

Original Research Article

Comparative effect of fluoxetine and imipramine on anxiety-like phenotypes in social defeat stress model in mice

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Abstract

Purpose: To evaluate the comparative effect of fluoxetine and imipramine on socially defeated mice.

Methods: Twenty-eight mice were divided into four groups of seven each as follows: 1 - Control (distilled water), 2 - untreated social defeat stress (SDS), 3 - SDS + fluoxetine (10 mg/kg), and 4 - SDS + imipramine (10 mg/kg). Anxiety was created via social defeat stress for 30 days; thereafter, animals were treated orally with fluoxetine (10 mg/kg) and imipramine (10 mg/kg) for 14 consecutive days. Twenty-four hours after the last administration, different behavioural tests, including elevated plus maze test and light and dark box test, were conducted, after which the animals were sacrificed by decapitation and brain regions (prefrontal cortex, dentate gyrus, amygdala and striatum) were obtained for analysis (superoxide dismutase, brain-derived neurotrophic factor, interleukin-10 and interleukin-1 α).

Results: Fluoxetine displayed a stronger anxiolytic effect in the elevated plus maze test and time spent in the dark box compared to imipramine ($p < 0.05$). Both treatment groups seem to have an equivalent effect on superoxide dismutase (SOD) and malondialdehyde (MDA) levels in both the prefrontal cortex and hippocampus. Neuronal density counts in the hippocampus (dentate gyrus), striatum, and amygdala exhibited a mirror effect for both treated groups. Imipramine boosted levels of interleukin-10 considerably ($p < 0.05$) compared to fluoxetine, but both drugs showed equivalent effect on brain-derived neurotrophic factor, nuclear factor kappa-B, and interleukin-1 α .

Conclusion: Although both drugs utilized for the study are antidepressants, outcomes from this study have demonstrated that both drugs have anti-anxiety-like effects, with fluoxetine displaying a better effect. However, both treatment groups (imipramine and fluoxetine) had equivalent effects in most of the studies undertaken.

Keywords: Anxiety, Social defeat stress, Fluoxetine, Imipramine

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INTRODUCTION

Anxiety disorders affect people of all ages and are among the most common mental health

conditions in the world [1]. Adolescents are more susceptible to developing anxiety disorders, according to a report from a prior study. The incidence of anxiety disorders in adolescents is

high; estimates of the lifetime prevalence rate range from 15 to 30 % worldwide [2]. Academic performance is adversely affected by anxiety because it impairs focus, memory, and problem-solving skills [2].

Adolescents with anxiety disorders are less able to establish and maintain peer relationships. Isolation and heightened susceptibility to other mental health issues, such as depression, may ensue from this [3]. Long-term anxiety has been linked to hypothalamic-pituitary-adrenal (HPA) axis dysregulation, which can lead to ironic stress and increased risk for cardiovascular illnesses in later life [4]. In early adulthood, adolescents with untreated anxiety were twice as likely to suffer from serious depressive illness [5]. Important neurodevelopmental alterations in the hippocampus and amygdala, which are involved in emotional regulation, are correlated with anxiety during adolescence.

Increased stress responses and difficulty managing fear have been linked to abnormal activity in these brain areas [6]. According to a comprehensive analysis by Han *et al* [7], adolescents who experienced bullying had twice the risk of developing anxiety as their peers who did not experience bullying. This study aims to evaluate the effectiveness of imipramine and fluoxetine as treatments for socially defeated mice, taking into account the detrimental effects of bullying-induced anxiety in teenagers.

EXPERIMENTAL

Animal

For the study, twenty-eight (28) male albino mice weighing 14 - 18 g were acquired from Delta State University's Central Animal House in Abraka. The animals were kept at room temperature (20 to 26 °C), allowed unlimited access to food and water, and allowed to acclimatize for two weeks. Delta State University's Faculty of Basic Medical Science Research and Ethics Committee in Abraka, Nigeria, provided ethical approval (no. RBC/FBMC/DELSU/24/550). The Guidelines for the Care and Use of Laboratory Animals were followed in every procedure [8].

Animals were randomly assigned to four groups (groups 1 - 4), comprising seven animals per group. Group 1: (control) received vehicle only, i.e., distilled water; Group 2: Induced with anxiety via social defeat stress (untreated SDS); Group 3: Induced with anxiety via social defeat stress and treated with fluoxetine (10 mg/kg) and Group 4: Induced with anxiety via social defeat stress

and treated with imipramine (10 mg/kg). Drug administration was done once daily orally using an oro-gastric syringe for 14 consecutive days.

Drug preparation

Fluoxetine and imipramine used for the study were bought from a pharmacy shop in Eku, Delta State. Fluoxetine and imipramine were manufactured by Flutex and Tofranil, respectively. Doses of fluoxetine (10 mg/kg) and imipramine (10 mg/kg) were prepared in stock solution. The doses administered were selected from previous literature [9].

Induction of anxiety

Prior to inducing anxiety in mice in groups 2, 3, and 4, ten additional adult male mice (residents) weighing between 22 and 27 grammes were acquired from the Central Animal House at Delta State University in Abraka. Anxiety was induced after 30 days of acclimatization for these 10 mice, who were housed in isolation in 10 different cages (singly). To elicit hostility in the mice kept individually, the ten mice had to be isolated. After the 30 days of isolation, a male intruder mouse from groups 2, 3, and 4 was placed in the resident cage. Both animals were allowed ten minutes of physical interaction, followed by a ten-minute' threat thereafter, intruder mice were returned to their home cages. The process was repeated seven days in a row. Animals in groups two, three, and four exhibited signs of anxiety, including recumbent posture, frequent distress calls, frozen behaviour, and submission. After the anxiety induction period, animals in groups 3 and 4 received oral treatments of fluoxetine (10 mg/kg) and imipramine (10 mg/kg) once daily for 14 days in a row. Following the last dose of imipramine and fluoxetine, behavioural tests such as the elevated plus maze were conducted in the different groups 24 hours later. Thereafter Light and dark phase test was conducted on animals in the different groups (groups 1 to 4).

At the end of the behavioral tests, animals were decapitated and different brain regions (prefrontal cortex, dentate gyrus, amygdala and striatum) were collected for biochemical and histological analysis.

Behavioural assays

Assessment of anxiety levels using the raised plus maze test

Elevated plus maze (EPM), an acknowledged test for anxiolytic and anxiogenic effects of a drug, was employed to determine prospective

anxiety levels alteration effects of fluoxetine and imipramine on the experimental mice. Briefly, the elevated plus maze equipment consists of two open arms and two closed arms and an elevation to a height of 25 cm above the floor level. Each mouse from all the treatment groups was positioned at the tip or end of an open arm, with the head directed towards the middle of the apparatus. The experimental animal was permitted to explore the maze for a duration of 5 min, during which the number of arm entries (either open or closed) and the time spent in open and closed arms were observed and recorded.

