Comparative Effects of Imipramine and Chlorpromazine on Neurobehavioural Parameters in Stressed Mice

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Abstract: *Comparative effect of imipramine (25mg/kg) and chlorpromazine (10mg/kg) on social behaviour, depression and anxiety on stressed mice was studied. This study compared the effects of these drugs on neurobehavioral parameters. Forty (40) male albino mice were used. They were divided into four (4) groups, n = 10, (control, stressed, stressed imipramine treated group, and stressed chlorpromazine treated group. The stress method used was total social isolation and unpredicted stress protocols. Only the mice in control group were put together in one single cage that was properly ventilated and the animals given free access to water and normal mice feed. The experiment lasted for forty two (42) days; the experimental groups were all stressed for 28 days after which drugs were administered for 14 days, and neurobehavioral test carried out within 24 hours after the last administration. The results showed significant decrease in social behavioural test (nesting) (P<0.001) in both stressed and stressed-chlorpromazine treated groups when compared to the control and stressed-imipramine treated groups, while the stressed-imipramine treated group showed significant decrease (P<0.05) when compared to the control. Also, the results obtained from the depression test showed significant decrease (P<0.001) in all the test groups when compared to the control. But the stressed-imipramine treated group showed significant improvement (increase) (P<0.05) when compared to both stressed and stressed-chlorpromazine treated groups. The anxiolytic test shows significant increase (P<0.01) in the grooming frequency of stressed and stressed-chlorpromazine groups in open field maze when compared to both control and stressed-imipramine treated groups. Stressed-imipramine treated group also showed significant increase (P<0.001) in grooming frequency when compared with the control. The neurobehavioral studies shows that stress can cause reduced social activities, depression symptoms and anxiety disorder, and imipramine can significantly restore some of these neurobehavioral activities better than chlorpromazine will do.*
Keywords: *Stress, Anxiety, Imipramine, Chlorpromazine, and Social Behaviour*

I. Introduction

Depression is a notorious and highly incapacitating neuropsychiatric problem exerting burden on the society. Its symptoms include loss of interest on pleasure, feeling of quilt or low self-worth, poor appetite, disturbed sleep, low energy and poor concentration. It is a multifaceted abnormality with heterogeneous pathology [1]

The World Health Organization (WHO) worldwide statistics in the year 2002 showed that 91 million people suffer behavioural disorders while 154 million suffer depression. The population of the depressed rose to more than 18% between 2005 and 2015 but lack of support and fear of stigmatization prevented many of them from receiving treatment. WHO statistics in 2017 also showed that 300 million people are depressed and it's a leading cause of poor health and disability [2]

In the African, it is estimated that one out of every six people suffer from some forms of depression (mental disorder), According to WHO (2017), Medical practitioners under the aegis of the Society of Family Physicians of Nigeria (SOFPON) had raised the alarm that 7 million Nigerians are living with depression. This has become even more serious as the rate suicide in Nigeria has recently been on the increase. SOFPON therefore called for a well-structured primary healthcare centres (PHC) to help detect and treat depression early before deterioration.

The rising cases of depression in Nigeria and Africa may be attributed to the economic hardship the people are faced, and it is feared that if the economy does not improve the figure may even go higher.

Social behaviour is behaviour among two or more organisms, typically from the same species, that are usually beneficial to one or more of the individuals. This is used in experiments to assay for behavioural disorders and depressive symptoms. Another way is to measure positive depressive symptoms in test such as the tail suspension test and forced swim test. [3]

Anxiety disorders are a group of mental disorders characterised by significant feeling of anxiety and fear. The feeling may cause physical symptoms such as increased heart rate. Elevated plus maze and open field maze are used for measuring positive anxiety in rodents Among the many ways of managing behavioural and anxiety disorders, antidepressants and antipsychotic drugs play a vital role for reason that they are more available and accessible to the poor that make the majority of the vulnerable population. Two of these drugs used in managing mental health related issues are imipramine and chlorpromazine [4]

The current study is aimed at comparing the effect of the imipramine And chlorpromazine in stress-induced mice in terms of social behaviour, depression, and anxiety with the view to determining which is more effective in this regard.

II. Materials And Method

Experimental Animal

Male Swiss mice weighing between 15-27g were obtained from the animal house of the Department of Physiology, University of Calabar. Animals were kept in standard laboratory conditions in a well-ventilated animal house, with a 12/12-hour light/dark cycle. Animal beddings were changed every other day and food administration and food trough cleaning was on daily basis. Animals were given food and clean drinking water *ad libitum*. Animals were allowed to acclimatize for two weeks.

All procedures in this study were in agreement with the Guide of Care on the use of Laboratory Animals and were approved by the ethics committee on animal experimentation of the University of Calabar, Nigeria.

Experimental Design

40 male mice were used for this study, they were randomly assigned into four groups (n= 10 in each group).

Group 1	-	Control group (food and water)
Group 2	-	stressed with no treatment
Group 3	-	stressed and treated with imipramine
Group 4	-	stressed and treated with chlorpromazine

Behavioural Assay

Nesting behaviour is an assay for social behaviour. Mice were housed individually and tested in their home cages. One hour before giving the mice nesting materials, all enrichment objects in the home cages of the mice were removed. About 3.0g of nesting material was supplied to each mouse in its home cage and allowed for 24 hours after which the nests were assessed and scored. Scoring was based on what was seen. In order not to destroy the nest so built, care was taken while bringing out the cage for observation. Scoring followed the nesting behaviour rating scale [5,6,7].

Scoring Requirements

1 Nestle not noticeably touched (90% or more intact).

2 Nestle partially torn (50 – 90% intact)

3.Nestle mostly shredded, often no identification nest site, 50% remains intact, but less than 90% is within a quarter of the cage floor (i.e. Not gathered into a nest site but spread throughout cage).

4.An identifiable, but flat nest, more than 90% of the nestle is torn, the nest is uneven, material is gathered into a nest within a quarter of the cage floor, but the nest is flat with walls higher than mouse body height for less than 50% of its circumference.

5. A (near) perfect nest, more than 90% of nestle is torn, nest is fairly even, the nest is a crater, with walls higher than the mouse body for more than 50% of its circumference.

Tail Suspension Test

It is a mouse behavioural test useful in the screening of potential antidepressant drugs, and assessing of other manipulations that are expected to affect depression related behaviours [8].

12cm paper tap was cut, with the middle made unstick by pasting it on any surface while the ends at least 2cm was sticky. A stopper was put through the tail of the animals and one sticky used to wrap round the tail of the animals, while the other end was attached to the tail suspension apparatus. The animal was allowed for 5 minutes (300sec). Video camera was used to capture the four animals under investigation at a time. The parameters that were determined include: Mobility, Immobility, Defecation, and Urination [8].

Swim Tests

Swim test is a rodent behavioural test used for evaluation of antidepressant drugs or experimental manipulations that are aimed at rendering or preventing depressive-like state. A transparent container containing

water and a thermometer was used to determine temperature and ensure that the water was at 30°c. The mice were then introduced into the water individually and allowed for 5 minutes.
- Latency time (the time it takes the animals to move their hind paws to the time it first stops).
- Mobility (the total time the mice used their hind paws to move before the expiration of the swim).
- Immobility (the total duration of inactivity).
- Defecation.

The animals will be made dry using paper towel before being returned to their home cages. **[9]**

Open field (animal test)

Open field test (OFT) is an experiment used to assay general locomotor activity levels and anxiety in rodents in scientific research and willingness to explore in rodents.

