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# N-acetylcysteine Alleviates Depression through Up-regulation of Synaptophysin, Inhibition of Reactivity Astrocytes, and Anhedonia in the Forced Swim Test Animal Model

**Adejoke E. Memudu**[1]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, Edo State University, Uzairue, Edo State, Nigeria

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## ABSTRACT

Depression is a mental disorder of global concern, with chronic psychological stress being one of the underlying predisposing factors. This study evaluated the role of the antioxidant, N-acetylcysteine (NAC), as an antidepressant using the forced swim test (FST) animal model. Thirty adult male Wistar rats (250 g average weight) were randomly grouped into six (n=5): Control (1 ml/day of normal saline); FST model; NAC (200 mg/kg/day); Fluoxetine (20 mg/kg/day); FST model treated with NAC (200 mg/kg/day), and FST model treated with Fluoxetine (20 mg/kg/day). All the treatments were orally. The FST, sucrose-preference test (SPT), and brain weights were assessed, and data analysed. The histo-architecture of the prefrontal cortex (PFC), as well as the immunohistochemistry of astrocytes and synaptophysin were also assessed. Findings showed that NAC prevented FST-induced depressive behaviour demonstrated by increased SPT and mobility time. NAC also prevented the FST-induced decreased brain weights and neuronal loss, reduced proliferation of reactive astrocytes, and diminished synaptophysin immunoreactivity in the PFC similar to that of fluoxetine, a standard antidepressant drug. NAC exhibited its neuroprotective mechanism via inhibiting the proliferation of reactive astrocytes, and protecting neurons and synapses from oxidative tissue damage induced by FST, hence, an increase in synaptophysin activity that culminated in increased neural activity, increased SPT, and reduced immobility time.

**Keywords:** *N-acetyl cysteine, Depression, Astrocytes, Synaptophysin, Anhedonia, Prefrontal cortex*

## INTRODUCTION

Depression may be caused by chronic psychological stress or trauma and is one of the leading causes of mortality worldwide, hence, a situation of global concern (Zhang et al. 2019). It is the most common neurodegenerative or psychiatric disorder (Ossig and Storch 2015). According to WHO (2017), people living with depressive disorder increased to 18% between 2005 and 2015. Furthermore, depression affects 4% of the global population taken to be about 322 million people (Furukawa et al. 2019).

The neuro-pathogenesis of depression is linked to neuro-inflammatory processes and oxidative stress (Kiraly et al. 2017). Glutamatergic dysfunction is also an important pathological mechanism in depressive disorders (Wright et al. 2016). Currently, N-acetylcysteine (NAC), an antioxidant supplement is being evaluated for its possible antidepressant therapy (Yang et al. 2018). It is a glutathione precursor and glutamate modulator with antioxidant and neurovascular-protective effects (Chen et al. 2016), and could ameliorate neuro-pathogenesis due to its ability to cross the blood-brain barrier (Pallanti et al. 2014). Within the brain, NAC being a precursor for glutathione synthetase prevents neuronal damage caused by reactive oxygen and nitrogen species

Correspondence: Adejoke E. Memudu, PhD; Department of Anatomy, Faculty of Basic Medical Sciences, Edo State University, Uzairue, Nigeria. Email: jokememudu@gmail.com; Phone: +234805194169; ORCID: 0000-0002-0204-8740

(Deepmala et al. 2015). NAC's mechanism of action for neuroprotection is linked to the regulation of neuroinflammation associated with neuronal dysfunction and neurodegeneration, thereby promoting neuro-regeneration of disrupted neurons, as well as correcting glutamate dysfunction linked to N-methyl-D-aspartate (Deepmala et al. 2015; Jakobsen et al. 2017).

Various studies reported that NAC's psychopharmacological effects are, an increase in neuronal connectivity in the brain, increase in the level of anti-inflammatory microglia (Bergold et al. 2012), and modulation of glutamate pathways in psychiatric conditions (McQueen et al. 2018; Mullier et al. 2019). NAC as an anti-inflammatory agent can be used as a treatment measure to ameliorate depression (Köhler et al. 2014), with a recommended oral dosage in humans per day being 2,000 - 2,400 mg (Ooi et al. 2018).

