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Research Article

N-Acetyl Cysteine (NAC) Enhances Synaptophysin Expression Which Alleviates Motor Dysfunction and Depressive Behavior in an Animal Model

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Abstract

Background: Depressive disorder affects millions of people worldwide and its pathogenesis is related to the loss of synaptic connections. The current treatment involves the use of fluoxetine which targets the monoaminergic system, this drug is reported to have a delayed response time., hence the need to discover new fast-acting drugs. This study was aimed at evaluating changes in anhedonia, food consumption, motor function, and synaptophysin expression following NAC administration in the Forced Swim Test (FST) animal model, used to screen antidepressant drugs using fluoxetine as standard.

Methodology: Thirty (30) adult male Wistar rats used for this study were divided into six groups (n = 5): A = Control, B = FST Model, C = 200mg/kg of NAC given orally, D = 20mg/kg fluoxetine given orally, E = FST Model + 200mg/kg of NAC given orally and F = FST Model + 20mg/kg fluoxetine given orally. The excised brain was fixed in 4% paraformaldehyde (4% PFA), tissue processing was done, paraffin-embedded brain tissues were sectioned, and immunohistochemical staining to express synaptophysin protein was done. Behavioral test data were analyzed using ANOVA and and tests for significance were done using post-hoc @$p < 0.05$.

Results: N-Acetyl Cysteine caused a significant increase in swimming time, mobility time, and a decline in immobility time as compared with the FST model. NAC prevented anhedonia demonstrated by an increase in sucrose as well as food consumption following exposure to FST post-NAC administration. There was a uniform expression of synaptic protein synaptophysin within the neuropil of the hippocampus and cerebellar cortex administered NAC and Fluoxetine as compared with the FST model.

Conclusion: These findings showed that NAC prevented FST induced depressive-like behavior in rats demonstrated by increased body weight, sucrose, and food consumption, increased mobility, and reduced immobility time attributed to a well-expressed synaptophysin activity indicator for neuronal connection or synaptic connection in the hippocampal CA1 horn and cerebellum (CB). Hence NAC has a antidepressant potential mediated by increasing synaptophysin expression.

Keywords: N-Acetyl Cysteine; depression, Synaptophysin; Sucrose Consumption; Forced Swim Test

Introduction

Oxidative stress is common to psychiatric disorders such as depression and bipolar disorders [1,2] characterized by elevation of inflammatory markers [3]. Depression is a neurodegenerative disorder associated with chronic psychological stress and it is of global concern because it is the leading cause of death [4]. According to the World Health Organization, depressive disorder is expected to increase to become the second contributor to the global burden by 2030 [5]. Loss of synapses is considered the earliest site of depression neuropathology [6]. A reduced synaptic activity causes detrimental effects on synapses and mood of animals demonstrated immunohistochemical by a decline in synaptophysin expression [7]. According to Heller [8] prefrontal cortex and CA1 horn of the hippocampus is essential in controlling mood and cognition while

the cerebellum is for coordinated movement. Aside from oxidative stress, [9] glutamatergic dysfunction linked with progress cell death is another important pathological mechanism in depressive disorders [10]. Of the 4% of the world's population affected with depression [11], only 50% are considered non-responsive satisfactorily to antidepressant treatment [12]. Hence there is an urgent necessity to develop or discover new and more effective therapies that target synaptic activity. N-acetylcysteine (NAC) is a strong antioxidant and acts as a glutathione precursor and a glutamate modulator [13]. Reports have it that NAC acts by modulating neuroinflammatory reactions, promoting neurogenesis while protecting neurons from damage [14]. N-acetylcysteine can cause an increase in neuron connectivity in the brain [15] thereby alleviating depressive-like behaviors. There is a need to evaluate N-acetylcysteine (NAC) as a possible antidepressant [16] since it has high bioavailability and can cross the blood-brain barrier [17] to cause an improvement in brain synaptic activity [15]. Animal models are very useful tools that are employed in investigating the etiology of depression, as well as progress in the development of effective and novel therapeutics for its treatment [18]. A valid rodent antidepressant treatment model will benefit by exhibiting stress-dependent behavioral responses [19] before translational research, i.e., more complex preclinical tests and clinical evaluation [21]. FST is used to screen the antidepressant effects of novel drugs [22]. This study aims to evaluate changes in synaptic activity in the CA1 horn and cerebellar cortex and assess neuro-behavioral changes in FST, food intake, and anhedonia to report NAC antidepressant effects.