Animals such as rats and mice display a natural aversion to brightly lit areas. They also have a drive to explore a perceived threatening stimulus. The result of these two conflicting drives is anxiety. Decreased anxiety leads to increased exploratory behaviour. Increased anxiety will result in less locomotor motion and preference for the edges of the field **[10].**

The open field is an arena with walls to prevent escape. Commonly, the field is marked with a grid and square crossings. The centre of the field is marked with a different colour to differentiate from the other squares. In the modern open field apparatus, infrared beams or video cameras with associated software can be used to automate the assessment process **[11].**

Behavioural patterns measured in the open field test include:
- Line crossing – Frequency with which the rodent crossed a grid line with all four paws
- Centre square entries – Frequency with which rodent entered centre square with all four paws
- Centre square duration – Duration of time spent in central square
- Rearing – Frequency with which the rodent stood on their hind legs in the field. This behaviour shows increased exploratory behaviour.
- Stretch attend postures – Frequency with which rodent demonstrated forward elongation of the head and shoulders followed by retraction to the original position. High frequency indicates high levels of anxiety.
- Defecation and urination – The frequency of defecation and urination is controversial. Some scientists argue that increase in defecation shows increased anxiety. Other scientists disagree and state that defecation and urination show signs of emotionality but cannot be assumed to be anxiety **[11].**

The relation between the OFT and other tests of exploratory activity (elevated plus maze and emergence) have been analysed in two mouse strains. Changes in these measures are often used to assess the sedative or stimulant effects of pharmacological agents. Newer attempts have been made to analyse the OFT by quantifying the animal's moment-by-moment developmental dynamics. A recent study was able to show that mouse exploratory behaviour consists of sequences of repeated motion: iterative processes that increase in extent and complexity, whose presumed function is a systematic active management of input acquired during the exploration of a novel environment. **[12]**

The elevated plus

The elevated maze has been described as a simple method for assessing anxiety responses of rodents by File and co-workers 1985. A task, using a Y-shaped apparatus that included an elevated open alley, which produced a strong approach–avoidance conflict, and an enclosed alley, which did not, was first described by Montgomery. This task was modified into an elevated maze with four arms (two open and two enclosed) that are arranged to form a plus shape and was described.. These authors described the assessment of anxiety behaviour of rodents by using the ratio of time spent on the open arms to the time spent on the closed arms. Unlike other behavioural assays used to assess anxiety responses that rely upon the presentation of noxious stimuli (i.e., electric shock, food/water deprivation, loud noises, exposure to predator odour, etc.) that typically produce a conditioned response, the elevated plus maze relies upon rodents' proclivity toward dark, enclosed spaces (approach) and an unconditioned fear of heights/open spaces (avoidance) **[13].**

There is great diversity in possible applications of the elevated plus maze. To name a few, pre-screening of newly developed pharmacological agents for treatment of anxiety-related disorders can be carried out. The anxiolytic and antigenic effects of pharmacological agents, drugs of abuse and hormones can be investigated. The effects of reproductive senescence/aging and/or pre-, peri- or postnatal exposure to various stressors can be assessed. Furthermore, beyond its utility as a model to detect anxiolytic effects of benzodiazepine-related compounds, the elevated plus maze can be used as a behavioural assay to study the brain sites (e.g., limbic regions, hippocampus, amygdala, dorsal raphe nucleus, etc.5,6) and mechanisms (e.g., GABA, glutamate, serotonin, hypothalamic–pituitary–adrenal axis neuromodulators, etc.1,3,7–12) underlying anxiety

behaviour. Indeed, the elevated plus maze has been used as a model of state, unconditioned anxiety for over two decades, and there are now over 2,000 papers related to this topic.

Behavioural responses in the elevated plus maze are easily accessed and quantified by an observer. Briefly, rodents are placed in the intersection of the four arms of the elevated plus maze and their behaviour is typically recorded for 5 min. This was based upon the early studies by Montgomery that revealed that rats demonstrated the most robust avoidance responses in the first 5 min after placement in the elevated open alleys. The behaviours that are typically recorded when rodents are in the elevated plus maze are the time spent and entries made on the open and closed arms. Behaviour in this task (i.e. activity in the open arms) reflects a conflict between the rodent's preference for protected areas (e.g. closed arms) and their innate motivation to explore novel environments. Anti-anxiety behaviour (increased open arm time and/or open arm entries) can be determined simultaneously with a measure of spontaneous motor activity (total and/or closed arm entries), albeit the arm entries made in the maze may not be an optimal measure of motor activity. Other ethological measures that can be observed in rodents in the maze are the number of rears, head dips, faecal boli, and freezing or stretched-attend postures.

Face validity of the elevated plus maze

The elevated plus maze has face validity, which is the ability of a task to appear to measure what it is supposed to measure. For instance, in the elevated plus maze, the anxiety or fear of open spaces/heights of rodents seems to be measured. In this task, the open arms are avoided and rodents spend the majority of the time in this task in the closed arms of the maze. Other anxiety-related behaviours of rodents, such as freezing/immobility and defecation, are increased on the open arms of the maze compared to the closed arms **[14].**

Construct validity of the elevated plus maze

The elevated plus maze has construct validity. Construct validity refers to whether an observable dependent variable, such as time spent in the open arms of the elevated plus maze, used measures an unobservable construct, such as anxiety. This is demonstrated by anxiogenic drugs reducing time spent on the open arms and anxiolytic drugs increasing the time spent on the open arms of the elevated plus maze **[14].**

Predictive validity of the elevated plus maze

The elevated plus maze has predictive validity, which is defined as the extent to which the dependent measure predicts behaviour on a related measure. We have shown that increased open arm activity occurs in rodents that also demonstrate increased central square entries in a brightly lit open field (Frye *et al.,* 2000). Furthermore, plasma corticosterone is increased with open arm exposure and is positively correlated with risk assessment behaviour (i.e., stretch-attend postures) in the elevated plus maze **[15,16].**

Effects of novel environment pre-exposure

Pre-exposure to a different novel environment, such as an open field or hole-board task, has been used in studies assessing anxiety behaviour of rodents. In some studies, exposure to a novel environment immediately before testing in the elevated plus maze increases motor activity in the elevated plus maze and a greater likelihood of entering the open arms of the maze **[14].** Although indices of anxiety behaviour in the elevated plus maze do not correlate with the amount of exploration in a hole-board Weiss 1998, using the hole-board task immediately before elevated plus maze testing can provide additional indices of activity and exploration (i.e., rearing and head dipping) **[14]** which are independent of plus maze exposure, to rule out changes in open arm exploration being due to changes in general activity and/or exploratory motivation. For instance, this method has been successfully used to demonstrate anxioselectivity of the long-lasting impact of experimental epilepsy on rodents and the impact of predator stress exposure (both experimental situations that can alter activity) on plus maze anxiety **[17]**

Ethical Approval: *Ethical approval was obtained from University of Calabar Medical School Ethical Committee for this research*

Statistical Analysis
The results obtained from the study were analysed with the aid of the computer software Micro Excel 2010 and \spss 17.1 for windows. Differences among groups were analysed using the one0-way analysis of variance (ANOVA), and the Lead square deviation (LSD) was used as a post hoc test to assay for differences between pairs of groups. Persons Chi square test was used to calculate the percentage alternation. Values for the result are expressed as mean $\pm$ SEM. The level of statistical significant difference was set at $p< 0.05$.

III.   Results

Anti-depressive and anti-psychotic test result

Comparison of the body weight change for control, stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg).

Body weight change for control, stressed, imipramine treated and chlorpromazine treated groups (x $\pm$ SEM) were 5.83$\pm$0.16, - 6.16$\pm$0.31, -0.18$\pm$0.09, and - 1.23$\pm$0.35, respectively. The results of body weight change in the stressed group, stressed treated with imipramine and stressed treated with chlorpromazine were significantly lower (p<0.001) when compared to the control group. However, the imipramine treated group showed significant decrease in weight loss (p<0.05) when compared to both the stressed group and the chlorpromazine treated group. Also, the chlorpromazine treated group showed significant decrease in weight loss (p< 0.0 5) when compared to the stressed untreated group.
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Comparison of latency to immobility in the forced swim test for control, stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg).