In preclinical drug screening, the forced swim test is adopted as the animal model for screening antidepressant-like activity (Slattery and Cryan 2012), where a decline in immobility time indicates that the compound/drug has antidepressant activity, while the sucrose-preference test (SPT) is a protocol to measure anhedonia characterized by loss of pleasure or interest (Ceren et al. 2018).

The prefrontal cortex (PFC) is an area commonly implicated in major depression disorders (Schubert et al. 2015; Fogaça and Duman 2019). The PFC plays an important role in the control of cognitive function and its neural connections with the amygdala, and has been implicated in depressive or mood disorders (Fogaça and Duman 2019). Woo et al. (2021) reported that exposure to stress can cause loss of synaptic connections in the PFC. It has been reported that brain samples of depressed individuals show low levels of synaptophysin protein involved in synaptic connections and formation (Holmes et al. 2019). This indicates a correlation between mood control and synaptic connections in the prefrontal cortex.

Synaptophysin is a membrane glycoprotein located in presynaptic vesicles of neurons and is involved in synaptic transmission, synaptic biogenesis, initiating neurotransmitter release, synaptic vesicle endocytosis, and synapse formation (Gudi et al. 2017). According to Holmes et al. (2019) and Ren and Guo (2021), a low level of synaptophysin is associated with loss of synaptic connectivity, as reported in depressive behaviour. Astrocytes are supporting glial cells which give structural and functional support to neurons, and its dysfunction is reported to be involved in the progression of depression (Zhang et al. 2020). Dolotov et al. (2022) reported stress induced proliferation and reactive astrocytes in the PFC of rodents. Hence, there is a correlation between synaptogenesis and proliferation of reactive astrocytes in depressive behaviour, i.e as reactive astrocytes proliferate, synaptophysin activity declines due to loss of synaptic connection associated with neuron dysfunction. Hence, this study was carried out to assess NAC antidepressant role by evaluating the changes in astrocytes and synaptophysin in the PFC, in addition to behavioural changes in the FST paradigm and anhedonia status in the animal FST model.

## MATERIALS AND METHODS

### Experimental Animals

Thirty adult male Wistar rats of average weight 250 g were obtained and housed in the Animal Facility of the Department of Anatomy, Bingham University, Karu, Nigeria. The rats were allowed to acclimatize for seven days before the commencement of the experiment. They were cared for according to the guidelines for the care and use of animals in research (National Research Council 2011), and ethical approval was sought from the Institutional Ethical Committee. The rats were housed in well-aerated metallic cages. They were fed with pelleted rat feed (Vital Feeds Limited, Nigeria); and water *ad libitum*, and maintained in standard pathogen-free laboratory conditions of 12 h light/dark cycle (lights on at 07:00 am), room temperature (37 ± 2°C), and 60 ± 5% relative humidity. The behavioural procedures were carried out from 08:00 a.m. to 12:00 p.m. in the test room within the animal facility.

### Experimental Design and Protocol

Fluoxetine (Medibios Laboratories PVT Ltd, India; 20 mg/kg daily; Ohira et al. 2019) was orally administered, while NAC (Swanson, USA; 200 mg/kg daily; Saraswathy et al. 2014) was also administered orally 60 min before the FST procedure.

### Experimental Animals Grouping

Group 1 served as the control given normal saline; group 2 was exposed to FST and the 30% sucrose solution to test for anhedonia; group 3 received 200 mg/kg NAC only; group 4 received fluoxetine (20 mg/kg) only; group 5 received 30% sucrose solution + FST and 200 mg/kg NAC; and group 6 received 30% sucrose solution + FST and fluoxetine 20 mg/kg. All the administrations were orally. Groups 2, 5 and 6 were exposed to the FST, while groups 1, 3 and 4 were not.

