

## Ameliorative effect of green coconut water on dexamethasone-induced depression-like behaviors in Sprague Dawley rats

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### Abstract

Depression is one of the major global neurodegenerative diseases. Despite the fact that a number of drugs and therapies have been described and used in its treatment, the incidence of prevalence has unexpectedly increased with time. There is a current drive to discover new treatment modalities or agents for depression to alleviate the negative effects it has on self and also on the society. Numerous factors, including psychological stress, oxidative stress, monoamine deficiency, and neuroinflammation have been linked to depression and consequently cause hyperactivation of the HPA-axis. Dexamethasone (DEX) is well known to induce depression-like behaviors in both experimental and human subjects. DEX has been shown to cause a reduction in neurogenesis and an increase in immobility time during forced swimming test (FST) and tail swimming test (TST). Green coconut water (GCW) is a natural product, rich source of antioxidant and has been found to provide remedy in oxidative stress and/or inflammatory-induced mediated diseases. Therefore, this study investigated the antidepressant potential of GCW in ameliorating depression-like behaviors caused by DEX administration in female Sprague Dawley rats. GCW exerts a substantial antidepressant effect as it was shown to increase hippocampal and prefrontal cortex tissue volume with reduction in neural degeneration when compared with the induced and recovery groups. GCW also showed complementary increase serum levels of monoamine neurotransmitters (Norepinephrine, Dopamine and Serotonin). There were evident increase in immobility time in the post treatment and recovery groups which were in contrast to the reduced immobility time in the induced group during FST and TST behavioral test evaluations. More importantly, GCW was shown to reduce malondialdehyde concentration and increased catalase, superoxide dismutase and glutathione peroxidase when compared with the induced group. Therefore, our study confirmed GCW as a potential natural therapeutic remedy with the capacity to eliminate the effect of oxidative stress induced by DEX administration leading to cascade of events underlining depression.

**Keywords:** Depression, Green coconut water, Dexamethasone, Oxidative stress, HPA-axis, Neuroinflammation, Monoamine neurotransmitters.

**Received** April 25, 2024; **Revised** June 26, 2024; **Accepted** July 3, 2024

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Publisher: *Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.*

## INTRODUCTION

Depression is a mental disorder characterized by the evidence of sadness, absence of pleasure and low self-esteem (Salik and Marwaha, 2022). About 21% of people worldwide suffer from this mood disorder, which is the most common and serious type of mental disease (Institute of Health Metrics and Evaluation, 2023). Approximately 280 million individuals worldwide suffer from depression, with women accounting for about 50% of cases (Institute of Health Metrics and Evaluation, 2023). Stressful life events, such as the death of a loved one, trauma, divorce, loneliness, and a lack of support can cause depression (Cui *et al.*, 2024). The pathophysiology of depression has been linked to several mechanisms through well-established pathways such as; neurotrophic factors, neuroendocrinology, neurochemical-induced monoamine breakdown, and neuroinflammation. (Tian *et al.*, 2022).

After multiple treatments, antidepressant medications currently have only been demonstrated to produce remission rates of roughly 56% (Fernandes *et al.*, 2024). Further complicating the management of this disorder is the fact that the majority of anti-depressant drugs now in the market have disturbing side effects such as; dry mouth, headaches, delayed onset of muscular action, and more (Niarchou *et al.*, 2024). The development of newer, more effective and tolerable agents is a pressing matter in neuropsychopharmacology. Green coconut water (GCW) is the water in an immature coconut fruit (Olayinka *et al.*, 2022). It is a natural, abundant source of minerals and electrolytes that help to increase energy level, essential for preserving the body's ideal fluid balance, and relieves a number of medical ailments (Olayinka *et al.*, 2022). GCW has high concentrations of phenolic chemicals, which are known to exhibit antioxidant property that can lower inflammation and prevent cells from oxidative stress. Hence several studies have found it more beneficial in enhancing metabolic activities and modulating biological processes in the body particularly protective effect on the brain by reducing inflammation and preventing cellular damage to brain cells (Tuyekar *et al.*, 2021). It is therefore important to investigate the antioxidant and anti-inflammatory effects of Green Coconut water on depression in the quest of discovering of a treatment agent.