In figure 2, latency to immobility in the forced swim test for the control, stressed, imipramine treated and chlorpromazine (x $\pm$ SEM) were 111.8$\pm$8.4, 200.3$\pm$7.3, 133.8$\pm$6.4, and 239.3$\pm$6.6, respectively. The results for latency to immobility in the forced swim test showed that the stressed group, the imipramine treated and the chlorpromazine treated were significantly lower (p<0.001) when compared to the control. Also, the imipramine treated group showed significant increase (p<0.05) when compared to the stressed group and the chlorpromazine treated group.
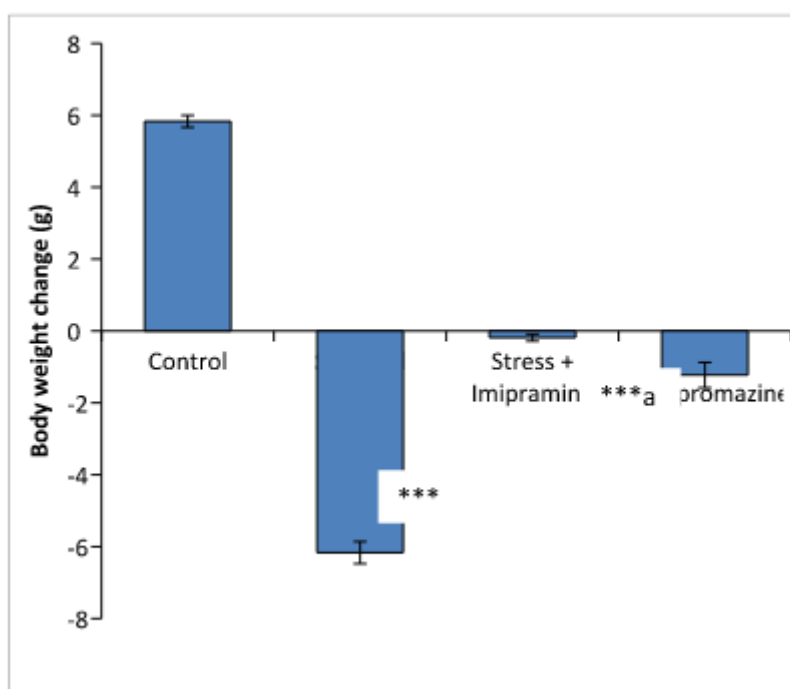
FIG. 1: Comparison of body weight change in stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). ***-significant at p< 0.001 vs control; a – significant at p<0.05 vs stressed group; b – significant at p< 0.05 vs imipramine
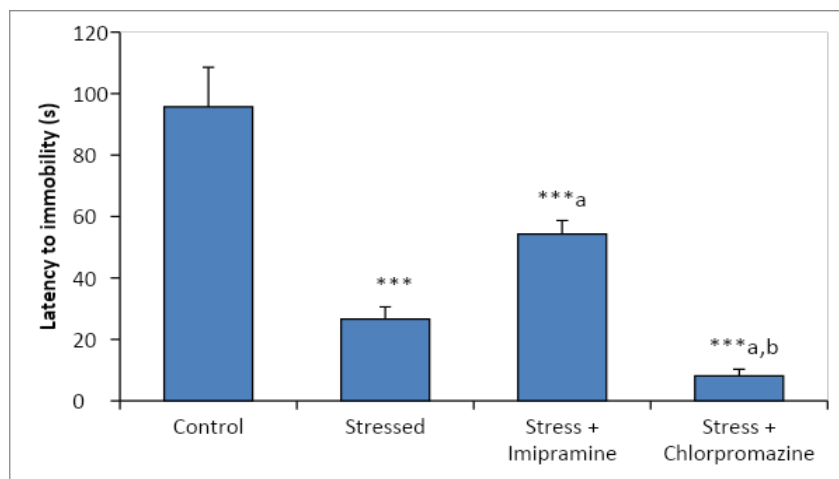
FIG. 2: Comparison of latency to immobility in the forced swim test for stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). ***-significant at p< 0.001 vs control; a – significant at p<0.05 vs stressed group; b – significant at p< 0.05 vs imipramine

Comparison of duration of immobility in the forced swim test for control, stressed CD 1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg).

Figure 3 shows comparison of duration of immobility for control, stressed, imipramine treated and chlorpromazine treated groups (X $\pm$ SEM) as of 111.8±8.4, 200.3±7.3, 133.8±6.4, and 239.3±6.6, respectively. The results for duration of immobility in the forced swim test showed that, the stressed group was significantly higher (p<0.001) when compared to the control group. The imipramine treated and chlorpromazine treated groups showed significant increase (P<0.05) when compared to the control group respectively. The stress group showed significant increase at (P<0.05) when compared to the imipramine treated group. Whereas, the chlorpromazine treated group showed significant increase at (P<0.05) when compared to the imipramine treated group.

Comparison of number of faecal boli in the forced swim test for control, stressed CD1 Mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg).

Figure 4, shows the number of faecal boli in the forced swim test for control, stressed, imipramine treated and chlorpromazine treated groups (x $\pm$ SEM) as 1.45±0.2, 2.35±0.16, 1.25±0.15, 2.±0.1 respectively. The result of faecal boli in the forced swim test showed significant increase (P<0.05) in stressed group when compared to the control group. Similarly, the stressed group showed significant increase (P<0.01) when compared to the control but the imipramine treated group showed significant decrease (P<0.05) when compared to the stress group. Equally, the chlorpromazine treated showed significant increase (P<0.05) when compared to the imipramine treated group.
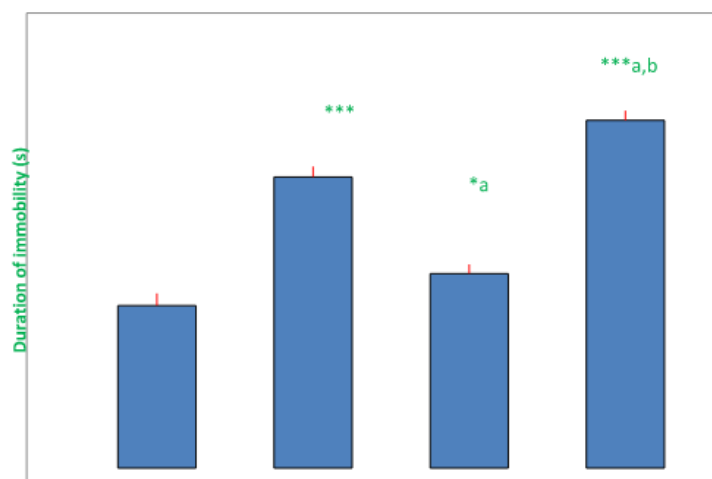

FIG. 3: Comparison of duration of immobility in the forced swim test for stressed CD1 mice treated with imipramine (…mg/kg) and chlorpromazine (…mg/kg). ***-significant at p< 0.001 vs control; * - significant at p< 0.05 vs control; a – significant at p<0.05 vs stressed group; b – significant at p< 0.05 vs imipramine
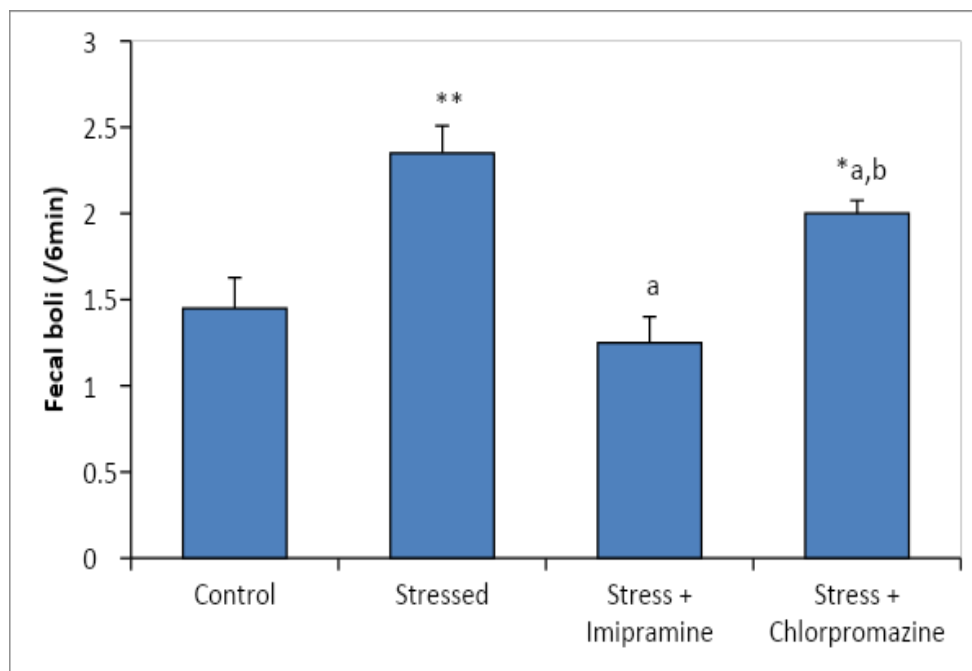
FIG. 4: Comparison of number of fecal boli in the forced swim test for stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). *-significant at p< 0.05 vs control; **-significant at p< 0.01 vs control; a – significant at p<0.05 vs stressed group; b – significant at p< 0.05 vs imipramine

Comparison of latency to immobility in the tail suspension test for control, stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg).

