal stands on its hind legs or leans against the wall with front paws.
3. Center square duration: Amount of time the mice spent in the central square.
4. Center square entries: Frequency with which the mouse enters the centre square with all four paws.
5. Grooming frequency: the number of times the animal spent licking or scratching itself while stationary.
6. Grooming duration: The period of time the animal spent licking or scratching itself while stationary.
7. Urination: Number of puddles or streaks of urine.
8. Defecation: Number of fecal boli produced.[13]

Elevated plus maze

5.2.1. Experimental procedure in the elevated plus maze

The mouse was picked by the base of its tail from the home cage and placed in the center square between the open and closed arms facing an open arm. Each mouse was allowed to explore the maze for 5 minutes and the behaviours scored within this period and then returned to its home cage. The apparatus was cleaned with 70% ethyl alcohol and then allowed to dry between tests in order to eliminate olfactory cue.

Behaviour scores in the elevated plus maze

1. Open arm entries: Frequency with which the animal entered the open arms with all four paws.
2. Closed arm entries: Frequency with which the mouse entered the closed arm with all four paws.
3. Open arm duration; length of time the animal spent in the open arms.
4. Closed arm duration: Length of time the animal spent in the open arms.
5. Head dipping: Frequency with which the animal lowered its head over the sides of the open arms toward the floor.
6. Rearing: Frequency with which the animal stands on its hind legs or leans against the wall with front paws.

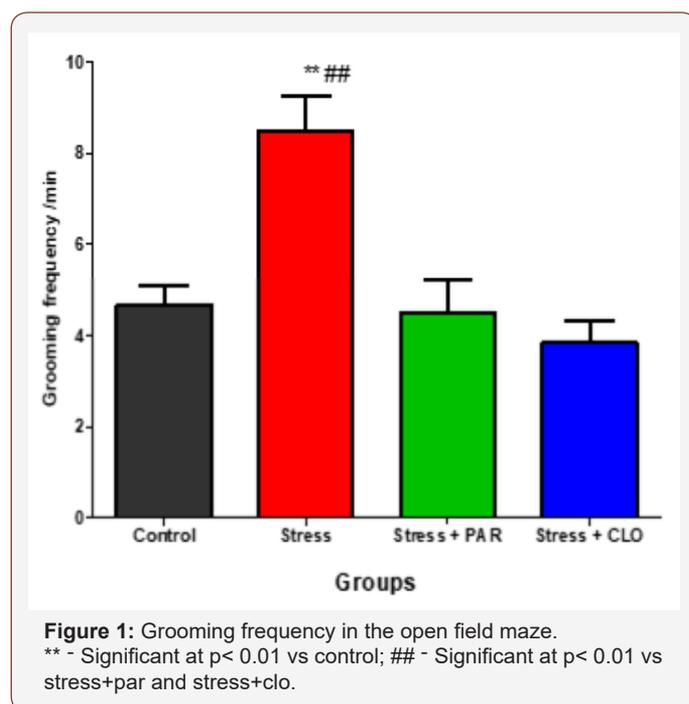
7. Grooming (Frequency and duration): The number and period of times the animal spends licking or scratching itself while stationary.
8. Urination: Number of puddles or streaks of urine.
9. Defecation: Number of fecal boli produced. [13-16]

Results

Frequency of grooming in the open field for control, stressed, stressed+paroxetine, and stressed+clomipramine groups

