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Activities of *Abelmoschus Esculentus* Seed Extract on the Structures and Hippocampus Associated Functions of Depressed Male Wistar Rat Induced by Chronic Stress

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ABSTRACT

Chronic stress if not manage properly results to major depression disorder which has profound impact on individual's life quality and deeply related to suicide death worldwide. This serious mental disorder is closely linked to the hippocampus due to it functionality in the regulation of cortisol production. *Abelmoschus Esculentus (A. Esculentus)* possess polyphenols, terpenoid and polysaccharides, indication the presence of anti-stress and antioxidant activities. This study examine if *A. Esculentus* can ameliorate stress-induced damages on the structure and hippocampus associated function, using fluoxetine treatment as a baseline. Forty-eight young male Wistar rats (80 ± 10 g) were divided into 6 groups (n= 8). Group-A received feed and water only, Group-B received 300 mg/kg of *A. Esculentus* and Group-C received 10 mg/kg of fluoxetine, for 14 days respectively, while Group-D were stressed for a period of 21 days, Group-E were stressed for 21 days followed with 300 mg/kg of *A. Esculentus* for 14 days and F were stressed for 21 days followed with 10 mg/kg of Fluoxetine for 14 days after. Chronic stress exposure (group-D) significantly depleted final body weight and increased the brain weight. It reduced lines crossed and middle squares crossed performance, while it increased the number of unaided rearing and immobility time performance significantly. GPx1 was significantly down-regulated and GSR obviously reduced. Further, it is noteworthy that chronic stress exposure caused histopathological changes in the hippocampus CA-1 area, presenting neurons with disorganized arrangement, apoptotic-like appearance, as well as shrunken and condensed cytoplasm. Glial fibrillary acidic protein (GFAP) staining demonstrated astrogliosis and KI-67 expression revealed high reduction in the degree of cell proliferation. Thus, treatment with *A. Esculentus improved hippocampal integrity by inhibiting the underlying mechanisms of stress induced damages in this study. Conclusively, o*ur findings put *A. Esculentus as a potential target for tackling stress-induced depression.*

Keywords: Anatomy, Neuroanatomy, Depression, Chronic stress, Hippocampus, *A. Esculentus,*

INTRODUCTION

Depression according to the World Health Organization (WHO) is a public mental diseases that presently affects over 300 Million people[1], having 1 in every 20 persons suffering from it with an increasing number of close to 800,000 suicide death yearly[2]. Both the disastrous constituents and the intellectual challenges of depression have deteriorated in the past decade. Nevertheless, depression is not just a form of extreme sadness, but it is a disorder that affects cognition, behavior, immune system and peripheral nervous system. [3,4]

The hippocampus is crucial in the formation of long term memories and spatial navigation; likewise it plays a pivotal role in emotional, adaptive, and reproductive behaviors.[5] Within the hippocampus, stress induced depression occurs because the hippocampus regulates the production of a hormone called cortisol.[6,7] The human body responds to stressful conditions during the time of depression both in physical and mental stress by activating the hypothalamic-pitutary axix (HPA) which then triggers the release of stress hormone known as cortisol, this cortisol provides the body with glucose by tapping into protein stores through gluconeogenesis.[8] This energy gotten can help an individual to fight stressor, but issues can occur when extreme volume of cortisol are been sent to the brain due to a stressful event and/or chemical imbalance in the body, leading to dysfunction of the stress system in the brain.[9] Increased cortisol levels can lead to hippocampal neurons shrinkage and inhibits neurogenesis thereby leading to hippocampal volume loss.[10,11]

Abelmoschus Esculentus (A. Esculentus) however is a flowering plant in the mallow family as well as an important vegetable having good dietary value with medicinal and industrial uses. [12,13] It has high percentage of phytochemical such as polyphenols, tannins, terpenoids, glycosides, isoquercitrin, Phenolic acids, flavonoids and quercetin-3-O-gentiobiose. The presence of polyphenols and polysaccharides indicates that *A. Esculentus* possess anti-stress activities.[13] likewise antioxidant agent with high therapeutic

efficacy due to high content of polyphenols and terpenoid.[12] Thus, this study examine if *A. Esculentus* can ameliorate stress-induced damages on the structure and hippocampus associated function, using fluoxetine treatment as a baseline.

MATERIALS AND METHODS

Substance procurement: Fresh *A. Esculentus* and Fluoxetine were procured from Babcock University farm house and Adel Pharmaceuticals, Lagos state, Nigeria respectively.

Substance extraction: Extraction procedure was done according to de Carvalho, et al., and Sami, et al., method.[14,15] In brief, *A. Esculentus* fruit were manually separated and the seeds were collected, sun-dried, and grounded to a powder form. The dried seed powder was soaked and thoroughly extracted using 1.7 liters of ethanol at room temperature (23 ± 1 ^{0}C) for 72 hours (3 days), following which the solution was filtered to remove residue while the filtrate was allowed to evaporate under mild sunlight until paste solution was obtain.