Assessment of anxiety levels using light/dark box

The light/dark transition test was utilized to further justify the putative anxiolytic activity of fluoxetine. Shortly, the device consisted of a rectangular box (45 × 27 × 27 cm), partitioned into two compartments joined by a 7.5 × 7.5 cm opening in the wall between the two chambers. The mouse from each group was deposited in the lit compartment of the box, and the number of entries and time spent in the light and dark compartments of the box will be taken and recorded within the length of a 5-minute session.

Biochemical analysis

Determination of superoxide dismutase (SOD) activity

The level of SOD activity was assessed. Briefly, 0.1 mL of brain supernatant was added to 2.6 mL of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer, and the reaction proceeded by the addition of 0.3 mL of freshly made 0.3 mM adrenaline to the mixture, which was swiftly mixed by inversion. The reference cuvette contained 2.6 mL of buffer, 0.3 mL of adrenaline, and 0.1 mL of distilled water. Then, an increase in absorbance at 480 nm was observed at 60-second intervals for 3 min. Superoxide dismutase (SOD) activity was expressed as units of adrenaline used per minute per mg protein.

Estimation of brain levels of malondialdehyde (MDA)

An aliquot of 0.4 mL of the supernatant was mixed with 1.6 mL of Tris-KCl buffer to which 0.5 mL of 30 % trichloroacetic acid was added. Then, 0.5 mL of 0.75 % thiobarbituric acid was added and placed in a water bath for 45 min at 80 °C, which was later chilled in ice and centrifuged at 3000 rpm for 15 min. The clear supernatant was

collected, and absorbance was measured against a reference blank of distilled water at 532 nm using a spectrophotometer. The MDA concentration was determined using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$, and values were represented as μmoles of MDA per gram of tissue.

Preparation of brain tissues for immunohistochemistry and histology

After the behavioural tests, mice ($n = 3$) in the respective groups were decapitated. The prefrontal cortex, hippocampus (dentate gyrus), striatum and amygdala were harvested and fixed with 10 % phosphate-buffered formaldehyde, used for perfusion. The brain regions were subjected to the routine method for paraffin wax embedment to obtain paraffin wax-embedded tissue blocks. Transverse sections (5 - 6 μm thick) of the hippocampus, striatum and amygdala were obtained with the aid of microtome (Leica, Germany) and the sections were fixed on glass slides.

Estimation of brain-derived neurotrophic factor levels, nuclear factor kappa-B levels, Interleukin 10 and Interleukin 1 α

Brain-derived neurotrophic factor (BDNF), Nuclear factor kappa-B assays, Interleukin 10, interleukin 1 α were estimated using quantitative sandwich enzyme immunoassay techniques based on manufacturers' protocols.

Statistical analysis

The data were displayed as mean $\pm$ standard error of mean (SEM). The outcomes were compared across groups using a one-way ANOVA, followed by Turkey's post hoc test. When $p < 0.05$, differences are regarded as significant. GraphPad Prism (Version 8.0) was the program used for statistical analysis.

RESULT

The effect of fluoxetine and imipramine on anxiety-like behaviour in SDS mice

The effect of fluoxetine and imipramine on anxiety-like behaviours, as measured by the time spent in the closed arm as seen in the elevated plus maze (EPM) and the time spent in the dark box as seen in the light/dark transition box, as shown in Figure 1 and Figure 2, respectively.

One-way ANOVA showed that there were significant differences between treatment groups. Untreated SDS mice spent more time on the

closed arm, as shown in Figure 1. There was a statistically significant difference ($p < 0.05$) in the time spent in the closed arm for untreated SDS mice compared to the control group, SDS + fluoxetine group and SDS + imipramine group. Also, animals in SDS + fluoxetine group spent less time in the closed arm compared to SDS + Imipramine group and the difference was statistically significant ($p < 0.05$; Figure 1).

The effect of fluoxetine and imipramine on light and dark phases is presented in Figure 2. From Figure 2, it was shown that untreated SDS mice spent a significant ($p < 0.05$) amount of time in the dark phase as compared to control, SDS + fluoxetine (10 mg/kg) and SDS + imipramine (10 mg/kg). Fluoxetine and imipramine were able to decrease the time that animals spend on the dark phase, with fluoxetine having a better reduction in time spent on the dark phase as compared to imipramine. There was no significant difference in the time spent in the dark

phase for SDS + fluoxetine group when compared to SDS + imipramine group (Figure 2).

The effect of fluoxetine and imipramine on antioxidant activity in SDS mice

The effect of fluoxetine and imipramine on antioxidant activity in the prefrontal cortex and hippocampus, as shown in Figure 3 and Figure 4, respectively. One-way ANOVA showed that there were significant differences between treatment groups in superoxide dismutase (SOD) and malondialdehyde in the prefrontal cortex and hippocampus when compared to untreated social defeat stress group. Post hoc analysis using Turkey's post hoc test revealed that untreated SDS group showed a significant ($p < 0.05$) decrease in SOD levels in the prefrontal cortex and hippocampus when compared to the control group.

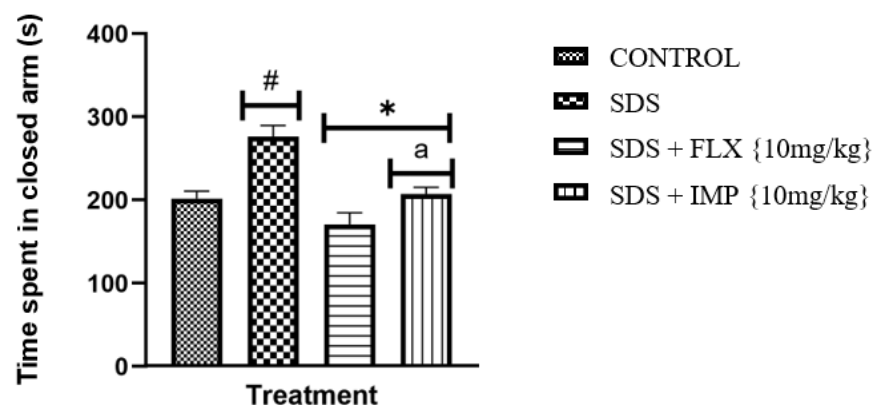


Figure 1: The effect of fluoxetine and imipramine on anxiety-like behaviour in SDS mice using the elevated plus maze (EPM). Values are mean $\pm$ SEM for 5 animals per group. [#] $P < 0.05$ vs control group, ^{*} $p < 0.05$ vs pathologic group. ^a $P < 0.05$ vs SDS + fluoxetine group