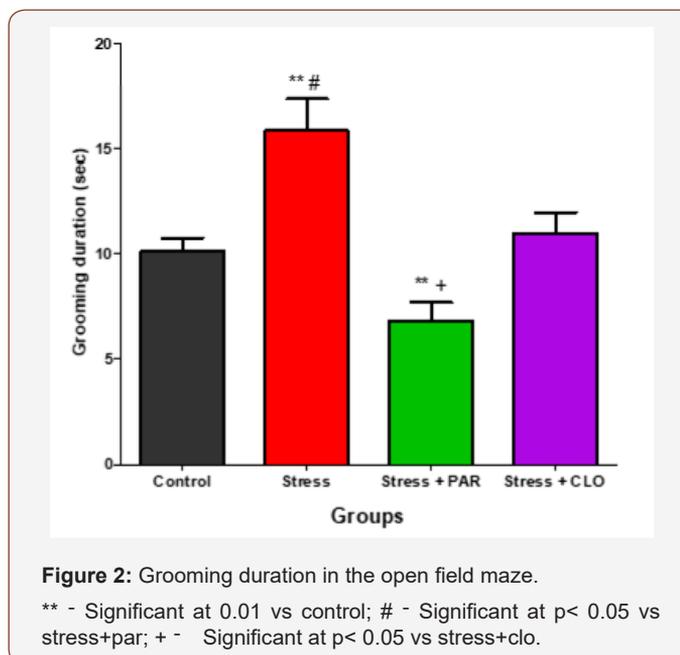
The grooming frequency in the open field for control, stressed, stressed+paroxetine and stressed+clomipramine were, 4.7 ± 0.42 , 8.5 ± 0.76 , 4.5 ± 0.72 , and 3.8 ± 0.48 respectively.

There was significant increase ($P < 0.01$) in stressed group compared to control, stressed+paroxetine, stressed+clomipramine groups ($P < 0.01$). The results showed no significant difference between stressed+paroxetine and stressed+clomipramine groups, although there was a significant decrease ($P < 0.01$) in both treated groups compared with the stressed group (Figure 1).



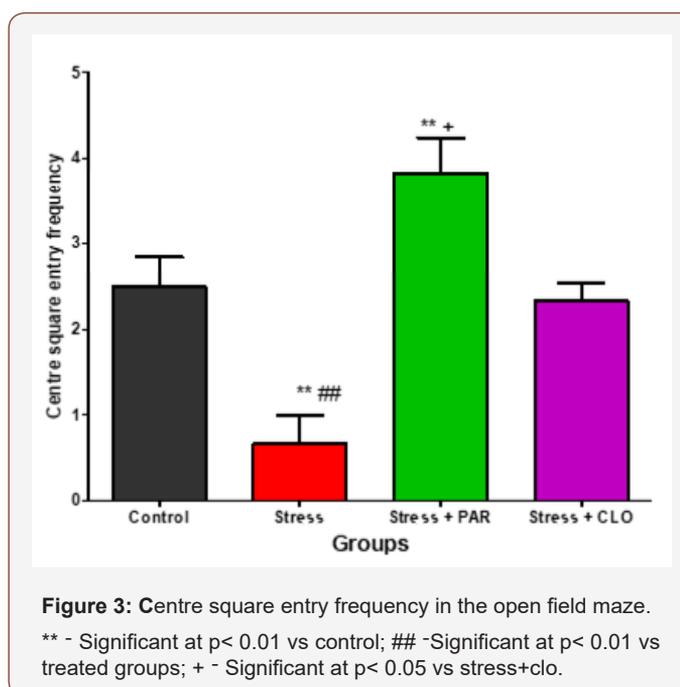
Grooming duration in the open field for control, Stressed, stressed+paroxetine, and stressed+clomipramine groups

The grooming duration in the open field for control, stressed, stressed+paroxetine, and stressed+clomipramine group of mice were, $10 \pm 0.6s$, $16 \pm 1.5s$, $6.8 \pm 0.87s$ and $11 \pm 0.97s$ respectively. The stressed group showed a significant increase ($p < 0.05$) compared to control, stressed+paroxetine, and stressed+clomipramine group. It was also observed that stressed mice treated with paroxetine spent lesser time grooming compared with stressed mice treated with clomipramine ($P < 0.05$) (Figure 2).



Centre square entry frequency in the open field for control, stressed, stressed+paroxetine and stressed+clomipramine group

The Centre Square Entry (CSE) frequency for the control, stressed, stressed+paroxetine, and stressed+clomipramine group of mice were 2.5 ± 0.34 , 0.67 ± 0.33 , 3.8 ± 0.40 , and 2.3 ± 0.21 respectively. The mice in the stressed group showed a significantly lower ($p < 0.01$) CSE compared to mice in the control, stressed+paroxetine and stressed+clomipramine groups. The stressed+paroxetine group showed a significant increase ($p < 0.01$) in CSE compared with control, but there was no significant difference in stressed+clomipramine group compared with control. Stressed+paroxetine showed a significant increase ($p < 0.05$) compared to stressed+clomipramine (Figure 3).