Rat care, management and ethical approval: Forty-eight (48) juvenile male Wistar rats (*Ratus norvegicus*) weighing between 80 ± 10 g were used in this experiment. The rats were gotten from the animal housing facility, Babcock University, Ilishan-remo, Ogun State. There were all bred, kept and cared for in the experimental animal laboratory, Babcock University according to the care and use of animals in research and teaching approved by the institute of laboratory Animal resources, National Research. The experimental animals were allowed to acclimatize for seven (7) days before the commencement of a pilot study. After the pilot study was carried out, research approval was gotten from the Babcock University Health Research Ethical Committee (BUHREC). The ethical clearance was given along with an assigned BUHREC no: 832/18, in accordance to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NRC, 2010).[16]

Experimental design and treatment duration: Rats were divided into 6 groups (n= 8), labeled A, B, C, D, E and F (see table 1). Group-A were given feed and water only, Group-B were given 300 mg/kg of *A. Esculentus* and Group-C were given 10 mg/kg of fluoxetine, both for 14 days respectively, while Group-D were stressed for a period of 21 days (see table 2). Group-E were stressed for 21 days followed with 300 mg/kg of *A. Esculentus* for 14 days and F were stressed for 21 days followed with 10 mg/kg of Fluoxetine for 14 days after. All substance administration was done orally using an oral cannula and activities involving the use, handling, treatment and management of rats were carried out in agreement with ethics and standard institutional research practices.

***Esculentus* treatment dose:** A stock solution of 1 g in 50 ml of distilled water was prepared, and rats were given 300 mg/kg from it.

Table 1: showing experimental design and treatment duration

Group (N=8)	Administration	Dosage (mg/kg)	Duration (Days)
A	Control	feed and water only	14
B	*A. Esculentus*	300 mg/kg	14
C	Fluoxetine	10 mg/kg	14
D	Chronic stress	CHS	21
E	Chronic stress + *A. Esculentus*	CHS + 300 mg/kg	35
F	Chronic Stress + Fluoxetine	CHS + 10 mg/kg	35

Table 2: showing weekly stress schedule for the 3 weeks duration

Day	Time	Activity
Monday	8 am – 8 pm	Water and Food deprivation
Tuesday	10 minutes in the morning, afternoon and evening	Constant shaking and sleep deprivation
Wednesday	24 hours	Food deprivation
Thursday	24 hours	Water deprivation
Friday	Week 1; for 5 minutes Week 2; for 10 minutes Week 3; for 20 minutes	Forced Swim Test
Saturday	7 pm – 7 am	Constant Illumination
Sunday	24 hours	Constant Darkness

Behavioural testing procedure: 24 hour after the end of the 14th and 35th day of treatment period, open field test (OFT) and forced swim test (FST) test[17] was conducted to examine rat activities across groups.

Sample collection and preparations: Rats were sacrificed through cervical dislocation method after behavioural test, and the thoracic cavity was dissected in order to have easy access to the rat heart. Blood was collected from the left ventricle of each rat's heart with syringes into heparinized bottles and immediately preserved in ice to prevent coagulation, which was used for hormonal analysis. Two animals from each group were perfused with 10% formol-saline and their brain were carefully excised, and fixed in 10% formalin for histological and immunohistological examination while the others animal in the same group were not perfused, rather their brain were placed in phosphate buffer, homogenized and used for relative gene expression; Glutathione peroxidase 1 (GPx-1) gene, *Glutathione* Reductase (GSR) gene, CAT and p53. The method used for conducting histological and immunohistological staining are with references to Omotoso et al., and slides prepared were viewed using a microscope connected to a computer with a digital camera.[18] All gene expression analysis in this study was done following manufacturer's protocol, similar to Omotuyi and colleagues.[19]

Statistical analysis: All data obtained from each group were presented and analyzed using one way analysis of variance (ANOVA) with GraphPad Prism 6.0 being the statistical software. Results were expressed as Mean±SEM in a tabular form and graph. Newman-Keuls post hoc test was used to compare the means thereby identifying differences with confidence interval at 95% such that in all cases a value of $p<0.05$ was considered significant.

RESULTS

Antioxidant activity of *A. Esculentus on weight changes and organ weight*: As noticed in figure 1A, there was no significant difference in rat initial body weight across all groups. During the course of this study (see figure 1B), the feeding pattern among rats exposed to chronic stress (group-D) reduced significantly when compared to control (group-A). However, a significant increase was seen in rats that received *A. Esculentus* as therapeutic treatment for chronic stress (group-E). Group-B, Group-C and Group-F also exhibited noticeable reduction which was not significant when compared to control. The weight of harvested organs (brain) was measured, which revealed a significant depletion and increase in group-C and group-D respectively when compared to control. Also, there is a noticeable depletion in the brain weights of groups-B, Group-E and Group-F when compared to group-A (control) as seen in figure 1C ($p \leq 0.05$).