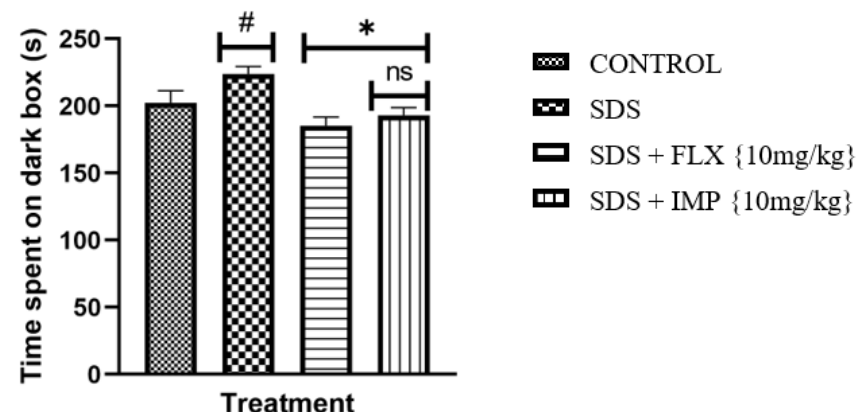


Figure 2: A graph showing the effect of fluoxetine and imipramine on the time spent in the dark box in SDS mice utilizing light and dark box model. Data are mean $\pm$ SEM for 5 animals per group. $P < 0.05$ vs pathologic group. [#] $P < 0.05$ for SDS (untreated) vs control. ^{*} $P < 0.05$ treated group vs SDS (untreated) group, while ^{ns} $P > 0.05$ denotes no statistically significant difference between SDS + fluoxetine group and SDS + imipramine group

Mean SOD value of SDS untreated was two-thirds of that of control group, SDS + fluoxetine and SDS + imipramine in the prefrontal cortex. Fluoxetine (10 mg/kg) and imipramine (10 mg/kg) had a significantly ($p < 0.05$) higher level of SOD in the prefrontal cortex and hippocampus. As shown in Figure 3, the difference in mean SOD levels between SDS + fluoxetine and SDS + imipramine was not statistically significant, as mean superoxide dismutase (SOD) values for SDS + fluoxetine and SDS + imipramine had almost the same mean values in the prefrontal cortex. In the hippocampus, there was a slight increase in SOD levels in SDS + fluoxetine group as compared to SDS + imipramine group, but the difference was not statistically significant (Figure 3).

One-way ANOVA showed that there were significant differences between treatment groups

in malondialdehyde (MDA) levels in the prefrontal cortex and hippocampus. Post hoc analysis using Turkey's post hoc test revealed that untreated SDS group showed a significant ($p < 0.05$) increase in MDA levels when compared to the control group. In both regions of the brain, there was a statistically significant increase in MDA level in SDS untreated group compared to SDS + fluoxetine and SDS + imipramine. In the prefrontal cortex, SDS + fluoxetine had reduced malondialdehyde (MDA) compared to SDS untreated mice. Animals in SDS + imipramine group had a better reduction in MDA compared to SDS + fluoxetine group. Although not statistically different ($p > 0.05$). While in the hippocampus, SDS + fluoxetine group had a better reduction in MDA level compared to SDS + imipramine group (Figure 4).

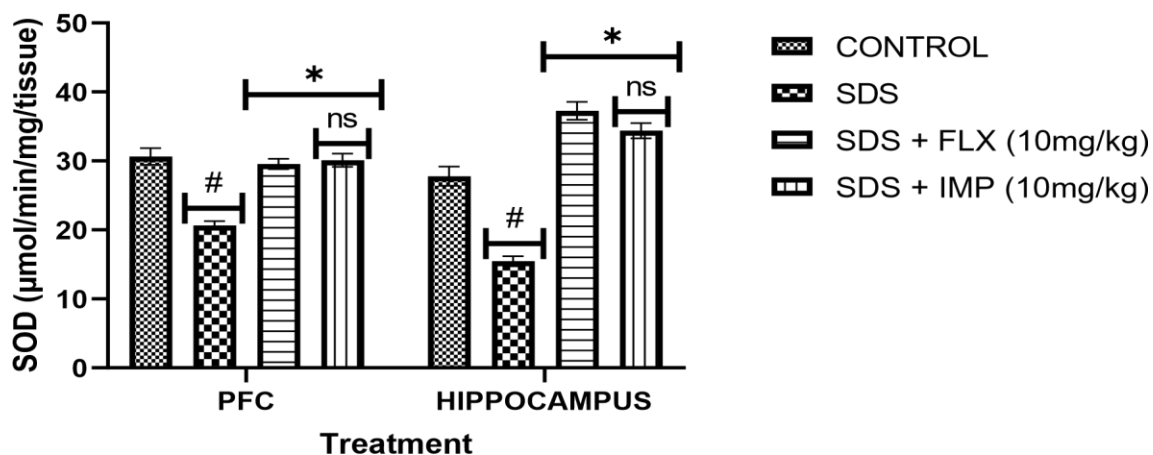


Figure 3: The effect of fluoxetine and imipramine on superoxide dismutase in SDS mice. # $P < 0.05$ for mean SOD of control (group 1) vs untreated SDS group. * $P < 0.05$ for mean SOD of SDS + fluoxetine and SOD + imipramine vs SDS untreated. $^{ns}P > 0.05$ between SDS + fluoxetine group and SDS + imipramine group

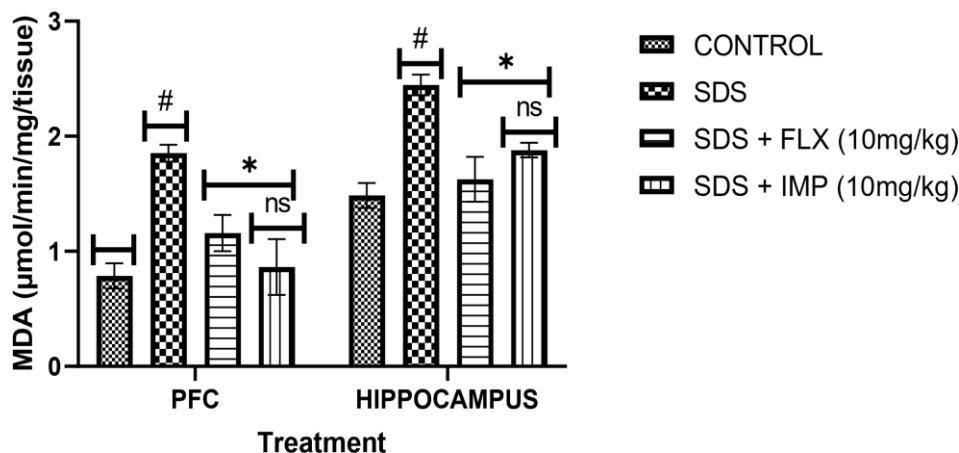


Figure 4: The effect of fluoxetine and imipramine on malondialdehyde in SDS mice. Data are presented as mean $\pm$ SEM for 5 animals per group. $P < 0.05$ vs pathologic group. # $P < 0.05$ between SDS (untreated) vs control. * $P < 0.05$ between treated group vs SDS (untreated) group, while $^{ns}P > 0.05$ between SDS + fluoxetine group and SDS + imipramine group

Effect of fluoxetine and imipramine on the mouse brain and essential proteins

The photomicrographs showed the effect of fluoxetine and imipramine on the dentate gyrus region of the hippocampus of SDS mice. Slide A revealed that there is no observable lesion in the dentate gyrus region of the hippocampus. Slide B revealed that there is atrophy of neurons in the dentate gyrus region of the hippocampus. Slide C revealed that there is no observable lesion in the dentate gyrus of the hippocampus. Slide D revealed that there is no observable lesion in the dentate gyrus of the hippocampus (Figure 5 and Figure 6). White arrows represent viable neurons, while black arrows represent atrophy of the neurons.