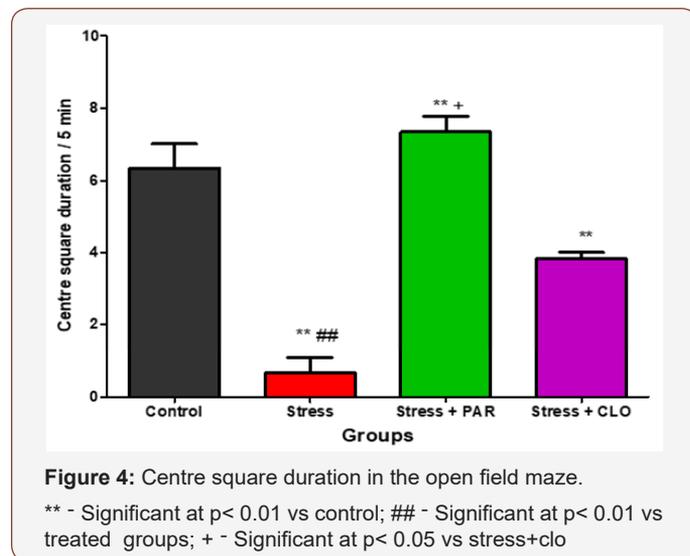


CSE duration in the open field for control, stressed, stressed+paroxetine, stressed+clomipramine groups

The CSE duration in the open field for control, stressed, stressed+paroxetine and stressed+clomipramine group of mice were $6.3 \pm 0.67s$, $0.67 \pm 0.42s$, $7.3 \pm 0.42s$, and $3.8 \pm 0.17s$ respectively.

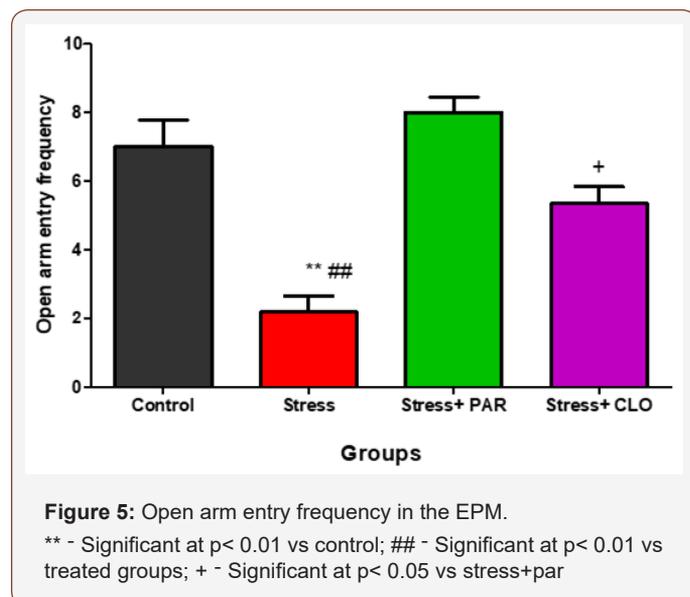
The stressed group was significantly lower ($p < 0.01$) when compared to control, stressed+paroxetine, and stressed+clomipramine groups.

However, stressed+paroxetine showed a significant increase in CSE duration compared with control ($p < 0.01$) and stressed+clomipramine group ($P < 0.05$) (Figure 4).



Open arm entry frequency in the EPM for control, stressed, stressed+paroxetine and stressed+clomipramine group

The open arm entry frequency in the EPM for control, stressed, stressed+paroxetine and stressed+clomipramine groups of mice were 7.0 ± 0.77 , 2.2 ± 0.48 , 8.0 ± 0.45 , and 5.3 ± 0.49 respectively. The stressed group had significantly lower ($p < 0.01$) values of open arm entry frequency compared to control, as well as the treated groups.