Materials and Methods

Experimental animals

Thirty (30) Adult Male Wistar rats with an average weight of 250 g used for this study were obtained from the Animal house attached to the department of Anatomy Bingham University, Karu, Nasarawa State Nigeria. The animals were cared for according to guidelines for care and use of animals in research [23] and the study approved by the departmental research and ethics committee. The rats were housed in well-aerated metallic cages, allowed seven days acclimatization before experimentation, and fed with rat pelleted feed (Vital Feeds Limited Nyanya, Nasarawa State) with water available *ad libitum*. They were maintained in standard pathogen-free (SPF) laboratory condition maintained at 12 hr light/dark cycle, temperature 37 ± 2°C and 60 ± 5% relative humidity (lights on at 07:00 am). The behavioral procedures were carried out in specially equipped rooms in the animal facility between 08:00 a.m. and 12:00 p.m.

Experimental drug of study and dose:

N- Acetyl Cysteine

N- Acetyl Cysteine; NAC (Swanson) was purchased from H-Medix Abuja, Nigeria. NAC was administered at 200mg/kg per day orally [12]. The administration was done an hour before the FST procedure [24,25].

Fluoxetine:

Fluoxetine (Medibios Laboratories PVT Ltd, India) purchased from H-Medix, Abuja, Nigeria was used for this study. This drug was administered orally using an oral cannula at a dose of 20mg/kg [26]. It is important to mention that fluoxetine recommended oral dose in rats is between 5 – 20 mg/kg administration [27,28].

Experimental design and protocol

Control animals received the same total volumes of vehicles by oral gavage

- **Group A:** Control was given 1 ml of normal saline
- **Group B:** the FST Animal Model
- **Group C:** the 200mg/kg orally treated NAC group
- **Group D:** the 20mg/kg daily oral fluoxetine treatment.
- **Group E:** the 200mg/kg orally treated NAC treated FST Animal Model
- **Group F:** the 20mg/kg daily oral fluoxetine treated FST animal Model

Behavioral Studies

The experimental animals were handled in compliance with the "Methods and welfare considerations in behavioral research with animals" [29] and Guidelines for the care and use of mammals in neuroscience and behavioral research [23]. The following behavior test was done, FST, cylindrical test for limb use; sucrose and water intake as well as food intake.

The forced swim test (FST)

It is commonly used to screen for antidepressant drugs because it can induce physiological stress via forced swimming and it gives a strong predictive validity that can reduce immobility time in

short-term tests [30]. The rats were placed individually in a transparent cylindrical tank (50 cm diameter, 60 cm height) filled with water (35.2˚C ± 1°C). The water was changed between the test session. Each rat received the study drugs an hour before the FST [25]. A pretest of 15 minutes (for habituations), then a test for 5 min following drug treatment [30,31]. Swim sessions were video recorded from the side, and immobility, swimming, and climbing behaviors were scored. The session was timed using a stopwatch. The 5 min test was scored by a trained blind observer [33]. All procedures were conducted between 8:00 am-12:00 pm.

- **Sucrose and Water in-take test:** In this study, the rats were habituated to a 1% sucrose solution for 48 hours before the test day. Rats were deprived of water and food for 12 hrs before exposure to identical bottles containing 100ml each of water and sucrose solution for an hour [34, 35]. Sucrose and water consumption were determined by measuring the change in the volume of fluid consumed [36]. The means of three measurements were taken as; Sucrose and water I:- measured 24 hours be¬fore 15 minutes of FST pre-test; Sucrose and Water II were measured 24 hours after 15 minutes of FST pretest, and Sucrose and water III were taken one hour after 5 minutes FST and drug administration.

- **Food Intake test:** The rats were food and water-deprived 12 hr before the test. They were then exposed to food for an hour according to the Razmjou., *et al.* [34] method. NAC was given 1 h before FST then weighed feed (50g of rat fed) were given and volume of food consumed was recorded an hour after exposure.