Photomicrograph of the effect of fluoxetine and imipramine on the striatum in SDS

The photomicrographs showed the effect of fluoxetine and imipramine on the striatum of SDS mice. Slide A revealed that there is no observable lesion in the stratum. Slide B revealed that there is acute neuronal necrosis in the striatum. Slide C revealed that there is pyknosis of neurons in the striatum. Slide D revealed that there is atrophy of neurons in the striatum (Figure 7 and Figure 8). White arrows represent viable neurons, yellow arrows represent neuronal necrosis, blue arrows represent pyknosis of neurons, and black arrows represent atrophy of neurons.

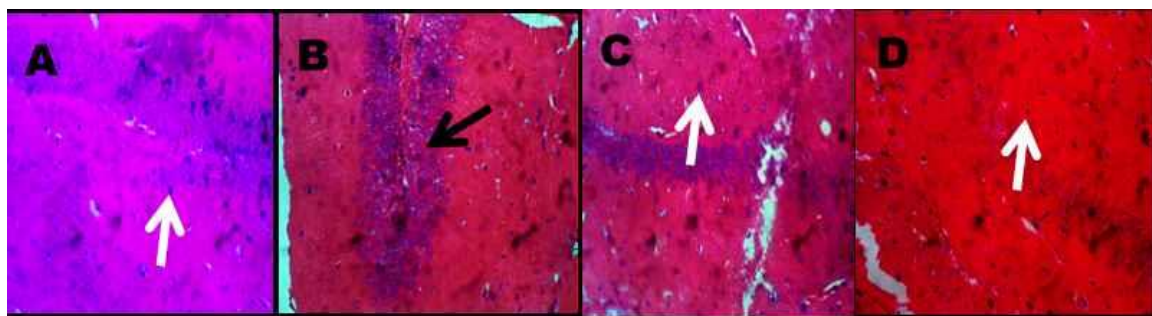


Figure 5: Representative photomicrographs (H & E-stained sections) of the effect of fluoxetine and imipramine on the dentate gyrus of SDS mice. Magnification = HE x400. A = Control, B = SDS, C = SDS + fluoxetine (10 mg/kg) and D = SDS + imipramine (10 mg/kg)

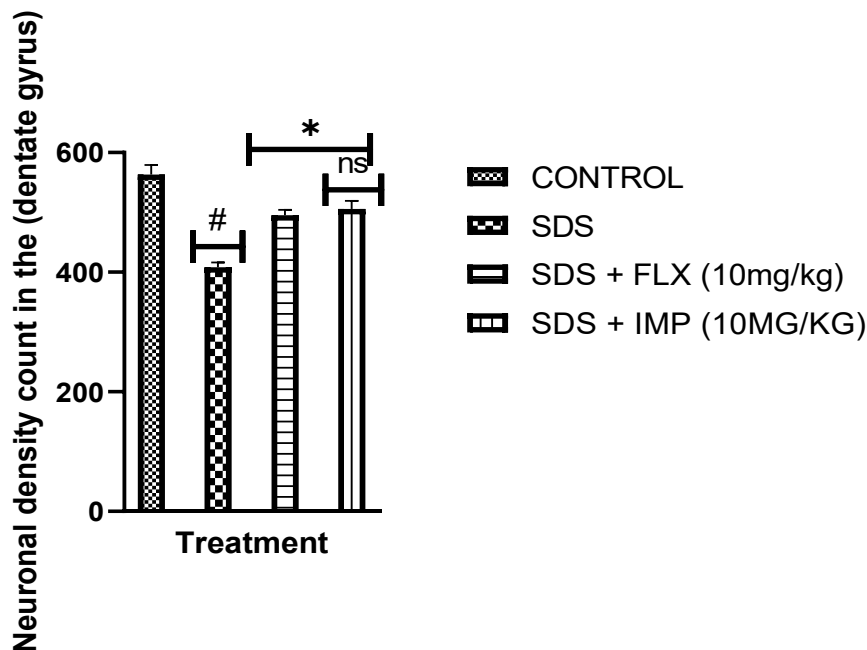


Figure 6: A graph showing the effect of fluoxetine and imipramine on hippocampal neuronal density count (dentate gyrus) in SDS mice. [#] $P < 0.05$ between SDS (untreated) and control. $*P < 0.05$ between treated group and SDS (untreated) group. ^{ns} $P > 0.05$ between SDS + fluoxetine group compared to SDS + imipramine group

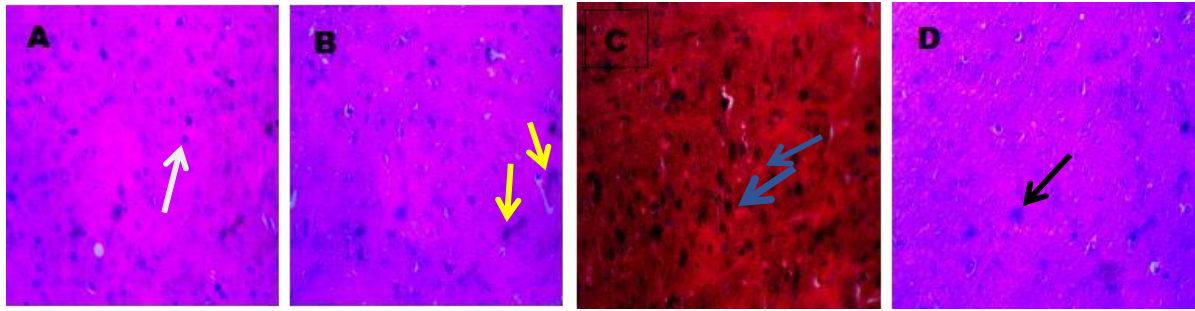


Figure 7: Representative photomicrographs (H & E-stained sections) of the effect of fluoxetine and imipramine on the striatum of SDS mice. Magnification = HE x400. A = Control, B = SDS, C = SDS + fluoxetine (10 mg/kg) and D = SDS + imipramine (10 mg/kg)