Figure 5, shows the latency to immobility in the tail suspension test for control, stressed imipramine treated and chlorpromazine treated groups (x $\pm$ SEM) as 39.2$\pm$2.16, 21.3$\pm$2.85, 35.8$\pm$1.4, 6.5$\pm$0.4, respectively. The results of latency to immobility in the tail suspension test showed significant increase (P<0.01) in the stressed group when compared to the control group. The chlorpromazine treated group also showed significant decrease (P<0.001) latency to immobility when compared to control group. But the imipramine treated group showed significant increase (P<0.05) when compared to stressed group. And, the chlorpromazine treated showed significant decrease (P<0.05) when compared to the imipramine group.

Comparison of duration of immobility in the tail suspension test for control stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg).

Figure 6, shows the duration of immobility in the tail suspension test for control, stressed, imipramine treated and chlorpromazine treated groups (x $\pm$ SEM) as 164.2$\pm$10.6, 222.8$\pm$9.7, 165.6$\pm$3.45, 222.8$\pm$9.76, respectively. The results for duration to immobility in the tail suspension test showed that the stressed group and the chlorpromazine treated group showed significant increase (P<0.05) when compared to control group and imipramine treated group respectively. Whereas the imipramine treated group showed no significant difference (P<0.05) when compared to the control group.
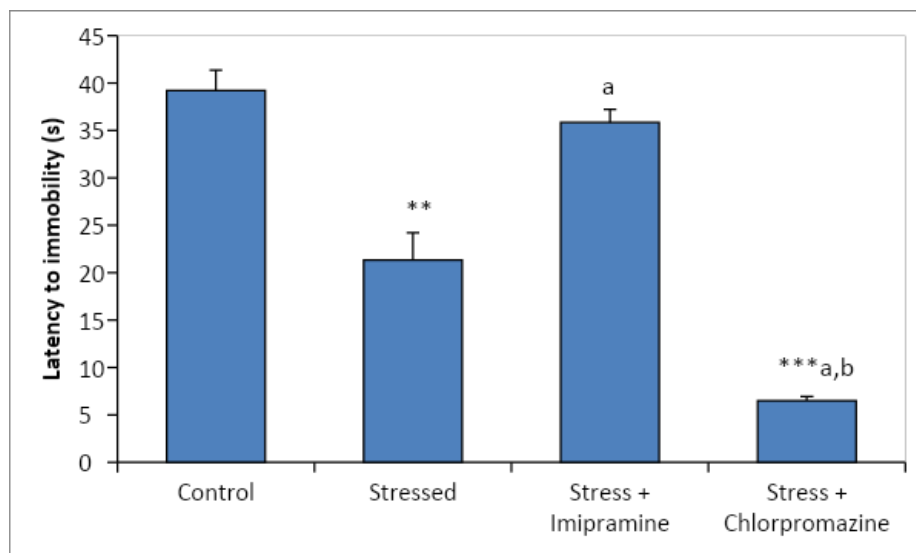
FIG. 5: Comparison of latency to immobility in the tail suspension test for stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). **-significant at p< 0.01 vs control; ***-significant at p< 0.001 vs control; a – significant at p<0.05 vs stressed group; b – significant at p< 0.05 vs imipramine
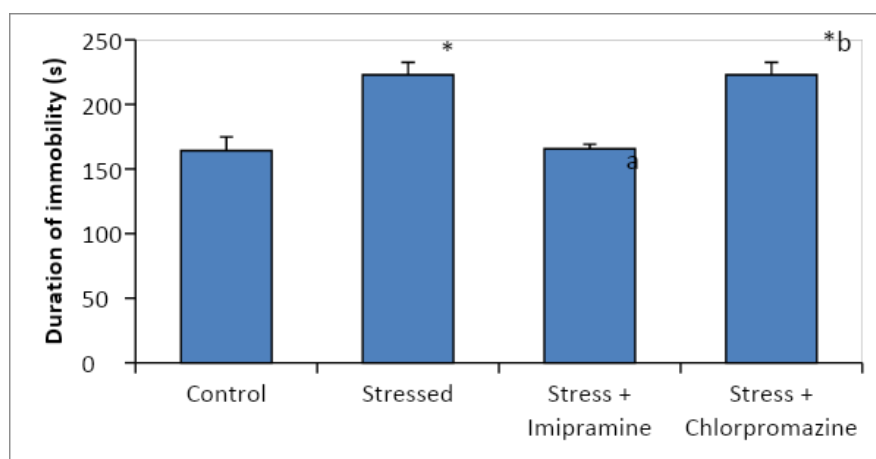

FIG. 6: Comparison of duration of immobility in the tail suspension test for stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). *-significant at p< 0.05 vs control; a – significant at p<0.05 vs stressed group; b – significant at p< 0.05 vs imipramine

Comparison of the number of faecal boli in the tail suspension test for control, stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg).

Figure 7, shows number of faecal boli in the tail suspension test for control, stressed, imipramine treated and chlorpromazine treated groups (x $\pm$ SEM) as 2.$\pm$0.25, 1$\pm$0, 1$\pm$0, 2$\pm$0.4 respectively. The result for number of faecal boli in the tail suspension test showed that the stressed group was significantly lower (P<0.01) when compared to the control. Similarly, the imipramine treated group showed significant decrease (P<0.01) when compared to control group. However, the chlorpromazine treated group showed significant increase (P<0.05) when compared to both the stressed and the imipramine treated groups.

Comparison of nesting score in stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg).