## MATERIALS AND METHODS

### Animal model

#### Population, source and maintenance of experimental animals

In this study, 30 female Sprague Dawley rats weighing 90 – 160 g were used. This study utilized female animals because the indicators of depression are more expressed in female (Mohammadi, *et al.*, 2023). The rats were obtained from a breeding stock named Priceless Test Animals in Ilogbo Eremi Oko-Afo, Badagry, Lagos and authenticated by the Department of Zoology of the University of Lagos. The experimental animals were kept in a temperature-controlled environment ( $22 \pm 3$  °C) with a regular 12:12 h light: dark cycle and were fed with commercially available standard rat chow and had free access to clean water. The animals were allowed to acclimatize for two weeks under standard laboratory conditions before experimentation. Over the course of the research, the animals' weights were checked and they were frequently observed for signs of distress. The experimental protocols were performed in compliance with the Animal Ethics Committee's guidelines of the College of Medicine, University of Lagos, Nigeria in accordance with the standard Guidelines of Animal Care and Use for laboratory investigations with ethical approval number (CMUL/ACUREC/11/23/1296).

#### Induction of depression-like behaviors

Depression-like behavior was experimentally induced with the administration of dexamethasone (DEX) at 0.75 mg/kg daily for 21 consecutive days (Alhaddad *et al.*, 2023). The drug was manufactured by Jiangu Pengyao Pharmaceutical Co., LTD. 10, Chaquan Road, Yixing Jiangsu, China and purchased from VIXA Pharmacy, Mushin, Lagos. The dose was given orally by forceful feeding using an oropharyngeal cannula, and it was determined using a simple proportion based on the weekly weights of the experimental animals. Three weeks after the administration of DEX, depression-like behavior was induced and established by increased immobility time through tail suspension test (TST) and forced swimming test (FST). The animals that displayed increased immobility time in FST and TST were used to stage depression.

## **Plant material**

### **Source of GCW**

The immature coconut fruits were harvested from coconut tree grown in Adejo farms, Badagry, Lagos, Nigeria. The average weight of the fruit was 500 g. The fruit was authenticated at the Department of Botany, University of Lagos with plant's ascension no LUH: 10127 by Dr. George Nodza.

### **Extraction and oral dose estimation of GCW**

The young coconut fruits were dehusked and cleaned with fresh water. The liquid endosperm of the coconut fruit was extracted through the germinal pores, then it was immediately transferred into an airtight bottle and kept at 4°C in the refrigerator. During the extraction process, care was taken to keep metal away from the coconut water and to keep particulates from getting into the water (Patel *et al.*, 2019). The phytochemical constituents of GCW includes flavonoids, tannins, phenols, anthocyanins, alkaloids and terpenoids (Anylam and Opara 2023). The administration of a dose level of 10ml/kg of body weight of GCW daily for 21 days based on dosage used in previous study on the anti-nociceptive, antioxidant and anti-inflammatory potentials of immature coconut water (Zulaikhah *et al.*, 2019).

### **Experimental design (animal distribution, events and durations)**

The animals were randomly divided into six experimental groups of five rats each. The animals in group 1, the control group received placebo orally for 21 days. The group 2 animals received DEX 0.75mg/kg daily for 21 days orally to induce depression. The animals in group 3 were post-treated with oral dose of GCW (10 ml/kg) for 21 days following experimental induction. Group 4 is the recovery group and animals were allowed to recover for additional 21 days from experimental induction of depression. The animals in group 5 were co-administered with DEX 0.75mg/kg and 10ml/kg GCW orally for 21 days. The animals in group 6, the positive control group received 10ml/kg GCW only orally for 21 days.