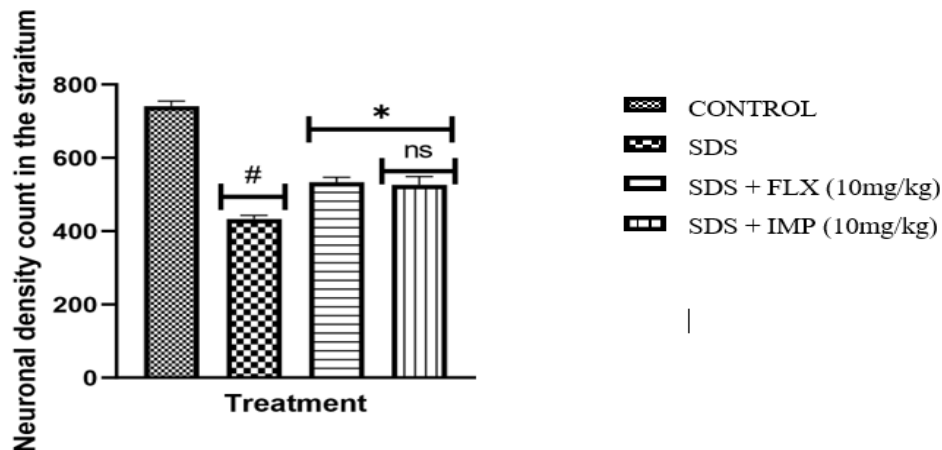


Figure 8: A graph showing the effect of fluoxetine and imipramine on striatum neuronal density count in SDS mice. Data are presented as mean $\pm$ SEM for 3 animals per group. $P < 0.05$ vs pathologic group. $*P < 0.05$ treated group vs SDS (untreated) group. $#P < 0.05$ SDS (untreated) vs control. $nsP > 0.05$ between SDS + fluoxetine group vs SDS + imipramine group

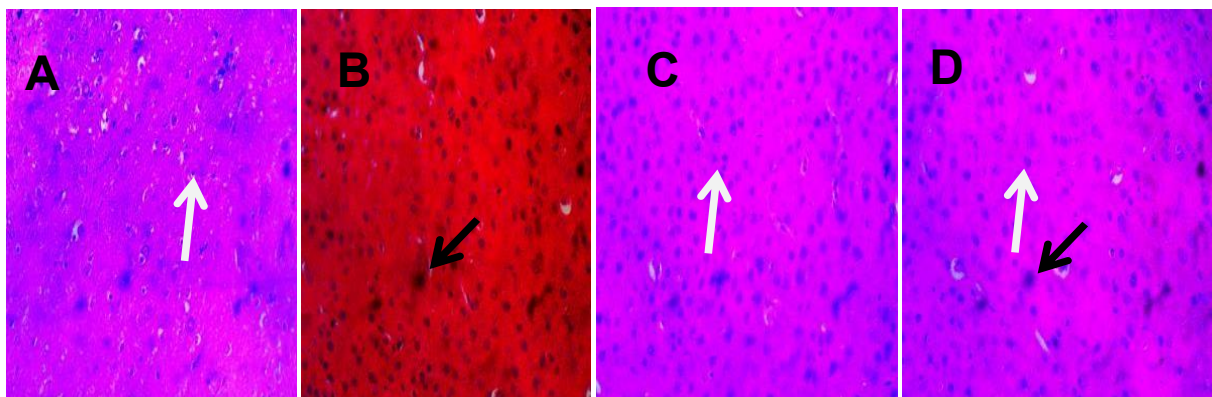


Figure 9: Representative photomicrographs (H & E-stained sections) of the effect of fluoxetine and imipramine on the amygdala of SDS mice. Magnification = HE x 400. A = Control, B = SDS, C = SDS + fluoxetine (10 mg/kg) and D = SDS + imipramine (10 mg/kg)

Photomicrograph of the effect of fluoxetine and imipramine on the amygdala in SDS mice

The photomicrographs showed the effect of fluoxetine and imipramine on the amygdala of SDS mice. In Figure 9, histological slides from the

control group, SDS + fluoxetine group and SDS + imipramine showed an observable lesion in the amygdala, apart from SDS group, which showed atrophy of neurons (Figure 9 and Figure 10). White arrows represent viable neurons, while black arrows denote atrophy of neurons.

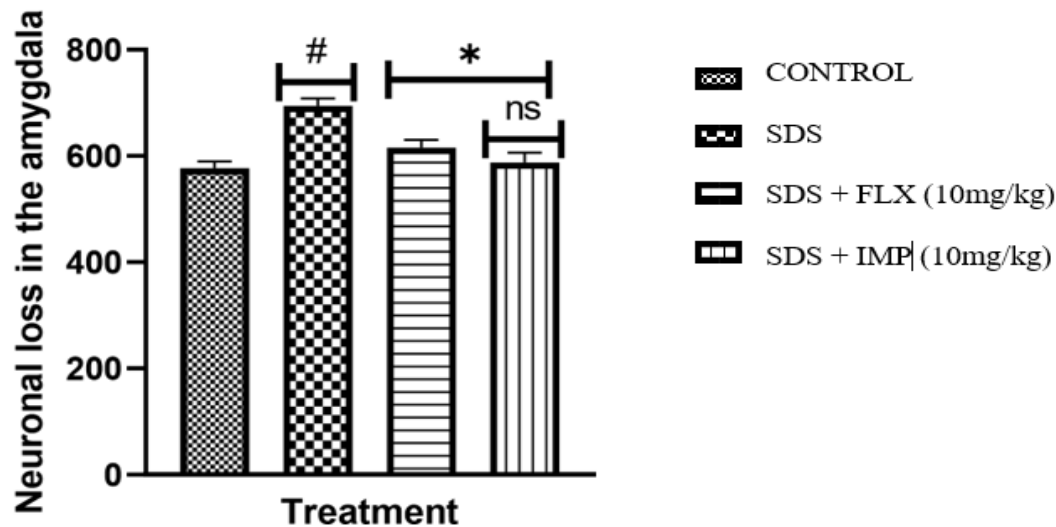


Figure 10: A graph showing the effect of fluoxetine and imipramine on the amygdala in SDS mice. Data are presented as mean $\pm$ SEM for 3 animals per group. $P < 0.05$ vs pathologic group. $*P < 0.05$ for SDS + fluoxetine group vs SDS (untreated) group. $\#P < 0.05$ SDS (untreated) vs control. $nsP > 0.05$ between SDS + fluoxetine group vs SDS + imipramine group