- **Cylindrical test:** It is also referred to as the limb-use test [37]. It had been used in rodents to evaluate limb use and asymmetries during exploratory activity. The rodent is placed in a transparent cylinder, and the exploratory activity was video-recorded. The time spent upright with the forelimbs simultaneously or independently (left or right forelimb) on the wall of the cylinder was recorded (as seen in figure 1). Rats with increased time spent are reported to have a good motor function whereas rats with reduced time spent had a poor motor function. This could be taken as a reliable test for neuroprotection study of drugs that avert neurodegeneration relating to motor dysfunction [38].

Figure 1: Diagram showing cylindrical test method applied during this study.

Experimental animals' euthanasia and tissues collection for preservation

Twenty -four hours after the last drug administration, the final body weights were taken using a weighing balance (OHAUS Pioneer™, India). The animals were randomly euthanized, decapitated via cervical dislocation [25]. The whole brain was then removed, wet weight was taken using an analytical weighing balance (OHAUS Pioneer™, India). and fixed for 24 hrs in 4% paraformaldehyde for tissue processing [39]. Coronal sections of the cerebellum and CA1 horn of the hippocampus were cut according to guidelines in "the rat brain in stereotaxic coordinates" for tissue processing and immunohistochemistry (IHC) for synaptophysin (p38) protein activity [39].

Histological tissue processing

The tissues excised were processed using an automated tissue processor (*LEICA TP* 1050) according to Bancroft and Gamble, [39] method. The paraffin section was cut sectioned using a Lecia rotary microtome set at 5 µm and stained with Synaptophysin (p38) immunohistochemistry.

Immunohistochemistry of synaptophysin a synaptic vesicle protein (p38)

Synaptophysin is a biomarker to detect axonal damage and synaptosomes in different neuropathological conditions in brain tissues post-fixed in 4% PFA and paraffin-embedded rat brain tissue and stained according to Sarnat., *et al*. [7] and Gudi., *et al*.

[40] methods. Using the following: primary antibody; monoclonal mouse anti-synaptophysin (MAB5258; Chemicon, Temecula, CA, USA and secondary antibody -biotinylated horse anti-mouse secondary antibody (Vector Labs, Burl-ingame, CA, USA), avidin-biotin complex linked to peroxidase (ABC Kit [Vectastain kit], Vector Laboratories, Burlingame, CA, USA) and 3,3′-diaminobenzidine (DAB) for chromogen development.

- **Tissue photomicrography:** Photomicrographs were taken using an Olympus (Tokyo, Japan) binocular Light microscope which was connected to a 5.0-megapixel Amscope camera (Amscope Inc., Irvine, CA, USA) with an objective lens of 10x. The images were captured at a magnification of X40 objective lenses and the phototube of the images was captured and stored using the joint photographic expert group (JPEG) format for analysis.

- **Statistical analysis:** Statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, Inc., LA Jolla, CA). Student's *t*-tests were used for all pairwise comparisons and one-way ANOVAs were used for all multiple comparisons followed by the *post hoc* Tukey test. For all analyses, differences were considered significant when *p*-values were lower than 0.05 and significant effects are indicated by asterisks (*$p < 0.05$). Data were expressed as mean ± standard deviation (SD).

Results

Body Weights of Experimental Animals

The initial body weights of experimental animals used were not proportionate as shown in figure 2. There was no significant change between initial and final body weights of control, FST, NAC treated and NAC treated FST group at $p < 0.05$. Fluoxetine (D) treated and fluoxetine treated FST induced depression (F) groups had a significant increase in final body weight as compared with its initial weight at $p < 0.05$. The final weight of FST antidepressant treatment groups showed that fluoxetine increased final body weight as compared with NAC treated FST (E) groups at $p < 0.05$.