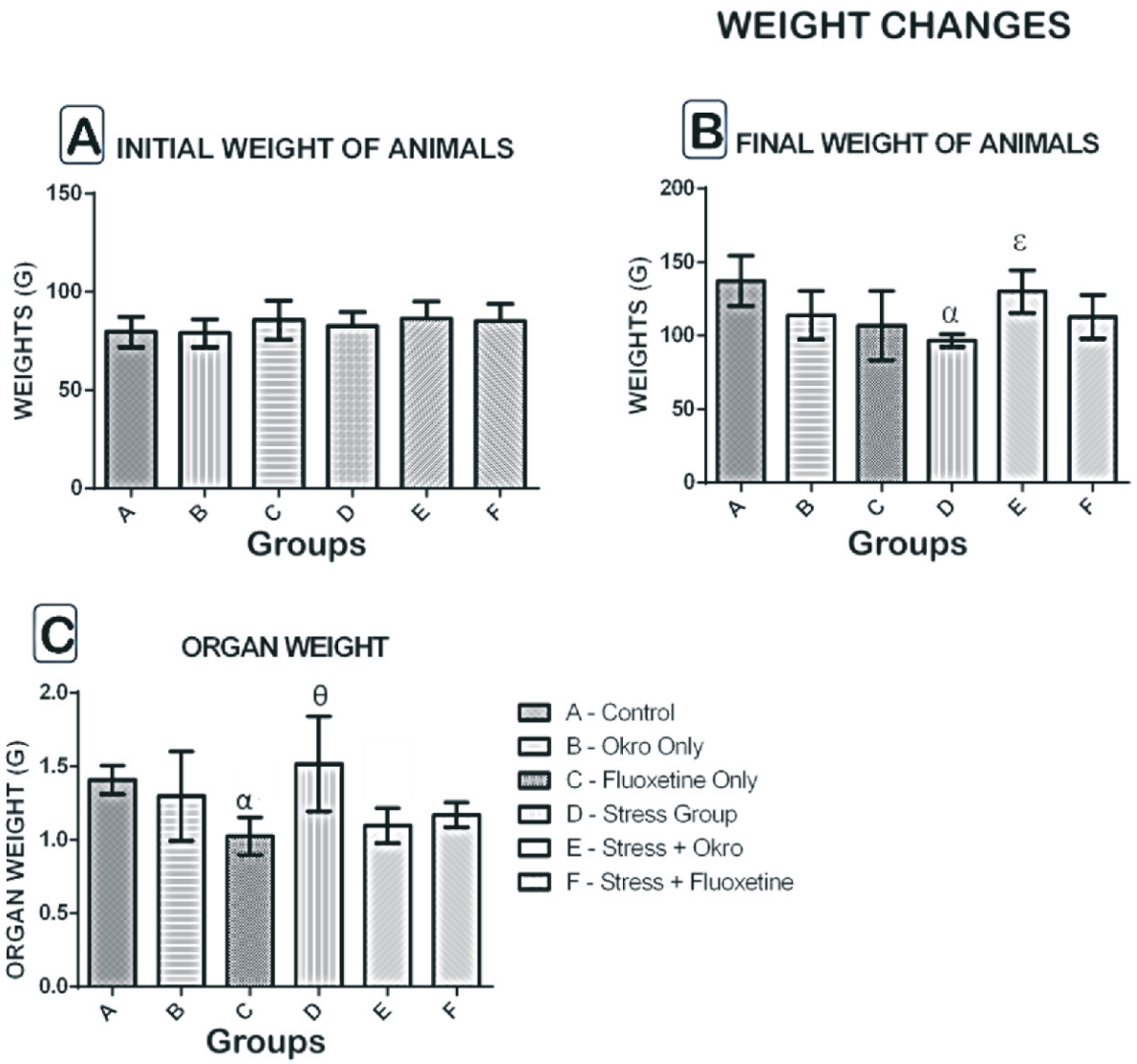


Figure 1: Bar chart showing (A) initial body weight; (B) final body weight; (C) organ weight of experimental animal. **α** = Indicates statistical significance when compared to group A; **θ** = Indicates statistical significance when compared to group C; **ε:** = Indicates statistical significance when compared to group D at $p < 0.05$.

Antioxidant activity of *A. Esculentus on* behavioural outcome: The number of lines crossed result from this study revealed an obvious decrease in group B and group C (54.34 ± 0.88 and 59.33 ± 3.48 respectively) when compares to group-A (85.33 ± 7.13). Group D (11.33 ± 1.202) was significantly lower when compared to group-A, group-B and group-C while group-E and group-F (31.67 ± 4.41 and 37.67 ± 3.38 respectively) was significantly lower when compared to group-A, and significantly higher when compared to group-D (see figure 2A). The number of unaided rearing (the occurrence with which the rat stands on their hind legs to explore) as seen in see figure 2B revealed a significant increase in group-D (8.33 ± 1.20) when compared to group-B (1.33 ± 0.33) and group-C (3.67 ± 0.33) and also obviously increased when compared to every other groups (group-A = 5.67 ± 0.67; group-E = 2.00 ± 0.58 and group-F = 3.00 ± 0.58). Also, middle square crossed performance in all groups when compared with group-A (8.00 ± 0.58) revealed a significant reduction. Group-B, group-C, group-E and group-F (3.00 ± 0.58, 2.00 ± 0.58, 2.33 ± 0.33 and 3.67 ± 0.88 respectively) were significantly higher when compared to group-D (0.00 ± 0.00). In furtherance, a significant increase in the immobility time of animals across groups when compared with group-A (68.00 ± 7.57) was seen. Group-D (198.7 ± 5.24) having the highest value was significantly higher than group-A and group-C (127.3 ± 3.71) while group-E and group-F (146.0 ± 4.58 and 143.0 ± 2.52) were significantly lower than group-D and group-B (165.7 ± 7.31) significantly increased when compare to group-A (see figure 2 D).