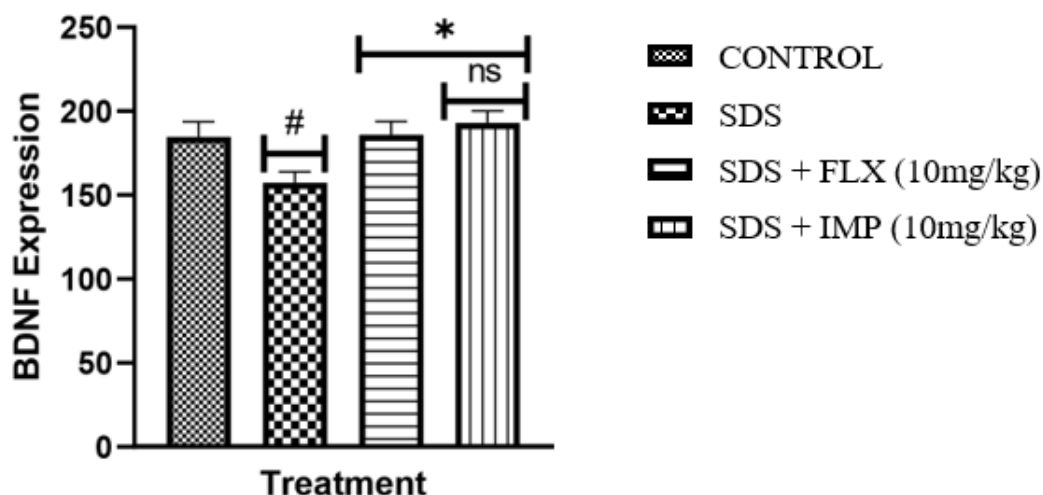


Figure 11: A graph showing the effect of fluoxetine and imipramine on the prefrontal cortex BDNF in SDS mice. Values are presented in mean $\pm$ SEM for 3 animals per group. $\#P < 0.05$ vs control group. $*P < 0.05$ for pathologic group (SDS untreated) vs SDS + fluoxetine and SDS + imipramine

Effect of fluoxetine and imipramine on the prefrontal cortex brain-derived neurotrophic factor (BDNF) in SDS mice

The effect of fluoxetine and imipramine on BDNF activity in the prefrontal cortex is shown in Figure 11. One-way ANOVA showed that there were significant differences between treatment groups in BDNF levels in the prefrontal cortex. Post hoc analysis using Turkey's post hoc test revealed that the untreated SDS group showed a significant ($p < 0.05$) decrease in BDNF levels when compared to the control group. Fluoxetine (10 mg/kg) and imipramine (10 mg/kg) produced a significant ($p < 0.05$) increase in BDNF levels when compared with the untreated SDS group.

There is no statistically significant difference between SDS + fluoxetine group and SDS + imipramine group (Figure 11).

Effect of fluoxetine and imipramine on the prefrontal cortex nuclear factor kappa B (NF- κ B) in SDS mice

The effect of fluoxetine and imipramine on NF- κ B activity in the prefrontal cortex is shown in Figure 12. One-way ANOVA showed that there were significant differences between treatment groups in NF- κ B levels in the prefrontal cortex. Post hoc analysis using Turkey's post hoc test revealed that the untreated SDS group showed a significant ($p < 0.05$) increase in NF- κ B levels

when compared to the control group, while (10mg/kg) and imipramine (10mg/kg) produced a significant ($p < 0.05$) decrease in NF-Kb levels when compared with untreated SDS group. However, comparing both treatment groups was not statistically significant ($p < 0.05$; Figure 12).

Effect of fluoxetine and imipramine on the prefrontal cortex Interleukin 10 (IL-10) in SDS mice

The effect of fluoxetine and imipramine on IL-10 activity in the prefrontal cortex is shown in Figure 13. One-way ANOVA showed that there were

significant differences between treatment groups in IL-10 levels in the prefrontal cortex. Post hoc analysis using Turkey's post hoc test revealed that the untreated SDS group showed a significant ($p < 0.05$) decrease in IL-10 levels when compared to the control group, while (10 mg/kg) and imipramine (10 mg/kg) produced a significant ($p < 0.05$) increase in IL-10 levels when compared with untreated SDS group. However, comparing both treatment groups, Imipramine increased the levels of IL-10 significantly ($p < 0.05$) compared to fluoxetine ($p < 0.05$; Figure 13).

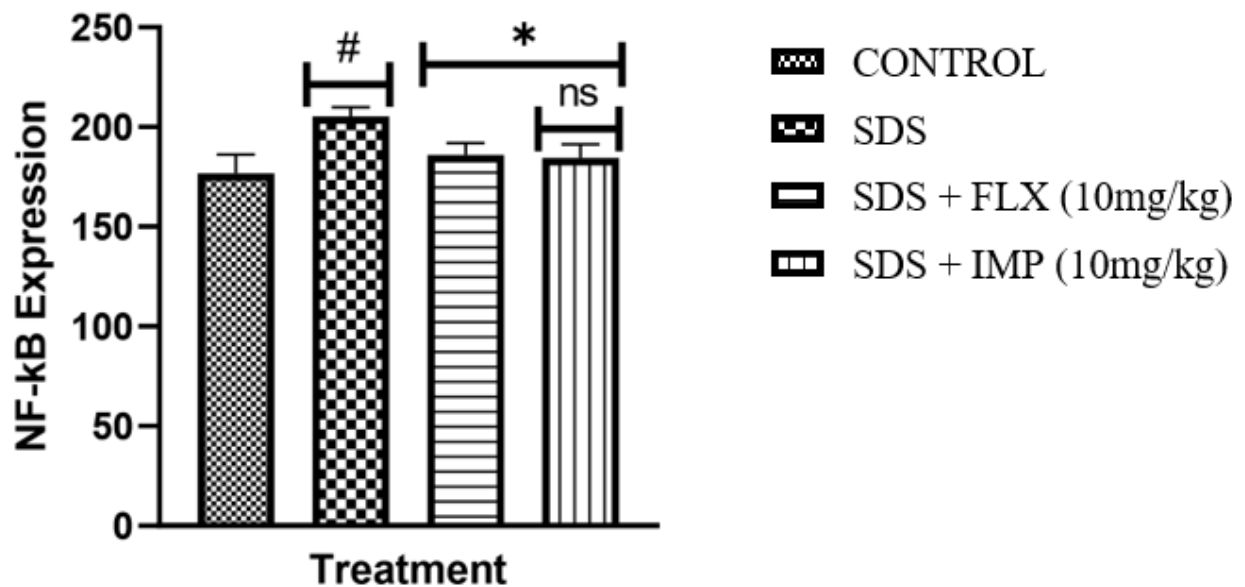


Figure 12: A graph showing the effect of fluoxetine and imipramine on the prefrontal cortex NF-kb in SDS mice. Values are presented in mean $\pm$ SEM for 3 animals per group. [#] $P < 0.05$ vs control group. ^{*} $P < 0.05$ for pathologic group (SDS untreated) vs SDS + fluoxetine and SDS + imipramine