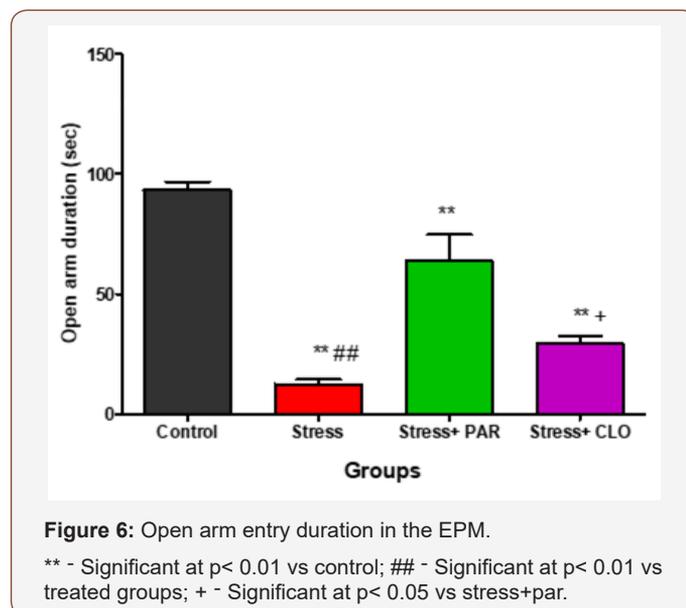
There was no significant difference in OEF in both stressed+paroxetine and stressed+clomipramine groups compared to the control group, but however, there was a significant increase ($p < 0.05$) in OEF in the stressed+paroxetine group compared to stressed+clomipramine group (Figure 5).

Open arm duration in the EPM for control, stressed, stressed+paroxetine and stressed+clomipramine group

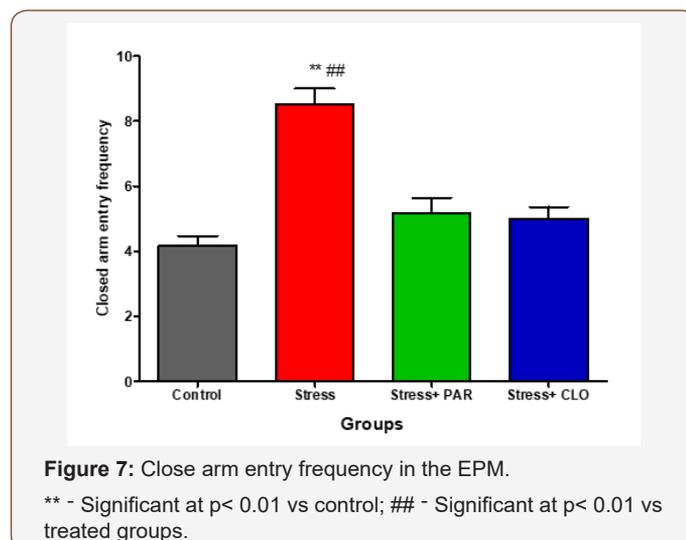
The time spent by the control, stressed, stressed+paroxetine, and stressed+clomipramine group of mice in the open arm were, $93 \pm 3.7s$, $13 \pm 1.9s$, $64 \pm 11s$, and 29 ± 3.3 respectively. Stressed, stressed+paroxetine and stressed+clomipramine groups of mice showed a significant decrease in OAED at $P < 0.01$ compared with control group of mice.

There was a significant increase ($p < 0.01$) in OAED in the stressed+paroxetine group of mice compared with stressed group.

The stressed+paroxetine group showed a significant increase ($p < 0.05$) in OAED compared to stressed+clomipramine group (Figure 6).