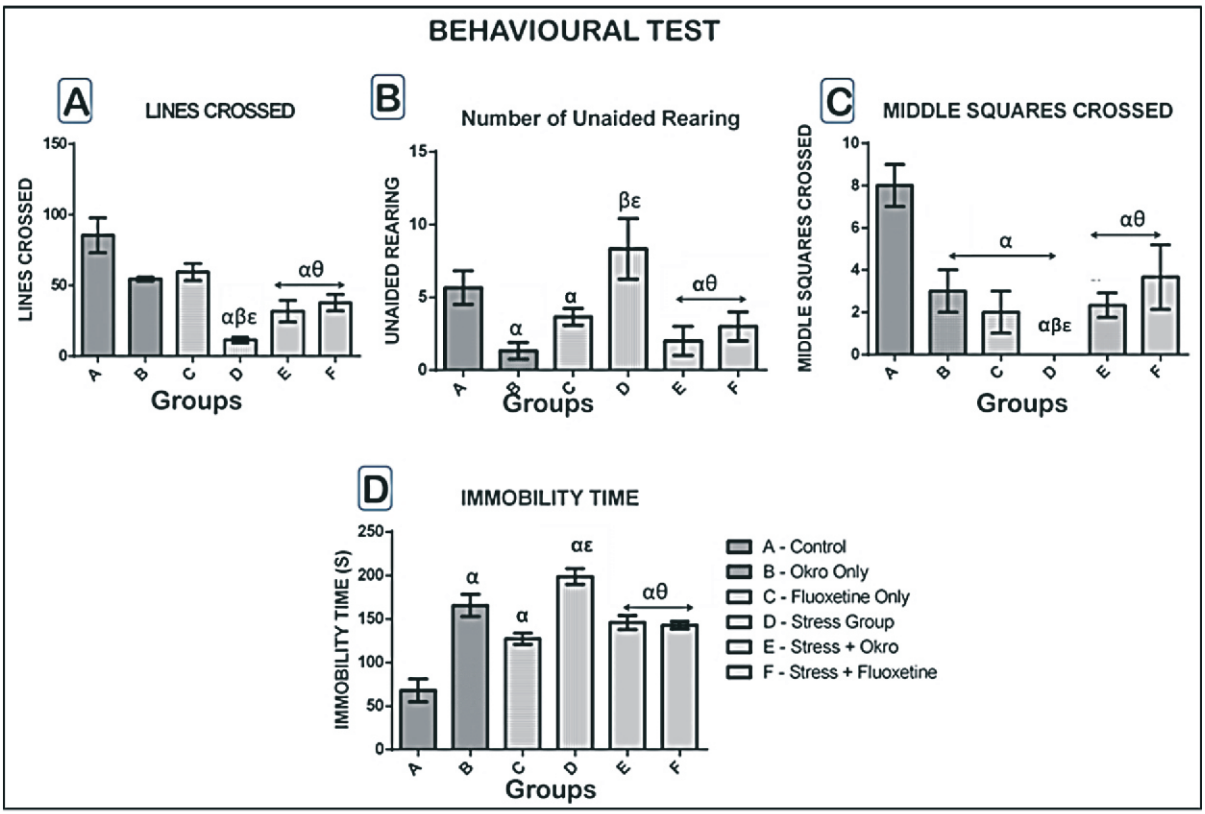
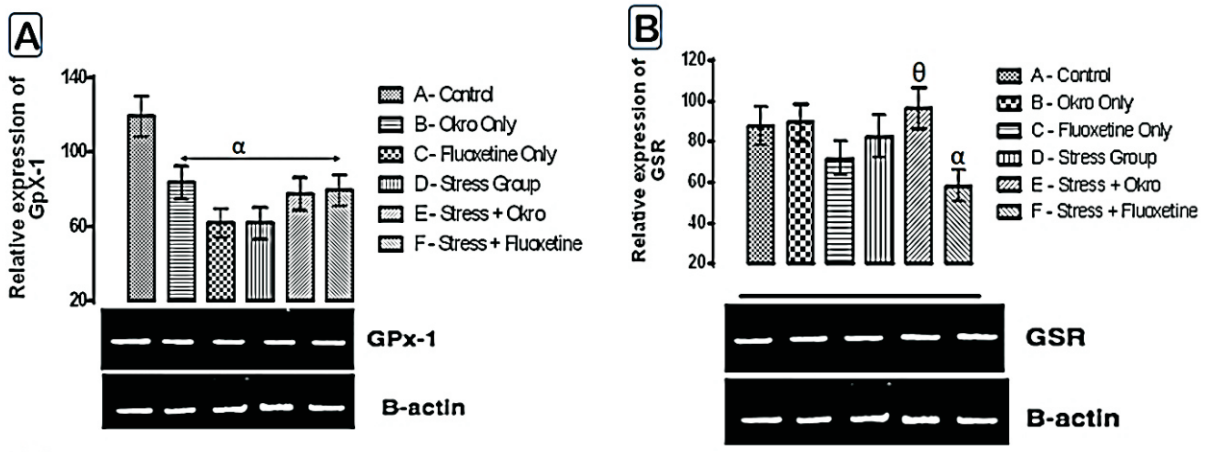


Figure 2: Bar chart showing (A) the number of lines crossed (B) unaided rearing (C) middle square entries in the open field test apparatus, and (D) immobility time in forced swim test apparatus. **α** = Indicates statistical significance when compared to group-A; **β** = Indicates statistical significance when compared to group-B; **ε** = Indicates statistical significance when compared to group-C; **θ** = Indicates statistical significance when compared to group-D at $p<0.05$.

Antioxidant activity of *A. Esculentus on* RNA gene expression: The relative expression of the **GPx-1** gene was significantly reduced across groups when compared to group-A: **α** = Indicates statistical significance when compared to group-A at $p<0.05$ (see figure 3A). In GSR gene expression: **α** = Indicates statistical significance when compared to group A, B, D and E; **θ** = Indicates statistical significance when compared to group-C at $p<0.05$ (see figure 3B).



3: Bar chart showing **(A)** the relative expressions of **GPx-1** gene **(B)** the relative expressions of *Glutathione* Reductase (GSR) gene isolated using mRNA from the hippocampus across various groups.

Antioxidant activity of *A. Esculentus* on the hippocampus histoarchitecture: This study provide the general histoarchitecture of the hippocampus stained with Haematoxylin and Eosin (H&E) in figure 4, showing the Dentate gyrus (DG), Cornus Ammonis-1 (CA-1), Cornus Ammonis-2 (CA-2) and Cornus Ammonis-3 (CA-3) at x40 magnification, of which this study further revealed the characteristic feature of CA-1 at x400 magnification (see figure 5). The control rat (group-A) showed normal neuronal cells (well-arranged) with distinctive cell layers, and stress exposure was shown to cause histopathological changes in the hippocampus CA-1 area in group-D, presenting neurons with disorganized arrangement, apoptotic-like appearance (red circle), as well as shrunken and condensed cytoplasm (red arrow). However, this feature were found to highly reduce in group-E and group-F, similar to control rat (group-A).