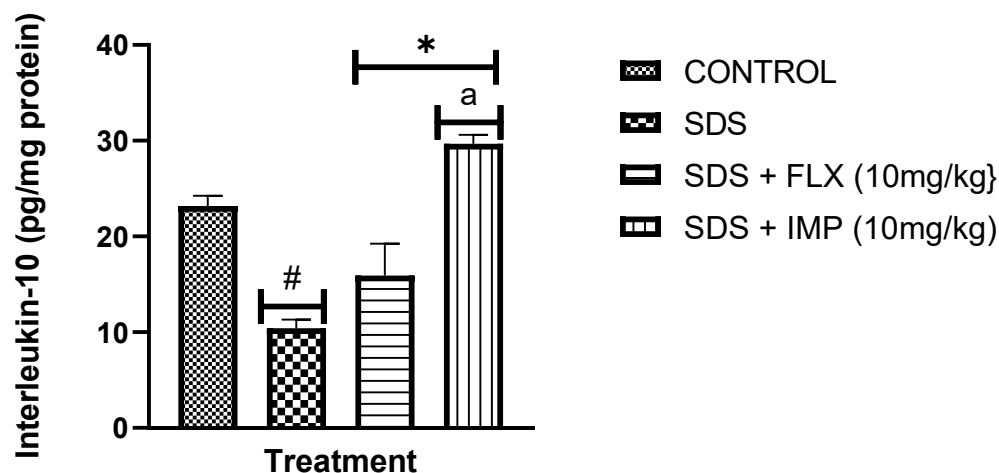


Figure 13: The effect of fluoxetine and imipramine on the prefrontal cortex levels of interleukin-10 in SDS mice. Values represent mean $\pm$ SEM for 5 animals per group. [#] $P < 0.05$ vs control group. ^{*} $P < 0.05$ vs pathologic group. ^a $P < 0.05$ vs SDS + fluoxetine group

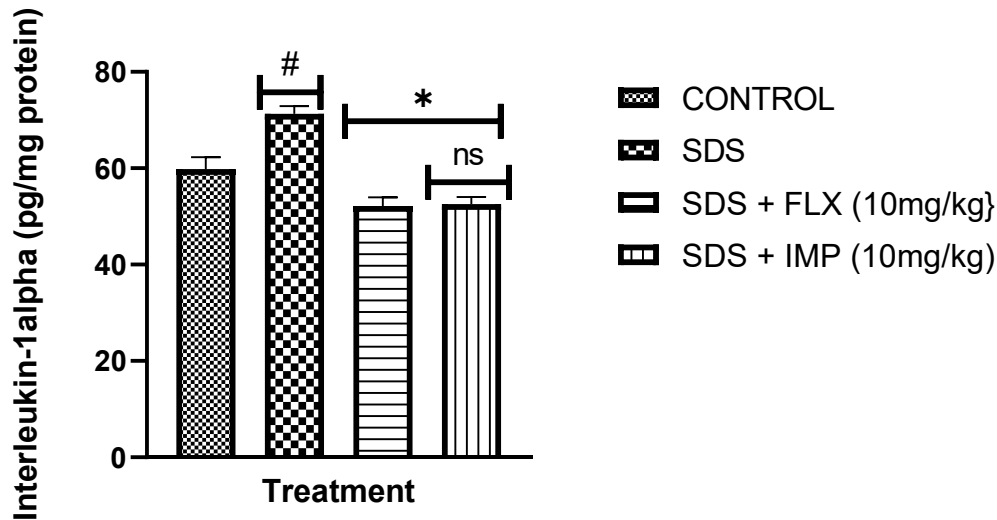


Figure 14: A graph showing the effect of fluoxetine and imipramine on the prefrontal cortex Interleukin-1alpha in SDS mice. Values are presented in mean $\pm$ SEM for 3 animals per group. [#] $P < 0.05$ vs control group. ^{*} $P < 0.05$ for pathologic group (SDS untreated) vs SDS + fluoxetine and SDS + imipramine

Effect of fluoxetine and imipramine on the prefrontal cortex Interleukin 1alpha (IL-1 α) in SDS mice

The effect of fluoxetine and imipramine on IL-1 α activity in the prefrontal cortex is shown in Figure 14. One-way ANOVA showed that there were significant differences between treatment groups in IL-1 α levels in the prefrontal cortex. Post hoc analysis using Turkey's post hoc test revealed that the untreated SDS group showed a significant ($p < 0.05$) increase in IL-1 α levels when compared to the control group, while (10 mg/kg) and imipramine (10 mg/kg) produced a significant ($p < 0.05$) decrease in IL-1 α levels when compared with untreated SDS group. However, comparing both treatment groups was not statistically significant ($p < 0.05$; Figure 14).

DISCUSSION

The elevated plus maze test compares the amount of time spent in open and closed arms, and is commonly used to examine anxiety-like behaviours in mouse models. Results from this study demonstrate that untreated SDS mice had a stronger preference for the closed arm of the elevated plus maze. The amount of time that animals spent in the closed arm was specifically reduced by fluoxetine and imipramine, with fluoxetine showing a significantly greater reduction than imipramine. Fluoxetine has been shown to have anxiolytic-like effects in earlier studies [10]. Because of its antihistamine and anticholinergic properties, imipramine has sedative effects in addition to its anxiolytic ones. As a result of sedation, animals may be less inclined to explore in the open arms and instead

stay in the more confined, "safe" closed arms. More time in the closed arms may result from imipramine's anxiolytic efficacy being diminished by the sedative effects.

The light-and-dark test uses mice's natural dislike of areas with bright lighting to analyse anxiety-like behaviours. Mice with elevated anxiety spend more time in the dark and avoid the light compartment more frequently. Untreated SDS mice in this study exhibit more time spent in the dark compartment and less exploratory activity in the light area, suggesting a component of anxiety [11]. This indicates a rise in avoidance and stress-related behaviours. Once more, imipramine and fluoxetine showed anxiolytic-like effects in reducing the amount of time spent in the dark phase. It appears that imipramine and fluoxetine have similar effects.

An imbalance between the oxidative and antioxidant systems is linked to anxiety, which increases oxidative stress, especially in parts of the brain like the hippocampus and prefrontal cortex. While the hippocampus is essential for memory and learning, the prefrontal cortex is crucial for executive functioning and emotion regulation. Because of their low levels of antioxidant defence and high metabolic activity, these areas are particularly susceptible to oxidative damage. The results of this investigation showed that, in comparison to control mice, untreated social defeat stress mice had significantly ($p < 0.05$) lower levels of superoxide dismutase (SOD) in their hippocampus and prefrontal cortex. Research has shown that individuals with anxiety exhibit reduced SOD activity, which worsens ROS

accumulation and causes cellular damage [12]. The study's findings showed that imipramine and fluoxetine effectively increased SOD levels in mice under social defeat stress. Despite the fact that both medications showed promise in raising SOD levels, their effects in the PFC and hippocampus are probably similar.