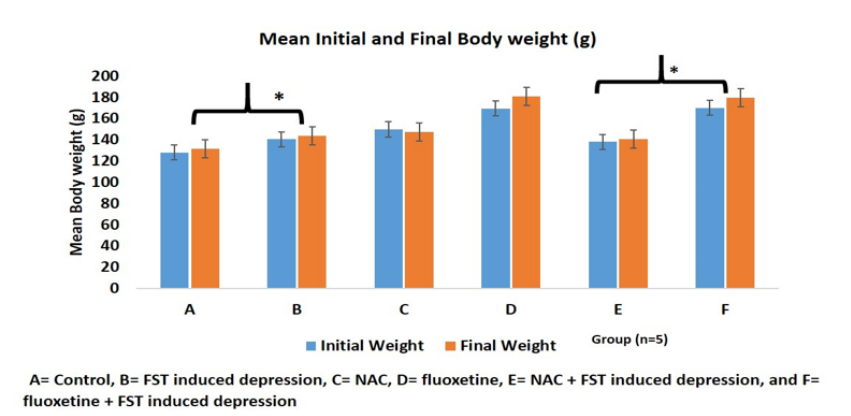


Figure 2: Graphical Representation of mean body weights of experimental Wistar rats used in this study. Data Analysed using one way ANOVA and expressed as Mean ± Standard Deviation (Mean ± SD). Statistical Significance is taken at $P < 0.05$ (*) using Tukey Post hoc test. Legend: A = Control, B = FST induced depression, C = NAC, D = fluoxetine, E = NAC + FST induced depression, and F = fluoxetine + FST induced depression.

Behavioral changes during FST behavioral despair test

Swimming time decreased significantly in the FST group when compared with NAC and Fluoxetine treated FST groups at $p < 0.05$ (*). NAC treated FST model had a significant increase in swimming time (*) as compared with Fluoxetine treated FST group at $p < 0.05$ using Tukey post hoc test (Figure 3). The immobility time for FST increased significantly as compared with NAC and Fluoxetine treated groups. There was no statistically significant difference in immobility time of NAC treated (E) and Fluoxetine (F) groups at $p < 0.05$ using Tukey post hoc test. The mobility time in FST model (B) decreased as compared with NAC treated FST model (E) and Fluoxetine (F) treated FST group at $p < 0.05$ using Tukey post hoc test. But NAC treated FST model (E) had a statistically significant increase (b) in mobility time at $p < 0.05$ using Tukey post hoc test as compared with Fluoxetine (F) treated FST group. Time spent climbing time increased in Fluoxetine (F) treated FST group as compared with NAC treated FST model (E) group at $p < 0.05$ Tukey post-hoc test. However, FST model (B) had a reduction in climbing time as compared to NAC treated FST model (E) and Fluoxetine (F) treated FST group at $p < 0.05$ using Tukey post hoc test.

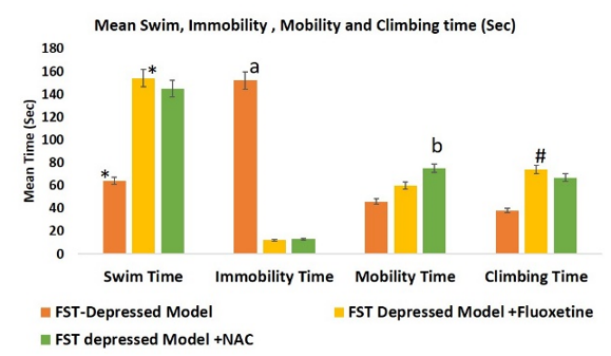


Figure 3: Graphical Representation of mean Swimming, immobility, mobility and climbing time of experimental Wistar rats used in this study. Data Analysed using one way ANOVA and expressed as Mean ± Standard Deviation (Mean ± SD). Statistical Significance is taken at $P < 0.05$ (*) using Tukey post hoc test for multiple comparison. Legend: A = Control, B = FST induced depression, C = NAC, D = fluoxetine, E = NAC + FST induced depression, and F = fluoxetine + FST induced depression.