Frequency of closed arm entry in the EPM



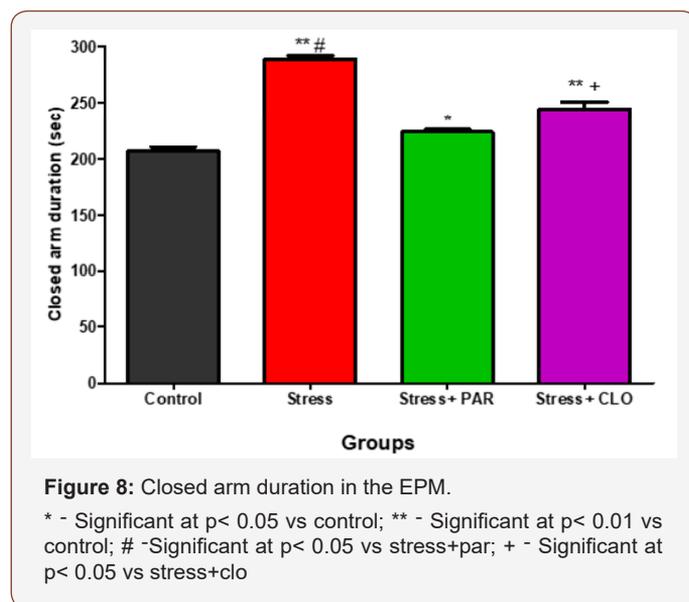
The closed arm entry frequency of the control, stressed, stressed+paroxetine and stressed+clomipramine groups of mice were, 4.2 ± 0.31 , 8.5 ± 0.5 , 5.2 ± 0.48 , and 5.0 ± 0.37 respectively. The closed arm entry frequency in the stressed group of mice was significantly higher ($p < 0.01$) compared with the control, stressed+paroxetine group, as well as stressed+clomipramine group.

It was observed that, there was no significant difference between the control and the two stressed-treated groups. There was also no significant difference between the two treated groups (Figure 7).

Closed arm duration in the EPM

The closed arm duration in the EPM for control, stressed, stressed+paroxetine and stressed+clomipramine groups of mice were, $207 \pm 3.7s$, $289 \pm 2.6s$, $224 \pm 2.6s$ and $244 \pm 6.7s$ respectively. The stressed and stressed+clomipramine groups of mice spent significantly higher time in the closed arm compared to the control at $p < 0.01$. Stressed+paroxetine showed a significant increase in CAD ($p < 0.05$) compared with control.

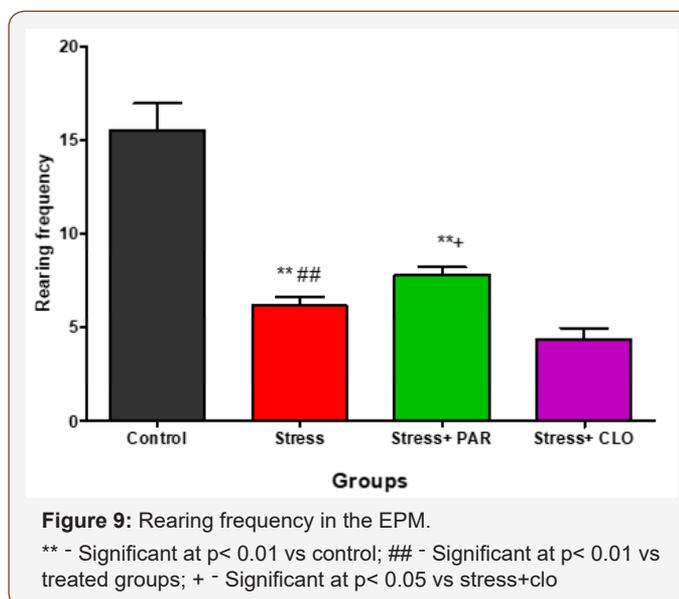
There was a significant increase ($p < 0.05$) in CAD in stressed group compared with stressed+paroxetine. Stressed+clomipramine groups of mice showed a significant increase ($p < 0.05$) in CAD compared with that of stressed+paroxetine (Figure 8).



Frequency of rearing in the EPM

The rearing frequency in the EPM for control, stressed, stressed+paroxetine, and stressed+clomipramine group of mice were, 16 ± 1.5 , 6.2 ± 0.48 , 7.8 ± 0.40 , and 4.3 ± 0.61 respectively. There was a significant decrease in rearing frequency in stressed, stressed+paroxetine, and stressed+clomipramine group of mice compared to the control group at $P < 0.01$.