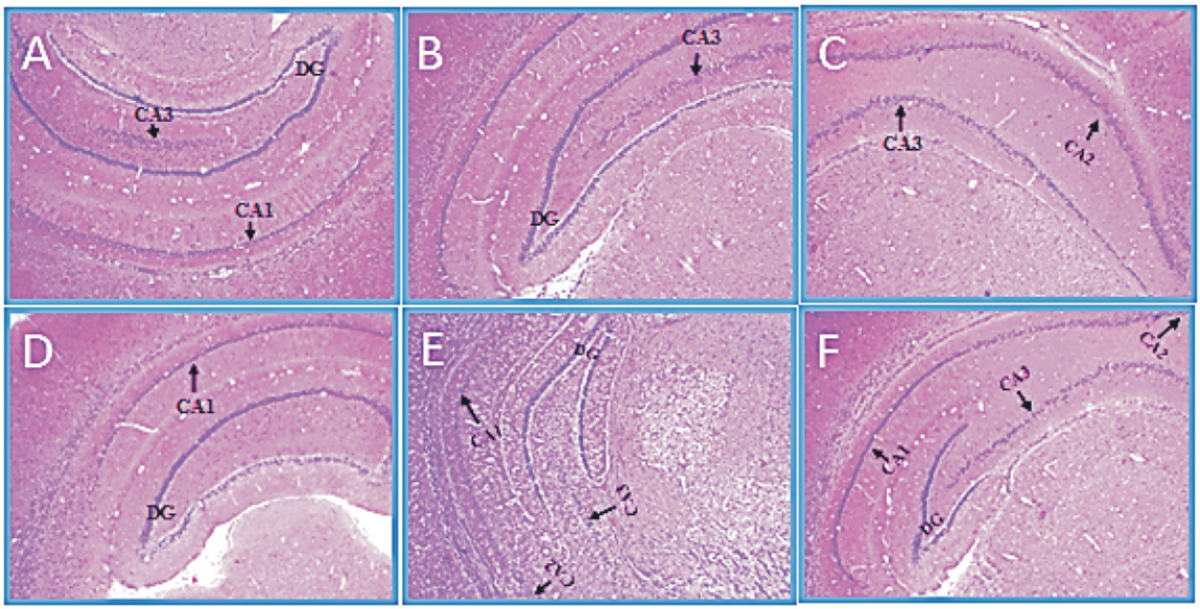


Figure 4: showing the general histoarchitecture of the hippocampus in Wistar rats (H&E) x40. (A) Control group; (B) ***A. Esculentus*** group; (C) Fluoxetine group; (D) Stress group; (E) Stress+*A. Esculentus group*; (F) Stress+Fluoxetine group. (CA1-3) Cornu Ammonis 1-3; (DG) Dentate gyrus.

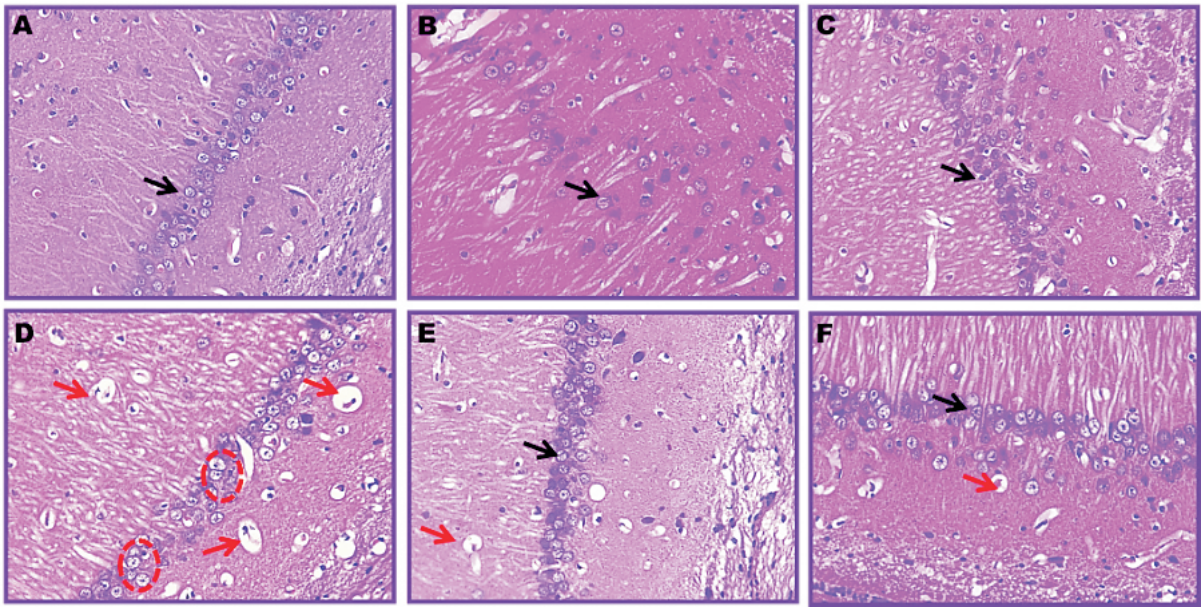


Figure 5: showing the hippocampal cornus ammonis 1 area (H&E) x400**, were** (A) Control group; (B) *A. Esculentus* group; (C) Fluoxetine group; (D) Stress group; (E) Stress+*A. Esculentus group*; (F) Stress+Fluoxetine group. Apoptotic-like appearance (red circle); shrunken and condensed cytoplasm (red arrow).