Cylindrical test for limb motor function

The cylindrical test is used to assess locomotion or motor function by recording time spent standing or upright leaning on the wall of the cylinder displayed in figure 4 in this study. The control group (A) had a significant increase in time spent as compared to the FST induced rats (B) at $p < 0.05$. The NAC treated (C) had an increase in time spent as compared with the fluoxetine (D) group at $p < 0.05$ using Tukey post hoc test of multiple comparisons. FST model (B) had a statistically significant decrease in time spent (motor function) as compared with the control (A), NAC treated (C), fluoxetine treated (D), as well as NAC (C) and fluoxetine (F), treated FST animal model at $p < 0.05$ using Tukey post hoc test. But NAC treated FST model (E) had a statistically significant increase in motor function as compared to Fluoxetine treated FST model (F) at $p < 0.05$

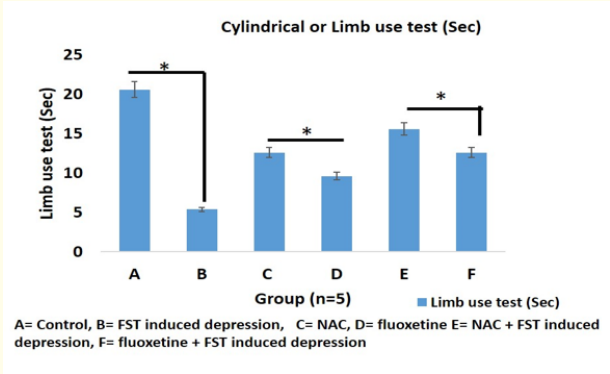


Figure 4: Graphical representation of mean cylindrical or limb use test of experimental Wistar rats used in this study to assess motor function. Data Analysed using one way ANOVA and expressed as Mean ± Standard Deviation (Mean ± SD). Statistical Significance is taken at $P < 0.05$ (*) using Tukey post hoc test for multiple comparison. Legend: A = Control, B = FST induced depression, C = NAC, D = fluoxetine, E = NAC + FST induced depression, and F = fluoxetine + FST induced depression.

NAC increased water and sucrose consumption

The FST model (B) had a significant decrease in sucrose II and water II consumption when compared with control at $p < 0.05$. Sucrose II consumption in fluoxetine (D) increased when compared with C, E, and F groups at $p < 0.05$. The antidepressant drug test session in Sucrose III showed that the control group had an in-

crease in sucrose consumption as compared to water at $p < 0.05$. Group B (FST model) had a significant decrease in water III and sucrose III consumption as compared with groups A, C, E, and F at $p < 0.05$. (Figure 5). Sucrose III consumption increased in NAC treated when compared with FST model (B) and fluoxetine treated (D) at $p < 0.05$. Water III increased in E and F when compared with B. However, there was no significant difference in water III consumption in NAC and fluoxetine treated FST model at $p < 0.05$ Tukey test.

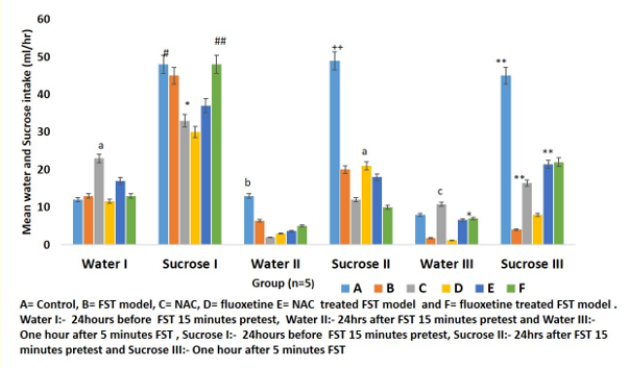


Figure 5: Graphical representation of mean water and sucrose intake (ml/hr) of experimental Wistar rats used in this study. Data Analysed using one way ANOVA and expressed as Mean ± Standard Deviation (Mean ± SD). Statistical Significance is taken at $P < 0.05$ (*) using Tukey post hoc test for multiple comparison. Legend: A = Control, B = FST induced depression, C = NAC, D = fluoxetine, E = NAC + FST induced depression, and F = fluoxetine + FST induced depression. Water and Sucrose, I: measured 24 hrs before FST pretest, water and sucrose II: measure 24 hrs after 15 minutes FST and Water and sucrose III: measured an hour after 5 minutes FST.

after 5 minutes FST shows that FST model (B) had a statistically significant decrease (**) in food consumed when compared with control (A), NAC treated (C), fluoxetine treated (D), NAC treated FST model (E) and fluoxetine treated FST model (F) at $p < 0.05$. But NAC treated FST model (E) and fluoxetine treated FST model (F) had a statistically significant (**) increase in food III consumed as compared with FST model (B) at $p < 0.05$ using Tukey test for multiple comparisons. However, there was no statistically significant difference between NAC treated FST model (E) and the fluoxetine treated FST model (F).