### **Forced swimming test (FST) and tail suspension test (TST)**

FST was performed as previously described by individually placing the animal in 2000 mL glass beakers with 10 cm of water, the animal was timed for 5 min to swim. The "duration of immobility" was evaluated and this referred to the time interval when mice were non-energetic and showed none of the escape-oriented behaviors like swimming, diving, jumping, rearing, or sniffing. The immobility period was recorded during the last 4 minutes (Rodrigues *et al.*, 2023). The TST was conducted by allowing the animal to rest for 24 hours following FST. The rats were suspended from the edge of a shelf that was 58cm above a table-top by adhesive tape that was positioned about 1cm from the tips of their tails. They were permitted to hang for a total of 6 minutes and the duration of immobility was recorded during the last 4 minutes of the test duration. Immobility was established with rats that hung passively and motionlessly (Rodrigues *et al.*, 2023).

### **Blood collection and sacrifice of experimental animals**

Blood samples were taken between 7-8 a.m. from the orbital venous sinus using a microhematocrit tube placed into the lateral canthus. The samples were then collected in an EDTA-coated capillary tube and centrifuged immediately. The plasma was separated until the biochemical assays were performed. This was followed with animal sacrifice by cervical dislocation. The prefrontal cortex and hippocampus were dissected, snap-frozen and kept at -80 °C for subsequent histological processing.

### **Histological processing**

The tissue samples were first dehydrated in graded ethanol. This was followed with tissue embedding in paraffin wax. Sections of 5- 6µ thickness were cut and stained with Hematoxylin and Eosin (Isaac *et al.*, 2023).

### **Antioxidants markers**

#### **Determination of Catalase (CAT)**

The blood samples were homogenized in 2.5 ml of 0.15 M potassium chloride. The supernatant from the homogenate was collected and stored at 20°C. Hydrogen peroxide was prepared with phosphate buffer; 0.2ml of sample was added to 1.8ml of 30mM of hydrogen peroxide substrate in a 2ml

curvette. The phosphate buffer was used as a blank. The absorbance for the test sample, blank and standard was read against a blank at 240nm at 30seconds interval for 1minute (Asmat *et al.*, 2016).

#### **Determination of superoxide dismutase (SOD)**

SOD activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by (Wang *et al.*, 2018). The reaction mixture, 2.95ml sodium carbonate buffer pH 10.2, .02ml of homogenate and 0.03ml of epinephrine in cuvette and enzyme activity was calculated by measuring the change in absorbance at 480nm for 5minutes.

#### **Determination of glutathione (GSH)**

The reduced GSH content as non-protein sulphhydryls was estimated by adding to the homogenate 10% trichloroacetic acid and centrifuged. 1.0ml of supernatant was treated with 0.5ml of Ellman's reagent (19.8mg of 5, 5-dithiobisnitro benzoic acid in 100ml of .1% sodium nitrate and 3ml of phosphate buffer. The absorbance was read at 412nm (Wiecek *et al.*, 2018).

#### **Determination of malondialdehyde (MDA)**

The MDA level was determined as described by Liu *et al.*, (2023). A 2ml of 0.375% trichloroacetic acid, thiobarbituric acid and hydrochloric acid were added to 1.0ml of the homogenate. This was mixed vigorously and heated for 15 minutes in a water bath at 80-90°C. The sample was cooled in ice-cold water again for 15 minutes at 1500 g and the tubes were placed in the photometer and absorbance was taken at 535nm against the reagent blank. The concentration was calculated using the molar absorptivity of malondialdehyde.

#### **Hormone assays of norepinephrine, dopamine and serotonin**

Phosphate comparison of the fluorescence of each sample to buffer (1 ml, pH 7.0) was added to the aliquot with standard curve of solutions prepared. The amount of norepinephrine, dopamine and serotonin were then established by comparing the fluorescence of each sample to a standard curve of known contents (Poojary *et al.*, 2020).