Immunohistochemistry finding: This procedure was done to examine the expression of glial fibrillary acidic protein (GFAP) as a strong marker for astrogliosis. Astrocytes expressions within the hippocampus of the group-A (control) appeared relatively normal, and similar finding was seen in other groups except in group-D rat, which exhibited high reactivity of GFAP when compared with others. In addition, this study revealed Ki-67 expression in the hippocampus. This protocol was carried out to demonstrate the degree of cell proliferation in the hippocampal cornu ammonis 1 (CA1) area. It was observed that, group-D rat significantly reduced in the degree of cell proliferation when compared with control and other groups.

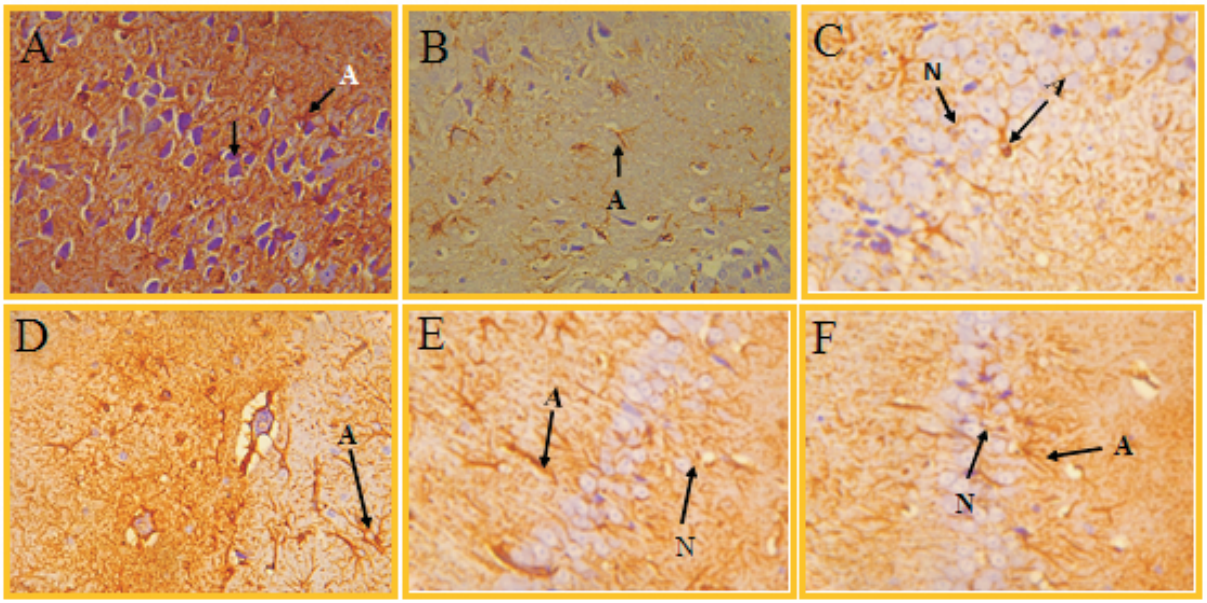


Figure 6: showing the expression of Glia Fibrillary Acidic Protein (GFAP) [x400]. (A) Control group; (B) *A. Esculentus* group; (C) Fluoxetine group; (D) Stress group; (E) Stress+*A. Esculentus group*; (F) Stress+Fluoxetine group. A- Astrocytes; – Neurons