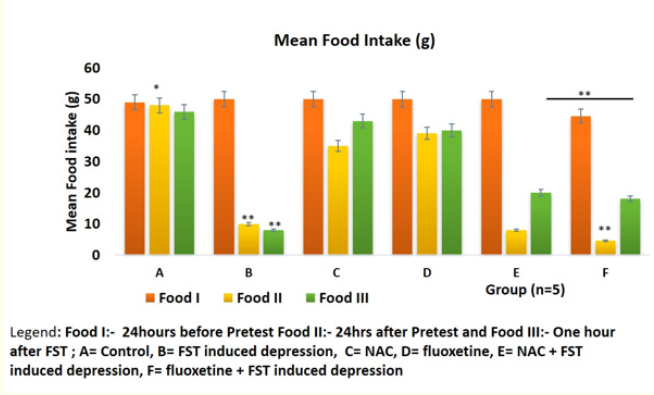


Figure 6: Graphical representation of mean food consumption (g) of experimental Wistar rats used in this study. Data Analysed using one way ANOVA and expressed as Mean ± Standard Deviation (Mean ± SD). Statistical Significance is taken at $P < 0.05$ (*) using Tukey post hoc test for multiple comparison. Legend: A = Control, B = FST induced depression, C = NAC, D = fluoxetine, E = NAC + FST induced depression, and F = fluoxetine + FST induced depression. Food I: measured 24 hrs before FST pretest, Food II: measure 24 hrs after 15 minutes FST and Food III: measured an hour after 5 minutes FST.

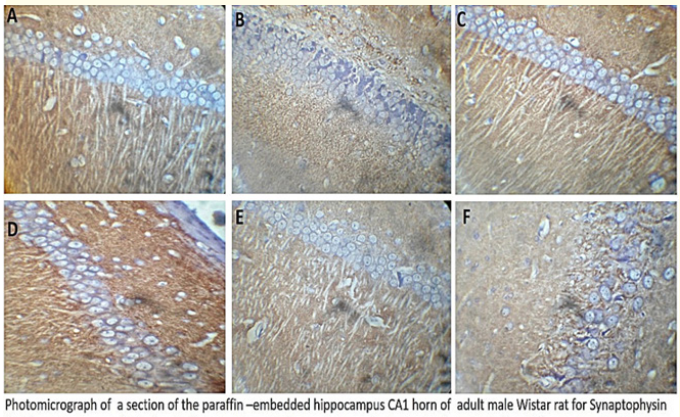
NAC increased food consumption

The control group (A) had a statistically significant increase in food II consumed when compared with groups B, C, D, E, and F at < 0.05 using Tukey test of multiple comparison FST model (B), NAC treated FST model (E) and fluoxetine treated FST model (F) had a significant reduction in food II consumed after 15 minutes FST as compared to the control group (A), NAC treated (C) and fluoxetine treated (D) at $p < 0.05$ (Figure 6). The food III consumed an hour

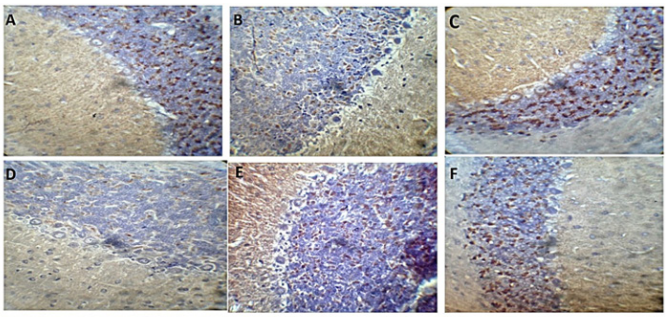
Immunohistochemical expression of synaptic protein synaptophysin

There was an accumulation of synaptophysin positive vesicles as seen in the brownish deposition within the neuropil of the gray matter of the hippocampal CA1 neurons (Figure 7A) and cerebellar cortex (Figure 7B) of the control group (A), NAC treated (C) and fluoxetine treated (D) rats. Synaptophysin immunoreactivity in the cerebellar cortex has uniformly intense reactivity in the cortices of

Groups A, C, D E, and F. The intensity of this pre-synaptic protein (p38) in the CA1 neurons was increased in groups A, C, D, E, and F, however, there was greater synaptophysin immunoreactivity in the CA1 sectors of Ammon horn of NAC and fluoxetine treated (C and D) as compared with B.