Figure 8, shows the nesting score in control, stressed, imipramine treated and chlorpromazine treated groups (X$\pm$ SEM) were 4.67$\pm$0.21, 1.83$\pm$0.31, 3.67$\pm$0.21 and 1.5$\pm$0.22 respectively. The nesting score results showed that the stressed group was significantly lower (P<0.001) when compared to the control group. But, the imipramine treated group was significantly higher (P<0.05) when compared to the stressed group. Also, the chlorpromazine treated group showed significantly decrease (P<0.05) when compared to the control group but significantly lower (P<0.05) than the imipramine treated group.
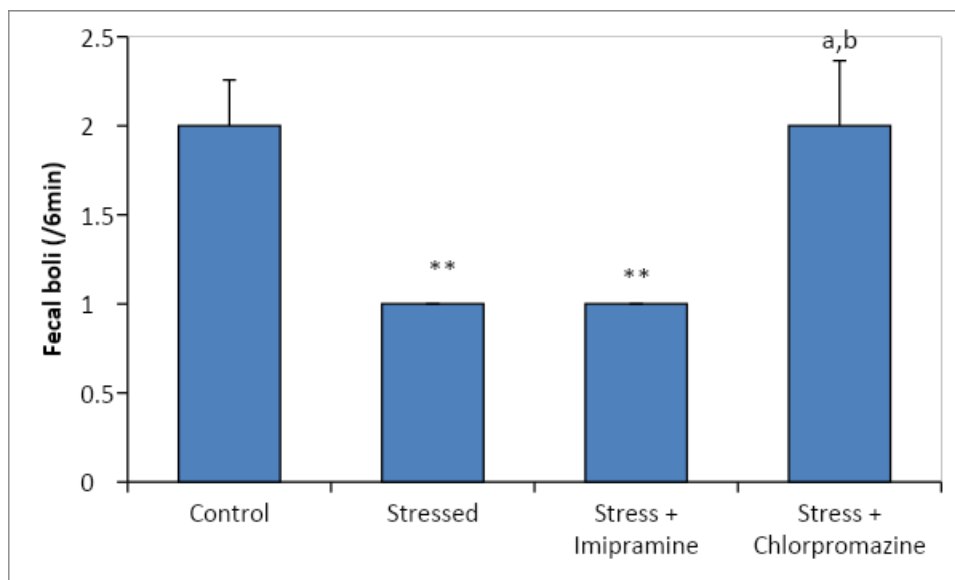
FIG. 7: Comparison of number of faecal boli in the tail suspension test for stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). **-significant at p< 0.01 vs control; a – significant at p<0.05 vs stressed group; b – significant at p< 0.05 vs imipramine
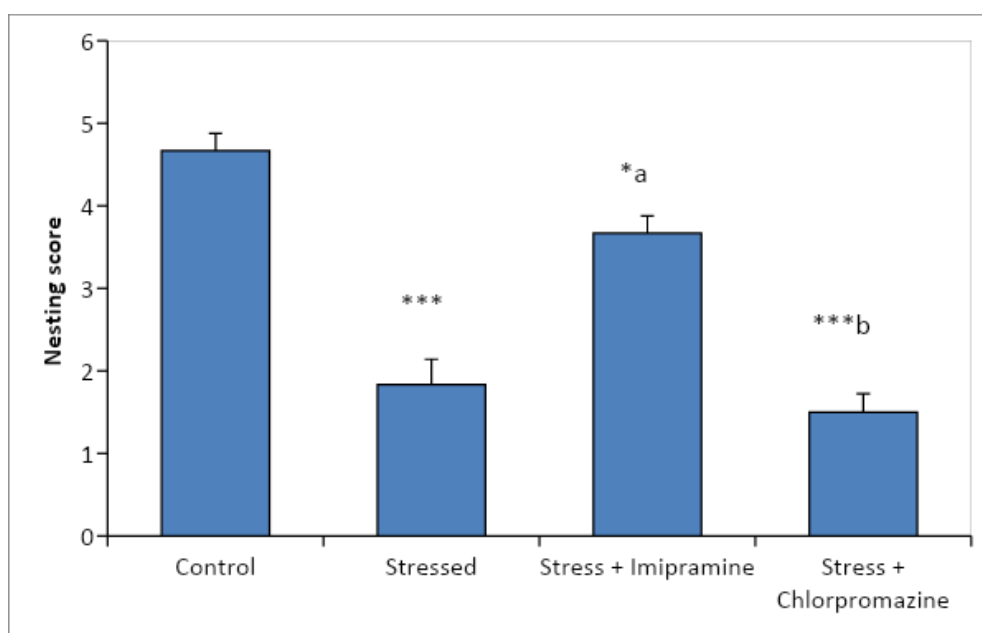

FIG. 8: Comparison of nesting score in stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). *-significant at p< 0.05 vs control; ***-significant at p< 0.001 vs control; a – significant at p<0.05 vs stressed group; b – significant at p< 0.05 vs imipramine

Comparison of frequency of grooming in the open field test for control stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg)

Figure 9, shows the frequency of grooming in the open filed test for control, stressed, imipramine treated and chlorpromazine treated groups (X± SEM) were 4.±0.2, 5.3±0.32, 2±0.4 and 5.3±0.3 respectively. The results for frequency for grooming in the open field test showed that both the stressed group and chlorpromazine treated group were significantly higher (P<0.01) when compared to the control. The imipramine treated group showed significant decrease (P<0.001) when compared to the control group. Similarly, the imipramine treated group showed significant decrease (P<0.05) when compared to the stressed group. Equally, the chlorpromazine treated group showed significant increase (P<0.05) when compared to the imipramine treated group.
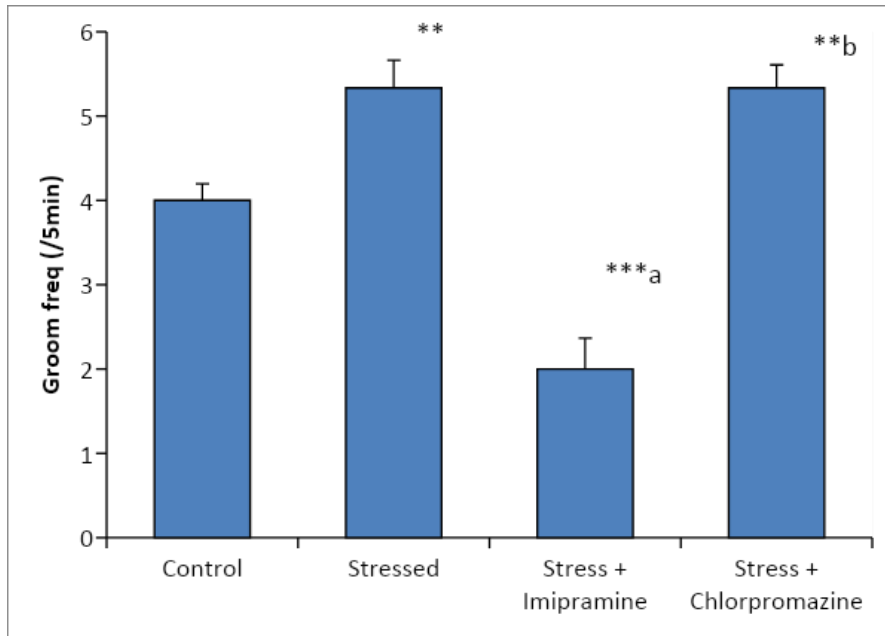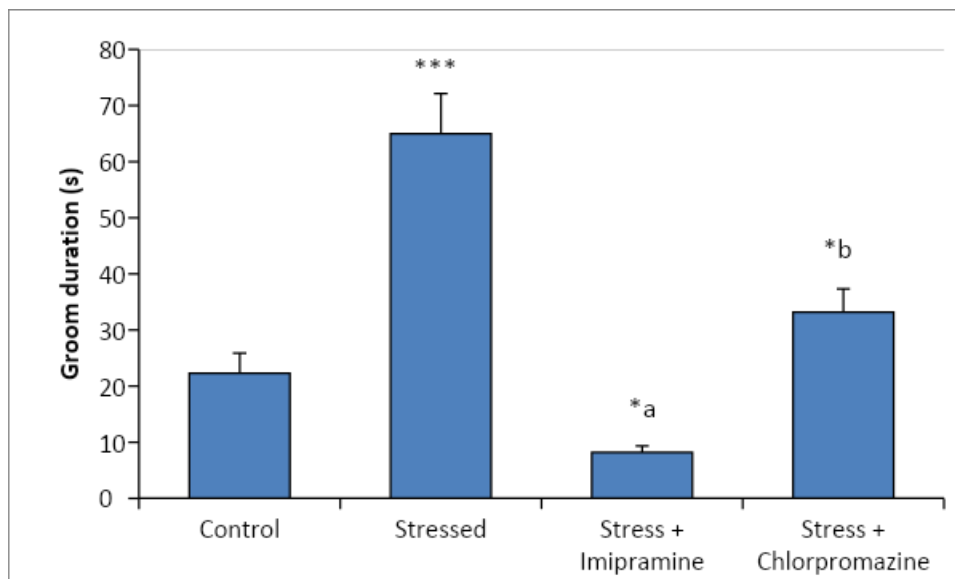
Comparison of duration of grooming in the open field test for stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg)

Figure 10, shows the duration of grooming in the open field test for control, stressed, imipramine treated and chlorpromazine (X± SEM) were 22.33±3.51, 65±7.15, 8.16±1.19 and 33.16±4.18 respectively. The results for duration of grooming in the open field test showed that the imipramine treated group was significantly lower (P<0.05) than both the control and the stressed group. The stressed group showed significant increase (P<0.001) when compared to the control. The chlorpromazine treated group showed significant increase (P<0.05) when compared to control and the imipramine treated group respectively.

FIG. 9: Comparison of frequency of grooming in the open field test for stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). **-significant at p< 0.01 vs control; ***-significant at p< 0.001 vs control; a – significant at p<0.05 vs stressed group; b – significant at p< 0.05 vs imipramine

FIG. 10: Comparison of duration of grooming in the open field test for stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). *-significant at p< 0.05 vs control; ***-significant at p< 0.001 vs control; a – significant at p<0.05 vs stressed group; b – significant at p< 0.05 vs imipramine

Comparison of frequency of freezing in the open field test for control, stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg)

Figure 11 shows, the frequency of freezing in the opening field test for control, stressed, imipramine treated and chlorpromazine treated ($X \pm$ SEM) were 1.45 ± 0.16, 3.5 ± 0.34, 2.6 ± 0.16 and 3 ± 0.16 respectively. The results for the frequency of freezing in the open field test showed that the stressed group was significantly higher ($P < 0.01$) when compared to the control group. Similarly, the imipramine treated group showed significant increase ($P < 0.001$) when compared to the control group. Also, the chlorpromazine treated group showed significant increase ($P < 0.05$) when compared to the control. The imipramine treated group showed significant decrease ($P < 0.05$) when compared to the stressed group. In addition, the chlorpromazine treated group showed significant increase ($P < 0.05$) when compared to the imipramine treated group.