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#### **Statistical analysis**

Behavioral tests were video-recorded and were visually analyzed using a video profiler. The study data were analyzed by Graph pad prism version 8 and they were expressed as mean  $\pm$  standard error of the mean (SEM). A one-way analysis of Variance (ANOVA) followed by Turkey's post hoc multiple- comparisons test was applied, and a p-value of < 0.05 was considered as statistically significant difference.

#### **RESULT**

##### **Effects of GCW on immobility time during FST and TST in DEX- treated rats**

The result revealed significant increase in immobility time during FSH and TST among the rats treated with DEXA only (group B) when compared to the control (group A) (Figure 1). There was decrease in immobility time during FST and TST among the rats that received combined administration of DEX and GCW (Group E) when compared with the control (group A) and GCW only group (group F) (Figure 1). Furthermore, the result also revealed that there was a significant decrease in immobility time during FST and TST in the rats that were post-treated with GCW (group C) compared with rats treated with DEX only (group B) (Figure 1).

#### **Histological studies**

##### **Effects of GCW on the histology of the hippocampal tissue of DEX-treated rats**

The photomicrograph of the hippocampus of the control group (group A) showed normal infoldings of the dentate gyrus, *cornu ammonis* and subiculum. More so, the three layers of the dentate gyrus -polymorphic layer, granule and molecular cell layer appeared normal and no evidence of neuronal loss or abnormalities (Figure 2). The photomicrograph of the hippocampus of the animals treated with DEX only (group B) revealed significant reduction in hippocampal tissue volume characterized by pyknosis, atrophy, shrinkage and degeneration of pyramidal neurons (Figure 2). However, in the group post-treated with GCW (groups C) showed better histological expression with increase in hippocampal tissue volume and increase in the number of the pyramidal neurons when compared with rats treated with DEX only (group B) (Figure 2). The recovery group (group D)

also showed increase in hippocampal tissue volume and increase in the number of pyramidal neurons but the expressions were not as significant as the post-treatment group. The group that was treated with combined administration of DEX and GCW (Group E) showed reduction in the hippocampal tissue volume, pyramidal neurons atrophy and shrinkage (Figure 2). The rats that received GCW only (group F) also showed normal histologic sections of the brain hippocampus with well-expressed infoldings of the dentate gyrus, *cornu ammonis* and normal subiculum with no evidence of neuronal loss.

#### **Effects of GCW on the histology of the prefrontal cortex in DEX-treated rats**

The photomicrographic expression of the prefrontal cortex of the control group (group A) showed frontal cortices of superior frontal gyrus, medial frontal gyrus, part of the inferior frontal gyrus and part of superior frontal gyrus and other neuronal deposits. The background of the neuropil appears normal with no induced neurodegeneration or other abnormalities (Figure 3). The photomicrograph of the prefrontal cortex of the rats treated with DEX only (group B) revealed significant reduction in prefrontal cortex tissue volume characterized by nucleolemma invaginations which imply shrinkage of the prefrontal nuclei and/or nuclear lysis and vesicular open-face nuclei. There were numerous degenerating neurons characterized by alterations in their shapes, shrinkage and dark-stained neurons with eosinophilic cytoplasm (Figure 3). However, in the group post-treated with GCW (groups C) showed better prefrontal cortex histology with reversal in the reduction of the hippocampal volume by increase in size of the shrinking nuclei and cytoplasm when compared with rats treated with DEX only (group B) (Figure 3). The recovery group (group D) also showed increase in tissue volume of the prefrontal cortices and increased in the number of the pyramidal neurons but the expressions were not as significant as the post-treatment group. The group that was treated with combined administration of DEX and GCW (group E) showed reduced prefrontal cortex tissue volume (Figure 3). The rats that received GCW only (group F) also showed histologic sections of the frontal cortices of the superior frontal gyrus, medial frontal gyrus, part of the inferior frontal gyrus and large portion of the superior frontal gyrus with no induced neurodegeneration or other abnormalities.