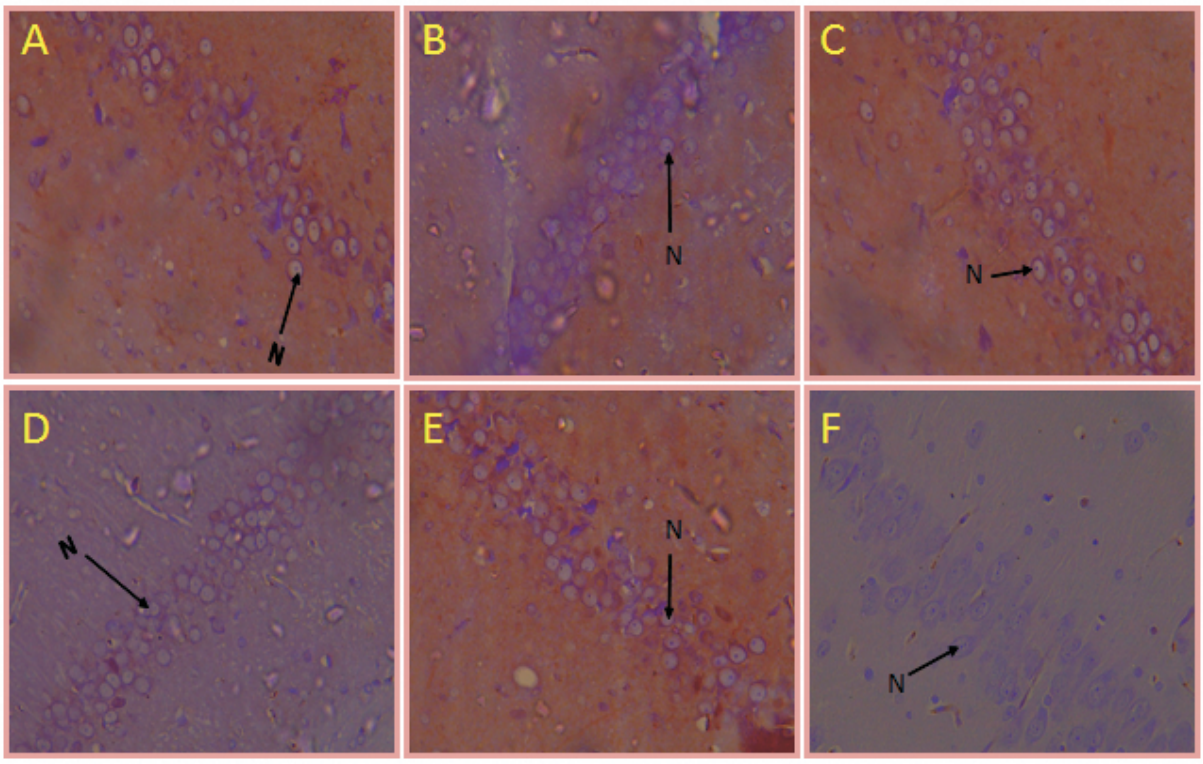


Figure 7: Ki-67 expression in the hippocampal Cornu Ammonis 1 (CA1) area [KI67 x400]. (A) Control group; (B) *A. Esculentus* group; (C) Fluoxetine group; (D) Stress group; (E) Stress+*A. Esculentus group*; (F) Stress+Fluoxetine group. – Neurons.

DISCUSSION

Chronic stress is a known contributing risk factor in developing psychiatric illnesses such as anxiety and depression.[20] Depression has been demonstrated to have clear relationship with neuronal and synaptic plasticity in different brain regions including the hippocampus.[20,21] Therefore, this study examined if *A. Esculentus* can ameliorate stress-induced damages on the structure and hippocampus associated function, owing to the search for antidepressant from natural plants product.

The results of this study shows that animals exposed to stress-induced depression resulted to significant decreased of body and increased brain weight which might be due to over exertion of the body high uptake of brain glucose and increased energy expenditure.[8] Smith & Wang, finding have provided evidence of decreased hippocampal volumes in depressed animal and human subjects which correlates to the result of our present study.[11] Treatment with *A. Esculentus* and flouxetine following stress-induced depression, significantly improve the body and brain weight (Fig. 1a-c). Fluoxetine is known to function by inhibiting the uptake of serotonin by nerve cells and it is prescription medicine used to treat major depressive disorder, bulimia nervosa (an eating disorder), obsessive-compulsive disorder, panic disorder, and premenstrual dysphoric disorder (PMDD).[22] The possible mechanism by which fluoxetine improved the body and brain weight in this present study might be due to its ability the stop serotonin reuptake, thereby inhibiting process that leads to over exertion, high uptake of brain glucose and excessive energy expenditure in stress-induced depression. *A. Esculentus* on the other hand contains phytochemical such as polyphenoids and flavonoids which possess antioxidant and anti-stress properties[12] that can inhibit stress-induced depression process and high polysaccharides content[13] that can make up for the high level of brain glucose uptake and increased energy expenditure caused by stress-induced depression. The above reason might be the underlying mechanism by which *A. Esculentus improved brain and body weight.*

The study of behavioral outcome in neurodegeneration in relation to behavioral sequel in therapeutic targets is a means and important measurement in assessing the efficacy of treatment.[18] Open field test and false swim test was used in this study to determine the exploratory and behavioral deficits among rats exposed to depression and possible therapeutic properties of *A. Esculentus* among the treatment groups. Higher exploratory and locomotive activities correlate lower anxiety and no depression in experimental animals.[23] Data from this study shows significant decreased of exploratory as well as locomotive activities of rats in the groups exposed to chronic stress as the number of line crossed, the middle square entry where significantly lower; while the immobility time and unaided rearing was higher significantly in relation to other experimental groups which is an indication of anxiety and depression. These results are in agreement with Patel et al., who reported increase immobility time among rats exposed to stress and subsequent depression.[24] *A. Esculentus* and fluoxetine intervention normalized the exploratory activities and prevent behavioral deficits due to stress-induced depression as the number of lines crossed, middle square entry, immobility time and unaided rearing where considerably similar to that of the control groups (Fig. 2a-d).

Normal metabolic processes often resulted to generation of free radicals; however there is a complex and effective balance that exists between these free radical generations and the antioxidant capability that mop up these free radicals.[18] When this balance or system is tempered with, it often results to massive production and retention of free radicals that leads to oxidative stress and subsequent cell death and axonal loss.[25] Mitochondria dysfunction and simultaneous oxidative stress has been linked by several authors as a key factors leading to neurodegeneration.[26] Chronic stress (sleep deprivation) a causative agent of depression has been linked as a risk factor for the development of several neurodegenerative diseases and mental diseases. Sleep has been hypothesized to possess antioxidant functions as free radicals generated during wakefulness are mopped up while sleeping, thus oxidative stress might be one of the underlying mechanisms of sleep deprivation in inducing its damages. Therefore, in this study gene expression of antioxidant enzymes (GPx and GSR) where investigated to shed light on the oxidative tendencies of chronic stress and mechanistic patterns of *A. Esculentus* in improving the antioxidative systems. Glutathione peroxidase (GPx) and glutathione reductase (GSR) closely related to the direct elimination of free radicals hence, they are considered fundanmental antioxidant enzymes.[27] While the former reduces lipid hydroperoxides to their corresponding alcohols and reduces free hydrogen peroxide to water the latter recycles oxidised glutathione back to the reduced form.[18,28] Thus, depletion of these enzymes results to a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide.[27]

In our present study chronic stress resulted to significant decrease in the gene expression of GPx and GSR in the hippocampal homogenate. This results confirms the findings of previous study that reported increase lipid perioxidation and oxidative stress marked by high level of malonyldialdehyde and decrease level of glutathione.[29] Sleep deprivation could results to increase metabolic rate leading to increase oxidative stress. Report of decrease memebrane fluidity in rat brain following sleep deprivation. Furthermore, deep destructive changes in the brain and erythrocyte mitochondria and decrease level of glutathione in the hippocampus of Wistar rats. Therefore, the mechanistic process by which sleep

deprivation induces depression is through the encouragement of free radical generation. Contraryly, interventional treatment with *A. Esculentus* caused a reversal to the deleterious effects induced as a result of sleep deprivation. Flavonoids exert a protective effect by directly scavenging ROS, by activating antioxidant enzymes or through metal chelating activity.[30] The ability of *A. Esculentus* to alleviate to the expression level of GPx and GSR genes can be attributed to the potential antioxidant properties of the flavonoids identify in *A. Esculentus* extract.