Comparison of duration of freezing in the open field test for control, stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg)

Figure 12 shows the duration of freezing in the open field test for control, stressed, imipramine treated, chlorpromazine treated at ($X \pm$ SEM) were 2 ± 0.9, 25 ± 3.7, 8 ± 1.7 and 6 ± 2.2 respectively. The results for duration of freezing in the open field test showed significant increase ($P < 0.001$) when compared to the control. However, both the imipramine and the chlorpromazine treated groups showed significant decrease ($P < 0.05$) when compared to the stressed group.
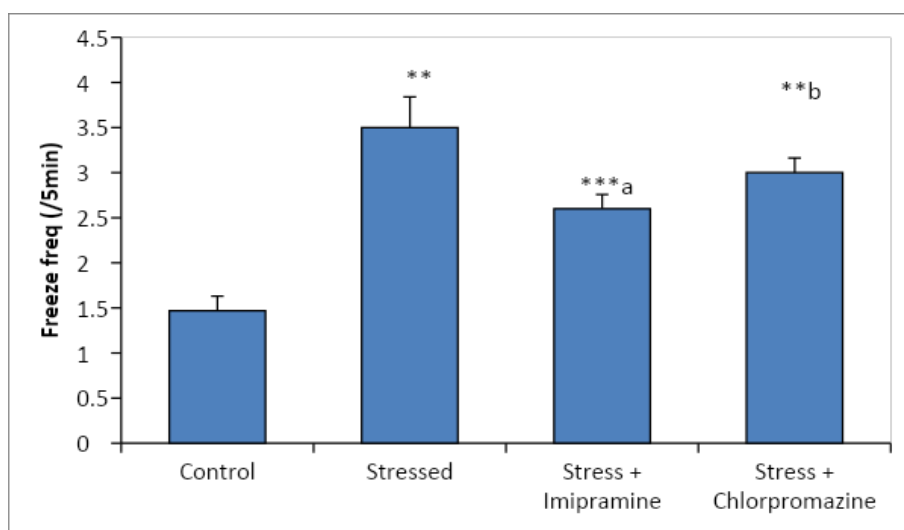

FIG. 11: Comparison of frequency of freezing in the open field test for stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). **-significant at p< 0.01 vs control; ***-significant at p< 0.001 vs control; a – significant at p<0.05 vs stressed group; b – significant at p< 0.05 vs imipramine
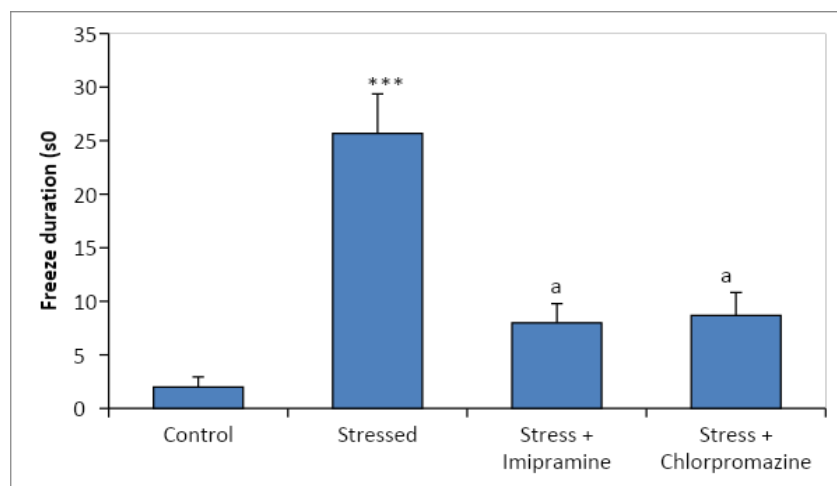

FIG. 12: Comparison of duration of freezing in the open field test for stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). ***-significant at p< 0.001 vs control; a – significant at p<0.05 vs stressed group

Comparison of frequency of open arms entry in the elevated plus maze test for control, stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg)

Figure 13, shows the frequency of open arm entry in elevated plus maze test for control, stressed, imipramine treated chlorpromazine treated (X± SEM) were 2.6±0.3, 1.5±0.4, 7.8±0.5 and 2±0.5 respectively. The results for the frequency of open arms entry in the elevated plus maze test showed that the stressed group was significantly lower (P<0.05) when compared to the control. But, the imipramine treated group showed significant increase (P<0.001) and (P<0.05) when compared to the control group and stressed group respectively. Whereas the chlorpromazine treated group showed significant decrease (P<0.05) when compared to the imipramine treated group.

Comparison of duration in open arms of the elevated plus maze test for control, stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg)

Figure 14 shows, the duration in open arm entry of the elevated plus maze test for control, stressed, imipramine treated and chlorpromazine treated groups (X± SEM) as 45±8.2, 19.6±3.4, 180±16.4 and 207±16.2 respectively. The results for duration in the open arm entry of the elevated plus maze test showed that the stressed group was significantly lower (P<0.05) when compared to the control group. The imipramine and chlorpromazine treated groups showed significant increase (P<0.001) when compared to the control group. Finally, the imipramine treated and the chlorpromazine treated group showed significant increase (P<0.05) when compared to the stressed group.
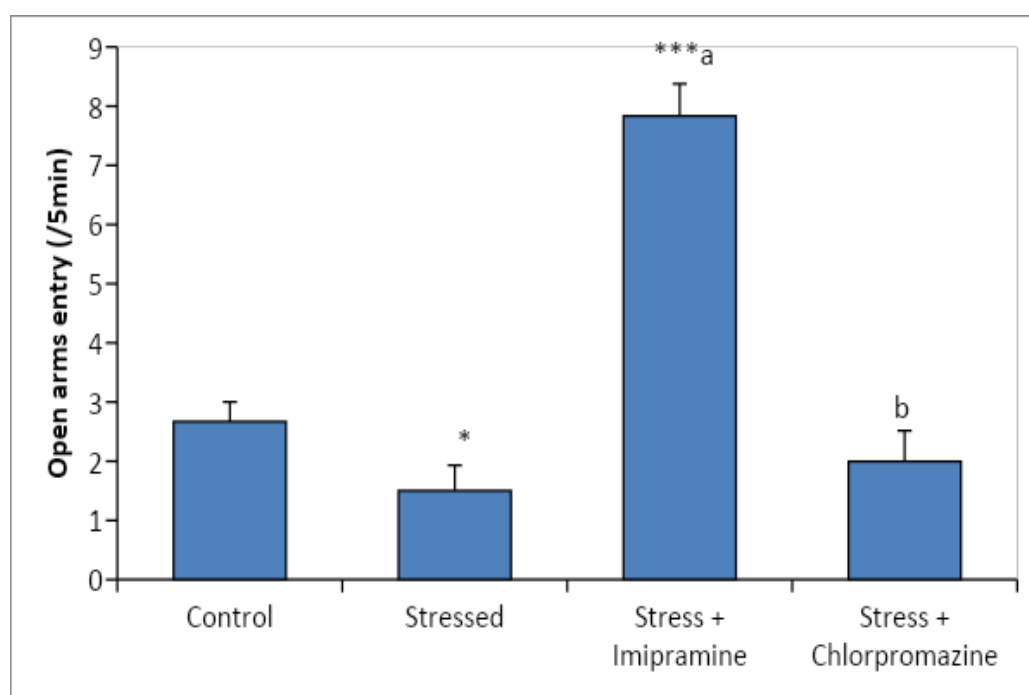