### Behavioural Studies

#### Forced Swim Test (FST)

The rats were placed individually in a transparent cylindrical tank (50 cm diameter, 60 cm height) containing water (35.2˚C ± 1°C), and changed after each test session. Each rat received the study drugs (NAC and fluoxetine, respectively) an hour before the FST (Porsolt et al. 2001). A pre-test of 15 min for habituation, and a 5 min test following drug treatment

was done (Stratinaki et al. 2013). Swim sessions were video recorded and the immobility, swimming, and climb behaviours were scored in each test session (Fischer et al. 2015) timed using a stopwatch. The 5 min test was scored by a trained blind observer (Castagné et al. 2010).

**Sucrose Preference and Water Intake Tests**

The rats were exposed to 1% sucrose solution for 48 h, and thereafter deprived of water and food for 12 h before the test day. Subsequently, the rats were exposed to an hour preference test by exposure to 100 mL of 30% sucrose solution and water delivered in identical bottles (Razmjou et al. 2015). The amount of sucrose and water consumed was determined by measuring the differences in the volume of the fluid (Çorumlu et al. 2015; Watson et al. 2020). Following Çorumlu et al. (2015), the remaining water and sucrose after exposure was weighed, and the sucrose preference (%) was determined with the formula:

$$\text{SPT}\ (\%) = \frac{\text{Volume of sucrose consumed (mL/h)}}{\text{Volume of water consumed+sucr} \quad \text{consumed(mL/h)}} \times 100$$

The volume of water and sucrose taken were measured as; first and second exposures to sucrose and water were done 24 h before the 15 min of FST pretests, while the third exposure to sucrose and water was done an hour after 5 min of the FST and the drugs' administration.

**Euthanasia and Prefrontal Cortex Excision**

The final body weights of the experimental rats were taken (OHAUS Pioneer™, India), and animals were euthanized via cervical dislocation and decapitated (Zaccarelli-Magalhães et al. 2019). Whole brains were excised and wet weight taken with OHAUS weighing balance and then fixed in 4% paraformaldehyde labelled sample bottle containing ready for tissue processing (Bancroft and Gamble 2008).

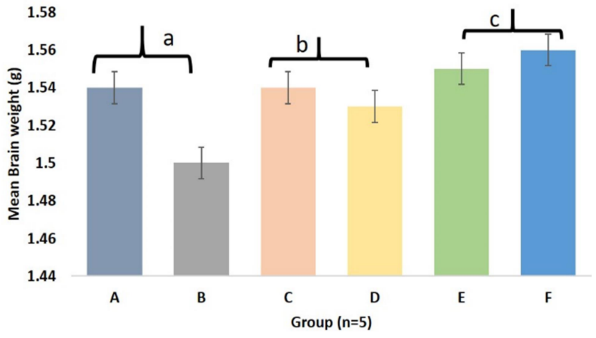


**Fig. 1: Mean brain weights of experimental Wistar rats**. Significant in Group A vs B (a), Group C vs D (b), and group F vs E (c) at P<0.05. A= Control, B= FST C= NAC, D= fluoxetine, E= NAC + FST, and F= fluoxetine + FST. p<0.05

**Prefrontal Cortex Histological Processing for Staining**

Coronal sections of the prefrontal cortex were dissected according to Paxinos and Watson (2007), and tissue processing done using an automated tissue processor (LEICA TP 1050) set to pass through dehydration in graded alcohols; clearing through xylene, and embedded in paraffin (Akinrinade et al. 2015). The embedded PFC tissues were sectioned using a rotary microtome (LEICA RM) set at 5 µm and mounted on glass slides for haematoxylin and eosin (H and E), as well as glial fibrillary acidic protein (GFAP) (Memudu et al. 2020) and synaptophysin (p38) immunohistochemistry staining procedures (Gudi et al. 2017).

**Immunohistochemical Staining for Astrocytes**

Astrocytes in the formaldehyde-fixed paraffin-embedded rat prefrontal cortex sections were examined using the GFAP immunohistochemical (IHC) protocol (Akinrinade et al. 2015). The Novocastra-mouse monoclonal (GFAP-antibody Leica Microsystems-Novocastra™ United Kingdom, 1:100 dilutions) and the Novocastra biotinylated secondary antibodies (biotinylated donkey anti-mouse IgG, 1:200) were used. The peroxidase-coupling was done using the avidin-biotin complex (ABC Kit, Vector Laboratories, Burlingame, CA, USA). The immunoreaction product was visualized with 3,3′-diaminobenzidine (DAB, Dako) for chromogen development. The counterstain was done using Mayer's haematoxylin and, mounted with distrene plasticizer xylene.