Photomicrograph of a section of the paraffin –embedded hippocampus CA1 horn of adult male Wistar rat for Synaptophysin (p38) protein immunohistochemical stain . Mag x 400 **Legend- Legend- : A= Control, B= FST induced depression, C= NAC, D= fluoxetine, E= NAC + FST induced depression, and F= fluoxetine + FST induced depression. Scale bar 100µm**



Photomicrograph of a section of the paraffin –embedded cerebellar cortex of adult male Wistar rat stained for Synaptophysin (p38) protein immunohistochemistry. Mag x 400 **Legend- Legend- : A= Control, B= FST induced depression, C= NAC, D= fluoxetine, E= NAC + FST induced depression, and F= fluoxetine + FST induced depression Scale bar 100µm**

Figure 7: Photomicrograph of a section of the formalin paraffin embedded prefrontal cortex (A), the hippocampal CA1 neurons (B) and cerebellar cortex (C) of adult male Wistar rats stained for synaptophysin (protein p38) immunohistochemistry immunohistochemical stain captured using objective lens of x40. Scale bar = 100µm. Legend: A = Control, B = FST induced depression, C = NAC, D = fluoxetine, E = NAC + FST induced depression, and F = fluoxetine + FST induced depression.

Discussion

The need to urgently develop or discover new and more effective therapeutic strategies for depression that target synaptic activity had undergone great research over the years. N-acetylcysteine (NAC) is a strong antioxidant and a glutamate modulator [13] that easily cross the blood-brain barrier [17] to improve brain synaptic activity [15].

In this study, the changes in the body due to depressive-like behavior affecting mood and appetite were studied. Fluoxetine caused an increase in final body weight when compared with initial body weight. The NAC treated groups had no significant change in initial and final body weights in this study. NAC had been reported to cause an insignificant change in the final body weight as compared to the control group [42] which supports this finding but contradicts the report made by Wright., *et al*. [10] that NAC causes a mild weight drop. FST model animal had no significant weight change, in this study, this could be linked to the short duration of this study. However, some studies have reported that FST mediated stress can cause loss of weight [43] while some reported a mild increase in body weight [43]. NAC has been reported to prevent stressed-induced body weight loss as compared to the stress model [44] which has a mild decline in body weight.

FST is used to create depressive-like behavior in the animal model used to screen drugs with antidepressant properties, based on assessing the immobility time of the animals [18]. In this study, the immobility time for the FST model increased significantly as compared with NAC and Fluoxetine treated FST groups. FST induced stress causes an increase in immobility time during FST in rats [18,22]. It has been reported that compounds with antidepressant potential could increase immobility, swimming, and/or climbing time during the FST [33]. In this study, NAC and Fluoxetine treated FST groups had a statistically significant reduction in immobility time. Fluoxetine has been reported to cause a significant increase in mobility time, decreases immobility [26], and increases swimming time which supports the report in this study.

In this study, the mobility time in the FST model decreased as compared with NAC and Fluoxetine treated FST groups. Swimming

time decreased significantly in the FST animal model when compared with NAC and Fluoxetine treated FST groups. NAC treated FST model had a significant increase in swimming time as com¬pared with Fluoxetine treated group. The effects of NAC on immo¬bility, swimming, and climbing time support Wright., *et al.* [10] report. The time spent climbing increased in the fluoxetine (F) treated FST group as compared with NAC treated FST model group, this finding support fluoxetine's reported ability to increase climbing time in FST [10] as compared with NAC treated FST model in this study. The antidepressant-like effect of NAC is dependent on glutamate transport [10], whereby NAC specifically increases glutamate in the synaptic cleft in a glutamate transporter-depen-dent manner [10].