FIG. 13: Comparison of frequency of open arms entry in the elevated plus maze test for stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). *-significant at p< 0.05 vs control; ***- significant at p< 0.001 vs control; a – significant at p<0.05 vs stressed group; b – significant at p< 0.05 vs imipramine.
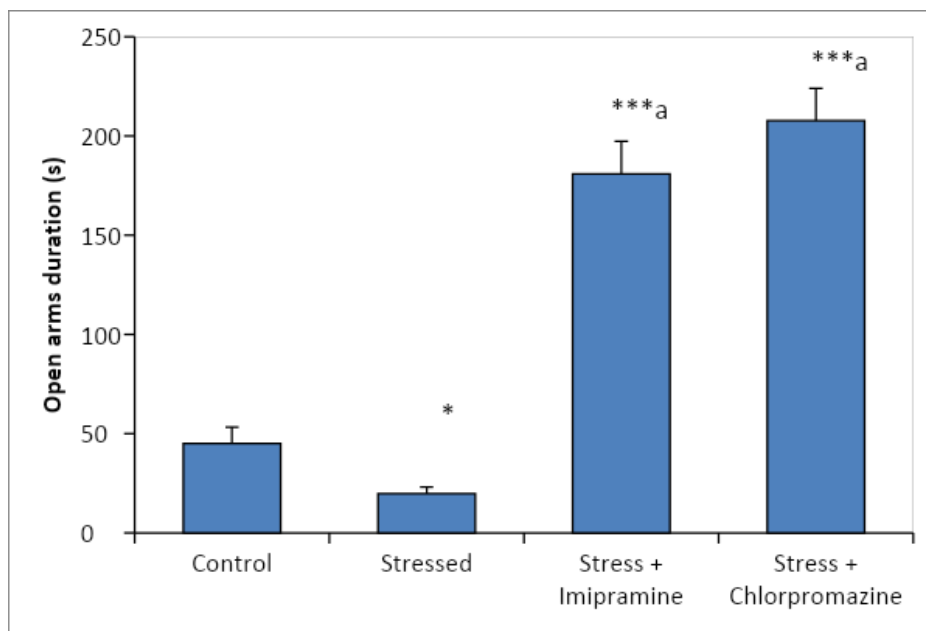
FIG. 14: Comparison of duration in open arms of the elevated plus maze test for stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). *-significant at p< 0.05 vs control; ***-significant at p< 0.001 vs control; a – significant at p<0.05 vs stressed group

Comparison of frequency of grooming in the elevated plus maze test for control, stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg)

Figure 15, shows the frequency of grooming in the elevated plus maze test for control, stressed, imipramize treated and chlorpromazine treated groups ($X\pm$ SEM) as 2.16 ± 0.3, 12 ± 1.3, 0.8 ± 0.31 and 25 ± 4.7 respectively. The results for the frequency of grooming in the elevated plus maze showed that the stressed group was significantly higher at $(P<0.01)$ when compared to the control group. While the chlorpromazine showed significant increase at $(P<0.001)$ when compared to the control group. But, the imipramine treated group showed significant decrease $(P<0.05)$ when compared to the stressed group. However, the chlorpromazine showed significant increase $(P<0.05)$ when compared to the imipramine treated group.

Comparison of duration of grooming in the elevated plus maze test for control stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg)

Figure 16, shows the duration of grooming in the elevated plus maze test for control, stressed, imipramine and chlorpromazine treated groups ($X\pm$ SEM) as 8.83 ± 12, 14.6 ± 1.2, 4.3 ± 1.02 and 15.3 ± 1.7 respectively. The results for duration of grooming in the elevated plus maze showed that the stressed group was significantly higher $(P<0.05)$ when compared to the control group. While the imipramine treated group showed significant decrease $(P<0.01)$ when compared to the control group. Also, the chlorpromazine treated group showed significant increase at $(P<0.05)$ when compared to the control. However, the imipramine treated group showed significant decrease $(P<0.05)$ when compared to the stressed group and the chlorpromazine treated group respectively.
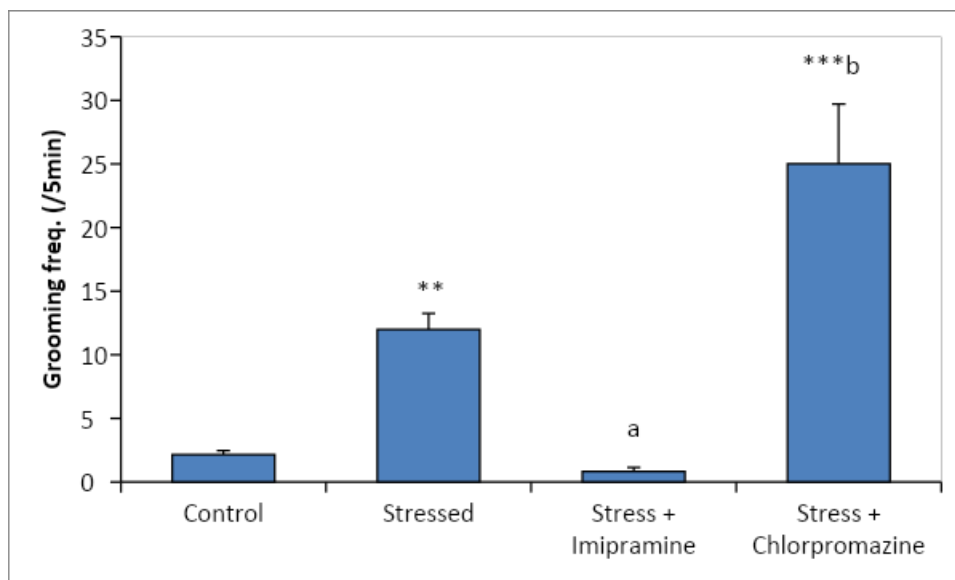
FIG. 15: Comparison of frequency of grooming in the elevated plus maze test for stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). **-significant at p< 0.01 vs control; ***-significant at p< 0.001 vs control; a – significant at p<0.05 vs stressed group; b – significant at p< 0.05 vs imipramine
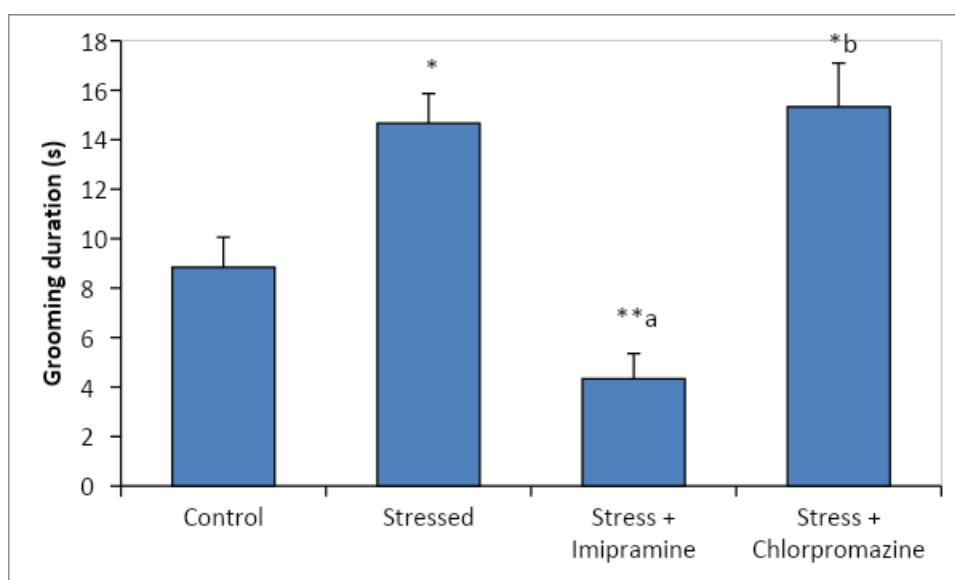

FIG. 16: Comparison of duration of grooming in the elevated plus maze test for stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). *-significant at p< 0.05 vs control; **-significant at p< 0.01 vs control; a – significant at p<0.05 vs stressed group; b – significant at p< 0.05 vs imipramine

Comparison of number of fecal boli in the elevated plus maze test for control, stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg)

Figure 17 , shows number of fecal boli in the elevated plus maze test for control, stressed, imipramine treated and chlorpromazine treated groups (X± SEM) as 0.64 ± 0.05, 19.8 ± 3.1, 8.3 ± 1.6 and 38 ± 6.2 respectively. The results of the fecal boli in the elevated plus maze showed that the stressed group was significantly higher (P<0.05) when compared to the control group. While the imipramine treated group equally showed significant increase (P<0.001) when compared to the control group. However, the imipramine treated group showed significant decrease at (P<0.05) when compared to the stressed group. Equally, the chlorpromazine treated group showed significant increase (P<0.05) when compared to the imipramine treated group.