**Immunohistochemical Staining for Synaptophysin a Synaptic Vesicle Protein (p38)**

A primary monoclonal antibody (synaptophysin rabbit monoclonal antibody, #MA5-14532), secondary antibody (biotinylated horse anti-mouse secondary antibody, 1:200, Vector Labs, Burlingame, CA, USA), avidin-biotin complex linked to peroxidase (ABC Kit [Vectastain kit], Vector Laboratories, Burlingame, CA, USA) and 3,3′-diaminobenzidine for chromogen and Mayer haematoxylin was applied according to Gudi et al. (2017).

Photomicrographs were taken using an Olympus compound light microscope (Olympus, Japan) connected to a digital camera (Amscope Inc., Irvine, CA, USA) with the objective lens ×10.

**Statistical Analysis**

Statistical analyses were done using GraphPad Prism 6 (GraphPad Software, Inc., USA). One-way analysis of variance was used for all multiple comparisons followed by the post hoc Tukey test. Statistics were significant when p-values were lower than 0.05. Data were expressed as mean ± standard error mean.

## RESULTS

### NAC Prevents FST-Induced Decline in Brain Weight

There was a statistically significant decrease in brain weight of the FST model group as compared to the control group at $p<0.05$. Groups treated with NAC had no significant difference in brain weight as compared with the control at $p>0.05$, but was significantly increased when compared with the fluoxetine-treated group at $p<0.05$. Fluoxetine-treated FST had increased mean brain weight as compared with NAC-treated FST group at $p<0.05$ (Fig. 1). There was no significant difference in brain weights of the NAC and control group at $p>0.05$.

### NAC Reversed FST-Mediated Decline in Immobility time

The immobility time for FST model increased significantly compared with NAC-treated FST model and fluoxetine-treated FST group at $p<0.05$ (Fig. 2). There was no significant difference in immobility time of NAC-treated FST model and fluoxetine-treated FST group at $p>0.05$. The mobility time in the FST model decreased compared with NAC-treated FST model and fluoxetine-treated FST group at $p<0.05$. But NAC-treated FST model had significantly increased mobility time at $p<0.05$ compared with fluoxetine-treated FST group.

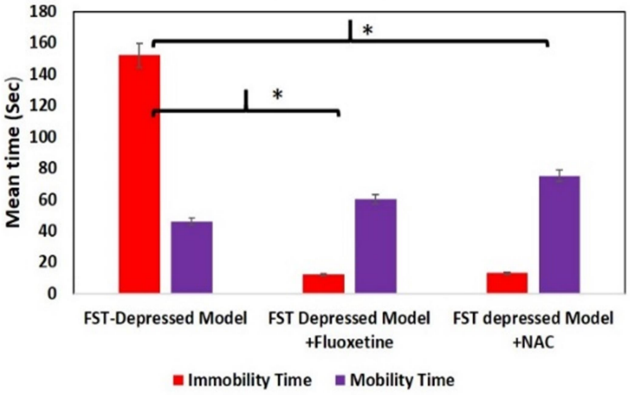


**Fig. 2: Mean immobility and mobility time of experimental Wistar rats.** * = significant immobility time in fluoxetine treated FST vs FST model, NAC treated vs FST. $p<0.05$

### NAC Prevented Anhedonia Effects of FST in Sucrose Preference Test

At first exposure to sucrose and water, the control, FST model, NAC-, and fluoxetine-treated FST models had significantly increased sucrose preference test compared with the NAC- and the fluoxetine-treated groups at $p<0.05$ (Fig. 3). At the second exposure to sucrose and water, the FST model, NAC-treated, fluoxetine-treated, NAC-treated FST model, and fluoxetine-treated FST model had significantly decreased SPT compared to the control group at $p<0.05$. However, there was no significant difference in the FST model, fluoxetine-treated and NAC-treated FST model at $p>0.05$. The third exposure to sucrose and water showed significantly declined SPT of the FST model compared to the other study groups at $p<0.05$. The NAC-treated FST and fluoxetine-treated FST models had significantly increased SPT compared with the FST model at $p<0.05$.