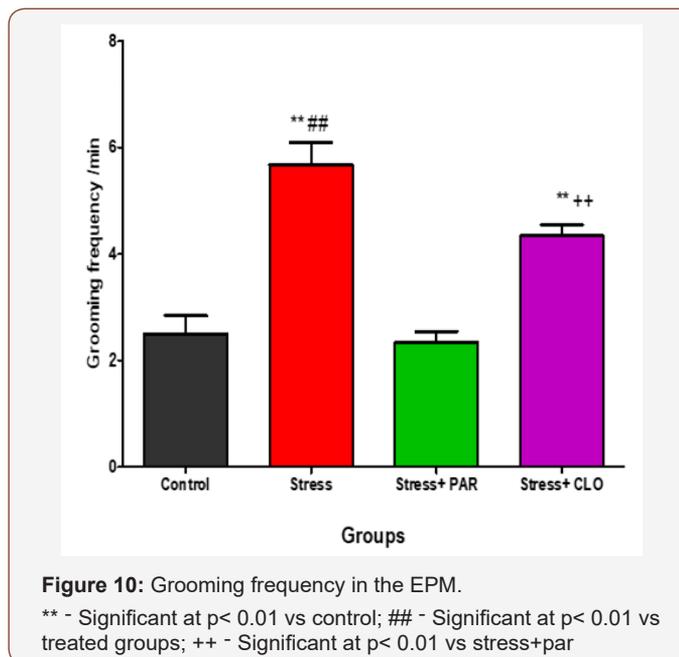
There was a significant increase ($p < 0.01$) in rearing frequency in stressed+paroxetine group of mice compared to that of stressed group and stressed+clomipramine group at $p < 0.01$ and $p < 0.05$ respectively. (Figure 9)



Grooming frequency in the EPM

The grooming frequency for control, stressed, stress+paroxetine, and stress+clomipramine group of mice were, 2.5 ± 0.34 , 5.7 ± 0.42 , 2.3 ± 0.21 and 4.3 ± 0.21 respectively. Stressed group of mice increased significantly ($p < 0.01$) compared to the control, stressed+paroxetine, and stressed+clomipramine groups. Stressed+paroxetine group of mice showed no significant difference compared to control. But however, stressed+clomipramine group of mice was significantly increased ($p < 0.01$) compared to control. Figure 10

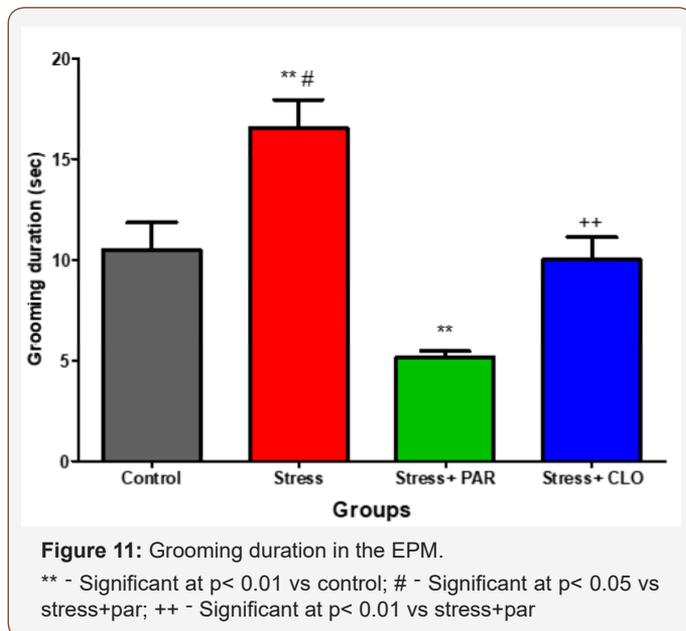
There was also a significant increase ($p < 0.01$) in the grooming frequency of the stressed-treated with clomipramine group of mice compared to stressed-treated with paroxetine group (Figure 10).