Higher levels of malondialdehyde (MDA) are one indicator of oxidative stress, which is linked to anxiety. Decreased executive function, affective regulation, and decision-making—all of which are frequently linked to anxiety—are linked to elevated MDA levels in the prefrontal cortex [13]. Higher MDA levels in anxiety also cause hippocampus shrinkage and cognitive deficits that are frequently linked to the condition [14]. The hippocampus is a region that is crucial for memory formation and stress responses. Once more, this study demonstrated that imipramine and fluoxetine had similar effects in the prefrontal cortex and hippocampus and were both capable of lowering MDA levels in both areas. There are several possible explanations for the significant ($p < 0.05$) reduction in MDA levels in the hippocampus and prefrontal cortex caused by imipramine and fluoxetine. Both medications have the potential to decrease oxidative stress and MDA levels in the brain by influencing mitochondrial activity and enhancing neurogenesis. Fluoxetine and imipramine may help shield the hippocampus and prefrontal cortex from oxidative damage by focusing on several oxidative stress-related pathways, which could improve the cognitive and affective effects of anxiety.

The results reveal that SDS caused atrophy in the hippocampus's dentate gyrus (DG). This is consistent with a previous report by Mazaheri *et al* [15], which showed that long-term stress, a major cause of anxiety, causes hippocampus shrinkage, decreased neurogenesis, and decreased neuronal density, particularly in the dentate gyrus region of the hippocampus. When fluoxetine and imipramine were administered, the negative effects of chronic stress disorder (SDS) on the dentate gyrus were reversed. This study is comparable to that of Chakrapani *et al* [16], who found that imipramine increases the expression of brain-derived neurotrophic factor (BDNF), which is linked to enhanced neurogenesis and an increase in neuronal density in the dentate gyrus.

A subcortical area of the forebrain, the striatum, plays a major role in cognitive, reward, and motor control functions. The striatum's histology and neuronal density are known to change as a result of anxiety, and this can exacerbate the motor and cognitive impairments associated with

anxiety disorders. The investigation's findings suggested that acute neuronal necrosis in the striatum was triggered by social defeat stress. This could be because stress causes glutamate to be released more often, which can overactivate striatal N-methyl-D-aspartate (NMDA) receptors. Calcium ions flood neurones as a result of this overactivation, starting a series of intracellular processes that encourage neuronal damage and death [17]. The striatal neuronal density count was affected in the same way by imipramine and fluoxetine. This study found that after taking fluoxetine, striatal neurones exhibited pyknosis, while after taking imipramine, striatal neurones showed atrophy. Higher serotonin levels by suppression of its reuptake may be the cause of neuronal pyknosis after taking fluoxetine medicine.

Results showed that the amygdala of mice under social defeat stress has accelerated neuronal death, while there was neuronal atrophy. The loss of neurones in the amygdala was lessened by the administration of imipramine and fluoxetine. Imipramine is frequently used to treat anxiety, but prolonged use may have adverse consequences and alter the body, such as causing neurons in the amygdala to shrink [18].

One important neurotrophin involved in synaptic plasticity, neuronal survival, and neurogenesis is brain-derived neurotrophic factor (BDNF). In comparison to both treated groups, untreated SDS mice exhibited a significant ($p < 0.05$) decrease in BDNF levels in the medial prefrontal cortex (mPFC). Anxiety is frequently associated with decreased BDNF levels in the mPFC, which leads to neuronal atrophy and the loss of synaptic connections [19]. Reduced synaptic plasticity and compromised cognitive and affective processing are linked to BDNF downregulation in the mPFC. When compared to the untreated SDS group, fluoxetine and imipramine both significantly ($p < 0.05$) increased BDNF levels in the mPFC (groups 3 and 4, respectively). In contrast to imipramine, which raises BDNF levels in the mPFC and promotes neurogenesis and synaptic remodelling, fluoxetine's therapeutic effects depend on this rise in BDNF since it aids in restoring the synaptic connections and neuronal density that are lost in anxiety.

Increased expressions of NF- κ B were identified in the mPFC of untreated SDS mice. NF- κ B is a transcription factor that plays a critical role in the modulation of inflammation and immunological responses. In anxiety, NF- κ B activity is often raised in the mPFC, leading to increased production of pro-inflammatory cytokines and

contributing to neuroinflammation. Increased expressions of NF- κ B in the mPFC are connected with neuronal damage, synaptic loss, and behavioural symptoms of anxiety. Both drugs revealed comparable reductions ($p < 0.05$) in NF- κ B expression in the mPFC. According to Irfan *et al* [20], both fluoxetine and imipramine have been demonstrated to limit NF- κ B activation in the mPFC, thus lowering the expression of pro-inflammatory cytokines and alleviating neuroinflammation. By inhibiting NF- κ B activity, these antidepressants can protect against brain injury and restore synaptic function.

Anxiety is linked to immune system disruption, which frequently results in decreased levels of cytokines that reduce inflammation, like interleukin-10 (IL-10). As a regulatory cytokine, IL-10 has neuroprotective and anti-inflammatory properties. Interleukin-10 levels were lower ($p < 0.05$) in untreated SDS animals than in control mice. This is comparable to recent studies that found that people with anxiety problems have been found to have reduced levels of IL-10, which can lead to a pro-inflammatory condition [21]. The protective impact of imipramine and fluoxetine in raising interleukin-10 levels was demonstrated in this investigation.

Results from this study contradict previous studies that suggested fluoxetine had a greater and more consistent effect on increasing IL-10 levels than imipramine [22], as imipramine appears to have a stronger effect ($p < 0.05$) on increasing interleukin-10 levels than fluoxetine.

An inflammatory and immunological response is linked to the pro-inflammatory cytokine interleukin-1 alpha (IL-1 α). Increased IL-1 α levels have been linked to neuroinflammation, which is a defining feature of many mental illnesses, such as anxiety and depression [22]. This study showed that untreated social-defeat stress resulted in higher levels ($p < 0.05$) of interleukin-1 alpha compared to the control. Common antidepressants, imipramine and fluoxetine, have an impact on cytokine levels, especially IL-1 α . However, depending on their target routes and modes of action, they have different impacts on IL-1 α . The study's findings showed that imipramine and fluoxetine both similarly reduced interleukin-1 alpha levels.

CONCLUSION

Bullying can generate neurochemical and behavioral changes. Although both medications utilized for the study are antidepressants, data from this study show that both drugs have anti-anxiety-like effects, with both treatment groups

having comparable effects in most of the studies undertaken.

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Ethical approval

Delta State University's Faculty of Basic Medical Science Research and Ethics Committee in Abraka, Nigeria, granted ethical approval (no. RBC/FBMC/DELSU/24/550).

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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