### NAC Protects the Pyramidal Cells of the PFC

The control group showed numerous pyramidal neurons with no abnormal histology. The FST model cortical neurons appeared necrotic with scanty and vacuole-filled neuropil. The neurons showed characteristics mild of neuron degeneration, pericellular spaces around the neurons, and homogenous cytoplasm with pruned apical and basal neuritis (Fig. 4). The NAC- and fluoxetine- treated groups showed numerous neurons with dense neuropil, absence of necrotic cells, and notable axonal and basal dendrite outgrowth as compared with the FST model. The NAC- and fluoxetine- treated FST models showed dense neuropil presence with the central nucleolus, neurites, and few necrotic cells. The number of necrotic or pyknotic cells appear reduced as compared to the FST-only model.

### NAC Reversed Proliferation of Reactive Astrocytes (Astrogliosis)

The control group showed well-expressed astrocytes demonstrated by GFAP expression in the astrocytic processes (Fig. 5). FST-only model expressed more reactive astrocytes when compared to the control group. NAC- and fluoxetine- treated groups expressed astrocytes mildly as compared with the FST model, but more as compared with the control. FST groups' treatment with NAC and fluoxetine had a decline in reactive astrocytes as compared with the FST model.

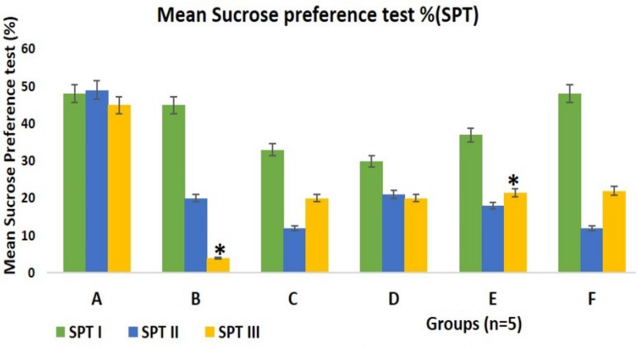


**Fig. 3: Mean percentage sucrose preference test (SPT) to assess anhedonia status of experimental Wistar rats.** A= Control, B= FST induced depression, C= NAC, D= fluoxetine, E= NAC + FST, and F= fluoxetine + FST. SPT I: measured 24 h before FST pretest, SPT II: measured 24 h after 15 min of FST, and SPT III: measured an hour after 5 min of FST. * $p<0.05$ = significant SPT of FST vs A, C, D and F; # Significant at NAC treated group (E) vs FST model group. $p<0.05$

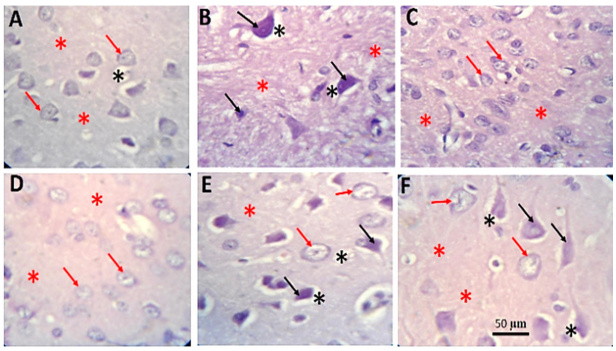


**Fig. 4: Section of the prefrontal cortex of adult male Wistar rats stained with haematoxylin and eosin.** A= Control, B= FST, C= NAC, D= fluoxetine, E= NAC + FST, and F= fluoxetine + FST. Red arrows = normal neurons, Black arrows = pyknotic or necrotic neurons, Red asterisks = neuropil and black asterisks = pericellular spaces. ×400; Scale bar =50µm.