Comparison of frequency of urination in the elevated plus maze test for control, stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg)

Figure 18 shows, frequency of urination in the elevated plus maze test for control, stressed, imipramine treated and chlorpromazine treated groups (X± SEM) were 1.16±0.16, 3.16±0.3, 1.5±0.3 and 3.5±0.4 respectively. The results for frequency of urination in the elevated plus maze showed that the imipramine treated group was significantly higher (P<0.05) when compared to the control group. The stressed and the chlorpromazine treated groups showed significant increase at (P<0.001) when compared to the control group. The imipramine treated showed significant decrease (P<0.05) when compared to the stressed group. However, the chlorpromazine treated group showed significant increase (p<0.05) when compared to the imipramine treated group.
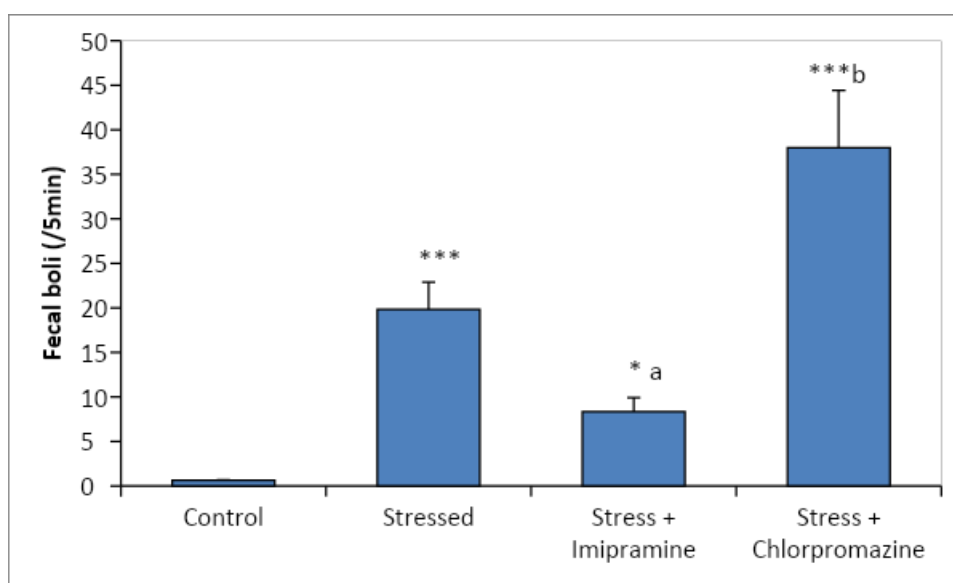

*FIG. 17: Comparison of number of faecal boli in the elevated plus maze test for stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). *-significant at p< 0.05 vs control; ***-significant at p< 0.001 vs control; a – significant at p<0.05 vs stressed group; b – significant at p< 0.05 vs imipramine*
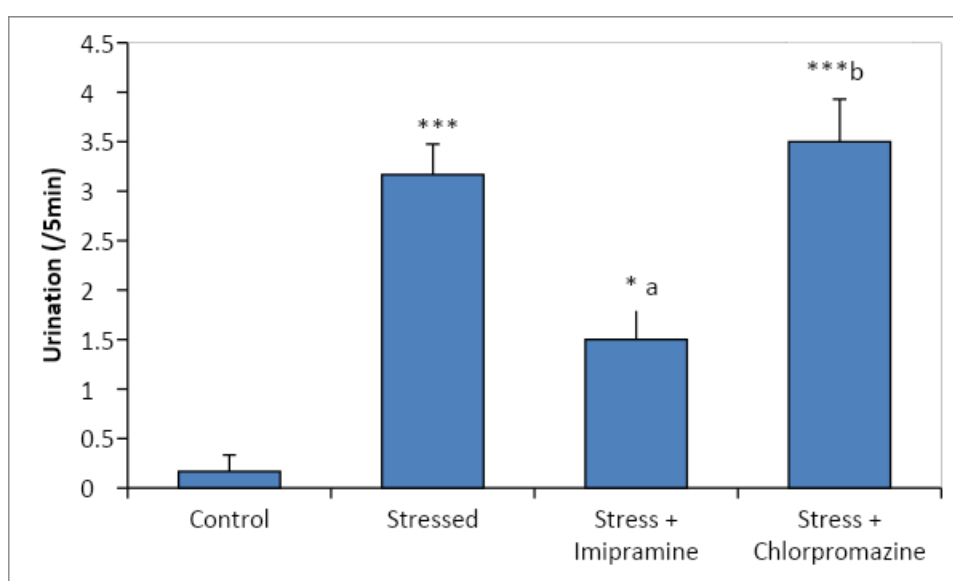

*FIG. 18: Comparison of frequency of urination in the elevated plus maze test for stressed CD1 mice treated with imipramine (25mg/kg) and chlorpromazine (10mg/kg). *-significant at p< 0.05 vs control; ***-significant at p< 0.001 vs control; a – significant at p<0.05 vs stressed group; b – significant at p< 0.05 vs imipramine*

IV. Discussion

Change in body weight

The results show that there was a drastic body weight loss in the stressed group compared to the control. These have substantial effect on both morbidity and mortality rate. Loss of weight in the stressed and stressed/treated groups shows that stress may have caused some physiological changes during the deprivation period that resulted in the weight loss. Also, results show that the two drugs could reverse stress-related weight loss, with imipramine being more effective in doing so when compared with chlorpromazine.

Social behaviour / depressive-like symptoms

A poor performance in the nesting task may indicate impairment in social relationship in the mice and perhaps a pointer to the presence of autistic behaviour. High level of nesting behaviour as indicated in the nesting score (grade) indicates increase social behaviour [18]

The result in the present experiment showed that the control group had significantly higher nesting score than other counterparts. The stressed group when compared to the control showed significant decrease, also the imipramine and chlorpromazine treated groups also showed significant decrease. However, the imipramine treated group showed significant increase when compared to the stressed and chlorpromazine-treated group. The chlorpromazine treated group showed no significant difference when compared to the stressed-untreated group.

This may indicate that imipramine improved social activities in stressed animal models, while chlorpromazine does not give any significant improvement. This may not be due to the stress effect but due to sedative effect of chlorpromazine which act by binding on the post synaptic neurons to block the dopamine and having effect on the basal ganglia to relax the muscles.

Considering depressive-like symptoms, the result showed that the stressed group was significantly higher when compared to the control group. Also, the imipramine treated group showed significant decrease in depressive-like symptom when compared to the control, but showed significant increase when compared to stressed group. Meanwhile, the chlorpromazine treated group showed significant increase when compared to control and all the treated groups, indicating that imipramine relieves from depression but chlorpromazine does not.

Anxiety

Freezing frequency and duration was significantly lower in the control group when compared to the stressed groups. Stressed/imipramine treated group also showed significantly lower frequency/duration of grooming when compared to chlorpromazine-treated and stressed-untreated groups. This shows that anxiety and fear was reduced in the control and imipramine treated groups when compared to the stressed and chlorpromazine-treated groups.

Generally, chronic stress leads to elevated hormones such as cortisol, the 'stress hormone'. But it reduces serotonin and other neurotransmitters in the brain, including dopamine. This has been linked to depression, anxiety/fear, and indeed low social behaviour [19].

V. Conclusion

The experiment shows that imipramine will reduce anxiety/fear, reduce depression-like symptoms and improve social behaviour in stressed mice better than chlorpromazine will do. This enhancing action of imipramine over chlorpromazine may have been mediated through the serotonergic pathway. Imipramine also has effect cortisol and consequently improving body weight after stress episode.

VI. Recommendation

Similar investigation may be carried out in human under strict medical watch, to determine if same observations will be made.

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