Grooming duration in the EPM

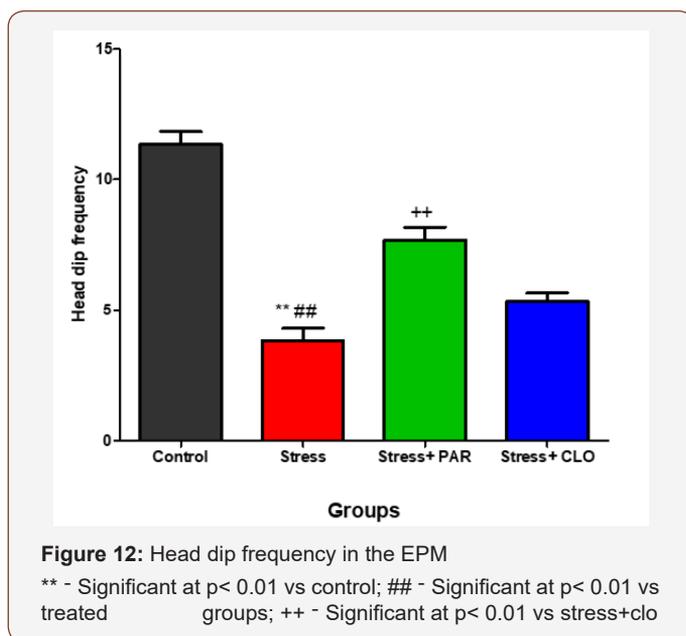
The grooming duration for the control, stressed, stressed+paroxetine, and stressed+clomipramine group of mice

were, 11 ± 1.4 s, 17 ± 1.4 s, 5.2 ± 0.31 s, and 10 ± 1.1 s respectively. The stressed group of mice showed a significant increase in the grooming time compared to control and stressed+paroxetine groups at $p < 0.01$ and $p < 0.05$ respectively. There was a significant decrease ($p < 0.01$) in the grooming frequency of the stressed+paroxetine group compared to stressed+clomipramine groups (Figure 11).



Frequency of head dip in the EPM

The frequency of head dip for the control, stressed, stressed+paroxetine, and stressed+clomipramine group of mice were, 11 ± 0.49 , 3.8 ± 0.48 , 7.7 ± 0.49 and 5.3 ± 0.33 respectively. Stressed group of mice showed a significantly decrease ($p < 0.01$) in head dip compared to control, stressed+paroxetine, as well as stressed+clomipramine group at. (Figure 12)



When comparing the stressed-treated groups, stressed+paroxetine group showed a significant increase ($p < 0.01$) in head dip frequency.

Discussion and Conclusion

Discussion

Anxiety is a neurobehaviour that is determined using both the open field apparatus and the elevated-plus maze, both of which were utilized in the course of this study. Behavior such as Grooming, Rearing, Head dip, Centre Square Entry (CSE) and Centre Square Duration (CSD) are all measures of anxiety [13,14]. An increase in Rearing, CSE, CSD and Head dip indicates decrease in the level of anxiety and vice-versa. An increase in Grooming frequency and Grooming duration both indicate an increase in the level of anxiety and vice-versa. There was a reported significant increase in anxiolytic effects of the stressed mice when compared with control mice, while the stress-treated with paroxetine and clomipramine mice both showed a decrease in anxiolytic effects compared to the stressed mice [14,15]. It was also further observed that, the stress-treated mice with paroxetine reduces anxiety significantly compared to the stress-treated mice with clomipramine. This reduction may be as a result of lack of anticholinergic effect of paroxetine which is one of the major advances of the SSRIs antidepressants. TCAs, apart from acting as strong inhibitors in the reuptake of both norepinephrine and serotonin, also blocks histaminic, cholinergic, and alpha 1-adrenergic receptor sites, and this lack of selectivity is what accounts for the unwanted side effects such as weight gain, dry mouth, constipation, drowsiness, and dizziness. Unlike the TCAs, SSRIs do not cause anticholinergic, hypotensive or sedating reactions, and are not associated with impaired cognitive function [14-16]. It can therefore be suggested that, paroxetine enhances endogenous acetylcholine in the dorsal hippocampus thereby reducing anxiety through the actions on nicotinic and muscarinic1 receptors.

Conclusion

Both Paroxetine and Clomipramine significantly reduce anxiety in stress. But Paroxetine reduces it significantly than clomipramine when compared. This hyper effect could be due to the selectivity properties of paroxetine which do not affect other neurotransmitters like histamine and acetylcholine, thereby causing the blockage of histaminic and cholinergic receptor sites. It may therefore be necessary to recommend paroxetine as the first line of treatment for enhancing minimizing anxiolytic effects in stress.

Acknowledgement

None.

Conflict of Interest

No conflict of interest.

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