### Immunohistochemical Expression of Synaptic Protein Synaptophysin

The immunoreactivity of synaptophysin protein was positive within the neuropil of the gray matter of the prefrontal cortex of the control group, NAC-treated and fluoxetine-treated groups (Fig. 6). The synaptophysin immunoreactivity was uniformly homogeneous throughout the PFC in the fluoxetine-treated group compared to the FST model.

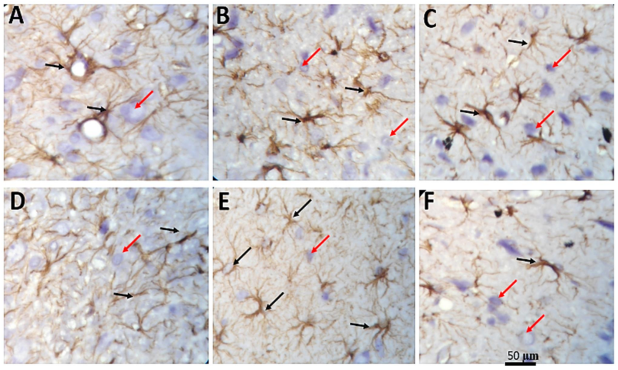


**Fig. 5: Section of the prefrontal cortex of adult male Wistar rats labelled for astrocytic GFAP expression.** A= Control, B= FST model, C= NAC, D= fluoxetine, E= NAC + FST, and F= fluoxetine + FST. Red arrows = normal pyramidal cell body; black arrows = astrocytic cytoplasmic processes (intermediate filaments). ×400; Scale bar =50µm.

## DISCUSSION

It is a common paradigm to screen for antidepressant drugs in animal models because they can induce physiological stress via forced swimming (Porsolt et al. 2001; Slattery and Cryan 2012). The present study evaluated the antidepressant potential of NAC using the FST model. In this study, there was significant decreased brain weights of FST-induced stressed rats as compared with the control, NAC-treated, and fluoxetine-treated groups. The decreased brain weight of the FST rats is slightly similar to Fortunato et al. (2010), who reported that chronic FST in rats leads to a mild decline in brain weight. However, the present study did not adopt prolonged chronic FST exposure since it was a short-term predictive validity test focussed on the potential of an antidepressant to reduce immobility time in the FST (Cryan et al. 2005). Nevertheless, Katz et al. (1981) reported that mild depression does not have much effect on brain weight. Similarly, Mohammed et al. (2019) reported that NAC-treated rats had no effect on brain weights compared with the control, which correlates with the report of the present study. Fluoxetine- and NAC- treated FST model rats had increased brain weights compared with the FST model rats. The reduced brain weight observed in the FST animal model in this study may be linked with a reduction in dendritic spine density (Kang et al. 2011; Penzes et al. 2011). The increase in brain weight of NAC- and fluoxetine-treated FST rats could be linked

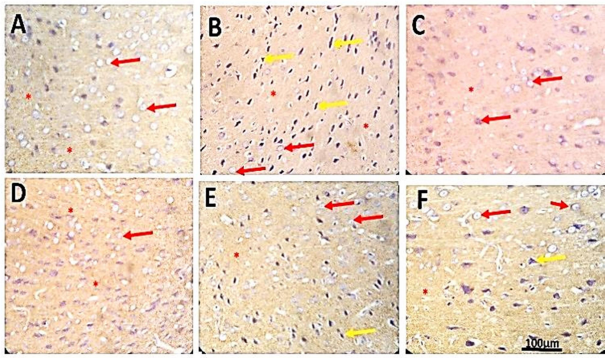


**Fig. 6: Section of the prefrontal cortex of adult male Wistar rats labelled for synaptophysin (protein p38).** A= Control, B= FST, C= NAC, D= fluoxetine, E= NAC + FST, and F= fluoxetine + FST. Red arrows= neuron cell body, Yellow arrows= necrotic/pkynotic neurons, red asterisk = immunoreactivity of the brownish coloration of synaptophysin protein within the neuropil, showing uniformly homogenous synaptophysin protein in the neuropil of A, C, D, E and F. ×400; Scale bar =100µm

with NAC and fluoxetine increasing synaptogenesis and increasing cortical spine density (Berk et al. 2014).