This anhedonia status in the FST model corresponds to a reduction in sucrose consumption (ml/hr) as observed in this study. NAC and fluoxetine reversed this anhedonia status of FST model rats in this study which supports Eagle., *et al*. 's [46] report. The FST model (B) had a significant decrease in sucrose and water II consumption when compared with control and fluoxetine [12,32]. A reduction in sucrose and water consumption corresponds to a depressive-like symptom [32], but NAC and fluoxetine reversed the decrease in water and sucrose consumption in FST induced stress.

The volume of food consumed an hour after 5 minutes FST shows that the FST model had a statistically significant decrease in food consumed when compared with control, NAC treated, fluoxetine treated, NAC treated and fluoxetine treated FST models in this study and this correlates with Arent., *et al*. [47]. But NAC and fluoxetine treated FST model (F) had a statistically significant increase in food consumed as compared with the FST model. This NAC effect on reversing the loss of appetite in FST supports Wright., *et al*. [10], and Arent., *et al*. [47] and findings.

Locomotion or motor activity was evaluated using the limb use /cylindrical test, a reliable test for motor dysfunction in a drug test [37,38]. In this study, FST model rats had a reduction in time spent in an upright position in the cylindrical test as compared with the control, this implies a decrease in motor function [12, 36] because FST causes a decline in motor activity. NAC and fluoxetine treated FST model increased motor function. But NAC had a significant increase in motor activity when compared with the fluoxetine treated FST model. Fluoxetine can reverse behavioral deficits such as motor dysfunction, mood caused by FST [48]. N-acetyl cysteine reverses motor dysfunction in the FST animal model by attenuating neuroinflammation associated with neuron dysfunction [10,14] and increasing neuron repair and connectivity [15].

The activity of synaptophysin protein (p38) which plays a critical role in synaptic plasticity or synaptogenesis [7], in the cerebellar cortex and hippocampal CA1 was considered for evaluation.

The loss of synaptic connection is associated with the pathogenesis of depression [35], hence it is important in this *in vivo* study to evaluate the correlation between changes in synaptic activity and behavior to define the relation between synaptic connection, the pathophysiology of the depression, and the therapeutics of antidepressant drugs.

In this present study, the control has an accumulation of synaptophysin positive vesicles as seen in the brownish deposition within the neuropil of the gray matter of the hippocampal CA1 neurons and the cerebellar cortex. The NAC treated FST model has an even spread of synaptophysin immunoreactivity in the gray matter as compared with the FST animal model. This decline in synaptophysin expression in the cerebellum and hippocampal CA neuronal tissue indicates a loss of synaptic connection [6,7]. However, NAC treatment protects the neurons from neurodegeneration [41] and loss of synaptic connection [49], which leads to the restoration of lost synaptophysin immunoreactivity in FST induced neurodegeneration associated with FST mediated oxidative tissue damage. In this study we compared NAC activity with fluoxetine a standard antidepressant drug [48], our findings indicate that fluoxetine increases synaptophysin activity in CA1 of the hippocampus which implies that fluoxetine influences synaptic changes [48] in the hippocampus and increases hippocampal cell proliferation as reported by Hajsza., *et al.* [50] by inducing an increase in pyramidal neuron synaptogenesis in the hippocampus.

Conclusion

N-Acetyl cysteine demonstrates its potential anti-depressant role seen in increased body weight, sucrose, and food consumption, increased mobility, and reduced immobility time which is linked to its ability to mediate synaptic connections in the cerebellum-hippocampal cortical neurons demonstrated by a uniform homogenous spread of synaptophysin protein activity indicator for neuronal connection or synaptic connection.

Ethics of the Study

All the procedures of this study were performed following the Guidelines for Care and Use of Laboratory Animals and National Institutes of Health as stipulated in by the National Research Council, (2011).

Conflicts of Interest

No conflict of interest

Funds

None.

Authors Contribution

Conception and design, analysis and interpretation of the data Drafting of the article, Critical revision of the article for important intellectual content, Provision of study materials, and Collection and assembly of data: AEM.

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