To further substantiate the therapeutic role of *A. Esculentus* against stress induced damages on the structure and hippocampus associated function, we studied the Histological changes of the hippocampus. Sleep affects the cognitive characteristics of every individual such as information encoding, stimulus detection, working memory, etc. while the deprivation of sleep has been shown to cause cognitive defects.[29] Sleep deprivation has been shown to cause destructive and instabilities in the structure and function of selective brain structures involved in cognitive processes.[31] Furthermore, a study carried using a potent noninvasive neurophysiological technique (TMS) specifically for evaluating primary motor cortex and corticospinal tract, reported significant alterations and variations in cortical excitability and functionality of primary motor cortex interneuronal circuitry which was identify in key characteristics of single and paired TMS techniques as a consequence of SD.[32,33] Thus aptly explain pathophysiological substrates of SD induced cognitive impairments. In this study, structural alterations where seen in CA1 regions of the hippocampus of rats exposed to sleep deprivation as neurons where characterized with disordered arrangement, apoptotic like appearance with shrunken and condensed cytoplasm which corroboratory to previous studies. D'Almeida et al demonstrated that thalamus and hypothalamus are more susceptible to free radical damage following sleep deprivation which might be probably due to their role in regulation of cortisol and low oxygen level. [6,7] Hippocampal neurons atrophy and decreased ERK1/2 MAP kinase activity, the hallmark of depression has been detected in post-mortem hippocampus of depressed person.[34] Furthermore, chronic stress has been shown to decrease hippocampal volume, expression of neurotrophic factors and total zinc level.[35,36] The aforementioned features of chronic stress can be regarded as the underlying mechanisms of SD in inducing its damages. Interestingly, *A. Esculentus* intervention following sleep deprivation restored the CA1 morphology which was closely similar to those of the control groups. Suggestively, the ability of *A. Esculentus* in restoring the CA1 morphology might be due to its rich content of phenols and flavonoids with antioxidant, anti-inflammatory and its ability to modulate and inhibits the release of cortisol in the hippocampus.

Glial fibrillary acidic protein (GFAP), a strong biomarker for astrogliosis demonstrated the expression astrocytes within the hippocampus across experimental groups. Normal astrocytic density, morphology and distribution was presented in the hippocampus of all experimental groups except for the stress-induced depression group, which exhibited high reactivity of GFAP when compared with others *(Fig. 4).* Studies have reported that social defeat-induced depression resulted to reduced neuronal activity in the medial PFC (mPFC) while astrocytic degeneration and glial loss induces symptoms and behavioral deficits associated to depression in mice and rats respectively.[37,38,39] Furthermore, hippocampal neuronal atrophy as well as neurochemical and structural changes has been documented among individuals that died has a results of depression[32] and these reports correlates with the findings of our current study. *A. Esculentus* and fluoxetine treatment was able to reverse the hippocampal changes caused by chronic stress-induced depression as hippocampal futures found in this group markedly similar to that of the control groups. The underlying mechanism of *A. Esculentus* and fluoxetine and efficacy presented in this study is suggestively due to their ability to inhibit free radical production leading to oxidative stress and serotonin re-uptake respectively.

The function of the hippocampus may partly rely on the generation, differentiation and integration of new cells in the dentate gyrus and these processes are altered by different harmful factors which includes chronic stress and glucocorticoids.[40] Chronic stress in the form of sleep deprivation has reported to inhibit hippocampal cell proliferation and neurogenesis leading impaired memory and learning.[41,42,43] Thus, in this study hippocampal cell proliferation was investigated using anti-KI67 immunostaining to investigate the proliferation patterns in the CA1 region exposed to chronic stress and *A. Esculentus* interventional treatments. Chronic stress exposure in this study resulted to reduced cellular proliferation in the hippocampal CA1 regions (Fig. 5). The results of this study agree with previous documentations that reported inhibition of cellular proliferation and hippocampal neurogenesis following exposure to chronic stress.[40,41] These inhibitory activities of chronic stress can due to increase activities neuronal toxins (such free radical, proinflammatory cytokines and glucocorticoids) and decreased ERK1/2 MAP kinase and BDNF activities as a results of stress.[40,41,44] In contrast, *A. Esculentus* interventional treatment improved cellular proliferation and subsequent neurogenesis in the CA1 hippocampal region after chronic stress exposure. The therapeutic activities of *A. Esculentus* in alleviating chronic stress induced effects might be due to its high antistress, antioxidant, anti-inflammatory and it ability to improve BDNF and decreased ERK1/2 MAP kinase activities.

CONCLUSION

our findings revealed behavioral, cellular and molecular alterations associated with stress-induced depressive damage on the hippocampus associated function; however, *A. Esculentus* intervention improved hippocampal functions. Despite the fact that *Esculentus* has shown alleviating properties when compared to fluoxetine, there might be need for a combine antidepressants *therapy in* managing chronic stress induced depression.

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Conflict of interest: None

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