FST is the most used antidepressant drug screening test in animal model, as a result of an immobility response induced by inescapable exposure to stress (Can et al. 2012). In the present study, the immobility time for FST model increased compared with the NAC-treated FST model and fluoxetine-treated FST groups. Kawaura et al. (2016) and Adu-Nti et al. (2019) reported similar results. Fluoxetine however, decreased the immobility time, which supports previous studies (Abdel-Salam et al. 2013). Furthermore, fluoxetine increased mobility time in the FST (Costescu et al. 2019), which the present study aligns. The potency of fluoxetine in the FST is based on its ability as a selective serotonin reuptake inhibi-

tor, demonstrated in alleviating behavioural depression, and possibly due to its ability to suppress cholinergic activities in the nucleus accumbens, or by inhibition of noradrenaline and dopamine reuptake. NAC on the other hand is a precursor for glutathione and glutamate synthesis that acts to protect the neurons (Wright et al. 2016). The antidepressant-like effect of NAC is dependent on its ability to modulate glutamate transport (McQueen et al. 2018), hence, increasing glutamate in the synaptic cleft, which helps to alleviate depressive behaviour (Wright et al. 2016).

Sucrose preference test (SPT) is a sensitive screening test as a measure of anhedonia in rodents, a decline in SPT shows a significant face validity for chronic stress and antidepressant treatment (Razmjou et al. 2015; Liu et al. 2018). The identical two bottles choice method of the SPT is significant in assessing anhedonia in a stress-mediated animal model of depression (Eagle et al. 2016). A reduced sucrose preference or consumption is used as an index of anhedonia in the FST animal model for depression (Çorumlu et al. 2015). According to Watson et al. (2020) anhedonia is a notable symptom of depression characterized by a loss of interest in usual activities that were pleasurable and rewarding. In the present study, all experimental animals showed a preference for sucrose compared to water (24 h before a 15 min FST test). In the FST-induced anhedonia, a decline in sucrose preference was observed 24 h after the 15 in FST when compared with the control and fluoxetine groups, and these correlate with previous findings (Dale et al. 2012; Browne and Lucki 2013). The test drug for antidepressant potential, NAC, and fluoxetine (a standard antidepressant drug) reversed the anhedonia effects of FST by sucrose preference increase compared with the non-treated, with reduced sucrose preference (Eagle et al. 2016).

Sucrose preference test and anhedonia are related to the reward circuit coordinated by the nucleus accumbens. The nucleus accumbens has both dopaminergic and glutamatergic afferent connections arising from the PFC, hippocampus and amygdala, and implicated in depressive disorder (Heshmati and Russo 2015; Schubert et al. 2015). NAC helps protect these neurons (Chen et al. 2016), hence, preserving the majority of the synapses within the nucleus accumbens (Heshmati and Russo 2015). NAC's alleviation of the anhedonia status in rats as in the present study may be via mediating glutamate activity in the synaptic clefts similar to the action of ketamine (Coyle and Law 2015). NAC motor dysfunction reversal in the FST animal model may also be by attenuating neuroinflammation linked to neuron malfunction, thereby increasing neuron repair and connectivity (Deepmala et al. 2015; Jakobsen et al. 2017).

The pyramidal neurons of the PFC are commonly implicated in depression (Schubert et al. 2015), with a reduction in these cells (Fogaça and Duman 2019). This afore-mentioned histopathological characterization in the PFC was demonstrated in the FST model of this present study. The FST PFC demonstrated numerous necrotic pyramidal neurons as compared with the control. This PFC characterization for neurodegeneration correlates with the findings of Réus et al. (2011) and Monteggia and Zarate (2015). However, the disrupted neuron integrity induced by FST was ameliorated by NAC. NAC- and fluoxetine- treated FST groups showed similar neuroprotective features. NAC-treatment reversed FST-induced oxidative stressed neuronal damage (Wright et al. 2016) because of its antioxidant potential (Berk et al. 2014; Chen et al. 2016), which is linked to its cysteine component, a precursor for glutathione in the brain (Pallanti et al. 2014; Yin et al. 2016). Fluoxetine's neuroprotective action may be linked to its ability to mediate neuron proliferation, repair, differentiation, protection, and regeneration of neurons (Surget et al. 2008).

GFAP is commonly used as an astrocyte marker, and is localized in the intermediate filaments. It expresses astrocyte cellular processes, and proliferates in neurodegenerative conditions due to inflammation (Gil-Martínez et al. 2018). Astrocytes play significant functions in neural activity in the brain, including the uptake of glutamate and glutamate and gamma-aminobutyric acid by specific transporters (Goubard et al. 2011), and the production of antioxidants. It also plays important role in neuropathology, hence, the need to target them as pharmacological therapy (Liu and Chopp 2016).

FST-induced oxidative stress is linked with astrocytic dysfunction, which affects its potential to detect or react to stress-mediated elevation in glutamate activity, disrupting neuronal homeostasis, and leading to hyperactivity of the N-methyl-D-aspartate receptors involved in the control of cognitive functions in the PFC (Finsterwald et al. 2015). The FST-induced PFC showed much astrocytic expression which implicates a neuroinflammatory response associated with the neuropathogenesis of depression, and is linked with increased deposition of inflammatory markers including interleukin IL-1β and IL-6 in the rat PFC (Kim et al. 2013). In the present study, NAC and NAC- and fluoxetine- treated FST attenuated PFC astrocytic proliferation as compared with FST only group. NAC can reverse neuroinflammation mediated by FST-induced oxidative stress (Berk et al. 2014). NAC's ability to ameliorate astrocyte proliferation may be by targeting astrocytic glutamate transporters to avert neurodegenerative disorders associated with excitotoxicity. Fluoxetine rapidly increases serotonin signalling *in vivo* (Ma et al. 2016), which explains its antidepressant activity in attenuating astrocytes proliferation, as demonstrated in the present study.

Synaptophysin is present in presynaptic vesicles of all neurons, and it is involved in synaptic biogenesis and transmission, initiation of neurotransmitter release, synaptic vesicle endocytosis and synapse

formation (Gudi et al. 2017). Synaptophysin is a biomarker to detect axonal damage in rat brain tissue according to Sarnat et al. (2010) and Gudi et al. (2017) methods, as well as demonstrates synaptic plasticity or synaptogenesis (Sarnat et al. 2010). It has been reported that the hallmark of depression neuropathology is linked to loss of synaptic activity (Sanacora 2012; Tizabi et al. 2012). Hence, it is important to define the relation between synaptic activity in the pathophysiology of the depression and therapeutics of antidepressant drugs. In the present study, the control had an accumulation of synaptophysin positive vesicles within the neuropil of the grey matter of the PFC, which declined in the FST model, indicating degenerating neuronal tissue. This result correlates with Sarnat et al. (2010) that reported a loss of synaptic vesicles or no synapses (Gudi et al. 2017). The PFC of the NAC- and fluoxetine-treated FST model showed uniformly intense and homogeneous synaptophysin immunoreactivity compared with the FST animal model. The present study supports the report of Karalija et al. (2012) that NAC restores the loss of synaptophysin in neurodegenerating tissue. Contrary to Pawluski et al. (2014) who reported that fluoxetine treatment decreased synaptophysin expression, fluoxetine increased synaptophysin expression which supports Larsen et al. (2008) report that fluoxetine affects synaptic changes and increases cell proliferation (Huang and Herbert 2006). Thus, NAC and fluoxetine have the potential to increase pyramidal spine formation (Hajszan et al. 2005).

## Conclusion

In conclusion, 200 mg/kg of NAC ameliorated FST-induced depressive-like behaviour in rats by attenuating reactive astrocytes proliferation, which protects against loss of neurons and increases synaptophysin activity translating to the alleviation of anhedonia and a reduced immobility time. These are indicators for effective antidepressant drugs.

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**Conflict of Interest**
None declared.

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**Authors Contribution**
Conceptualization and design of the study, formal analysis and writing of the draft, Data curation, Methodology, Validation, Analysis and interpretation of data, Writing - review and editing were all done by EAM.

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