#### **Effects of GCW on serum levels of CAT, SOD, GSH and MDA levels in DEX-treated rats**

The result revealed that rats treated with for the DEX only (group B) showed a significant decrease in CAT, SOD and GSH levels (0.88, 1.37 and 3.5) respectively and a significant increase in MDA level (29.04) when compared to the control group (group A) (1.67, 1.9, 3.47 and 10) respectively ( $P < 0.05$ ) (Figure 4). However, there was no change in GSH levels in these groups. The result revealed that there was an increase in the serum levels CAT, SOD and GSH levels (1.37, 1.9 and 3.54) respectively and a decreased serum level in MDA level (11.59) in the rats post-treated with GCW (groups C) when compared with rats treated with DEX only (0.88, 1.37, 3.5 and 29.04) respectively (group B) (Figure 4). Similarly, there was significant decrease in serum levels of CAT and SOD (1.0 and 1.58) respectively and an increased serum level of MDA (14.11) in the combined administration group treated with DEX and GCW (Group E) when compared with the control (group A) (1.67, 1.9 and 10) respectively and GCW only group (1.7, 1.91 and 10.55) respectively (group F) ( $P < 0.05$ ) (Figure 4). The rats that were treated with GCW only showed no significant difference in CAT, SOD, GSH and MDA levels when compared the control group (group A).

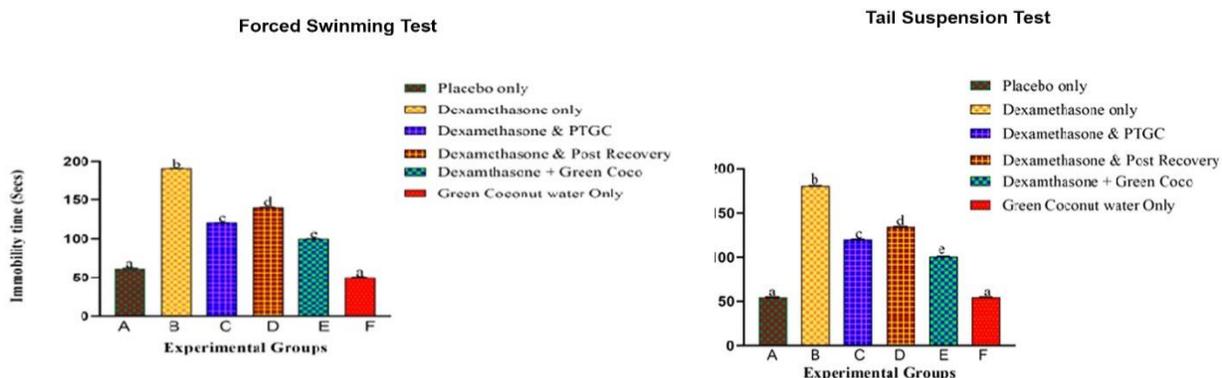
#### **Effects of GCW on serum levels of serotonin, norepinephrine and dopamine in DEX-treated rats.**

The result showed a significant decrease in serotonin, norepinephrine and dopamine serum levels in rats treated with DEX only (group B) when compared to the control group (group A) (Figure 5). There was significant increase observed in serotonin, norepinephrine and dopamine serum levels in the rats post-treated with GCW (group C) when compared with DEX only (group B) while the post-recovery group (group D) showed increase in serotonin, norepinephrine and dopamine serum levels that were not comparable with the post-treatment group ( $P < 0.05$ ) (Figure 5). The combined administration of DEX and GCW showed a significant reduction in serotonin, norepinephrine and dopamine serum levels when compared with the control (group A) and GCW only group (group F) (Figure 5). The group that received GCW only (Group F) showed a significant increase in serotonin, norepinephrine and dopamine serum levels comparable with the control group (group A) ( $P < 0.05$ ) (Figure 5).

### Effects of GCW on brain and total body weights in DEX-treated rats

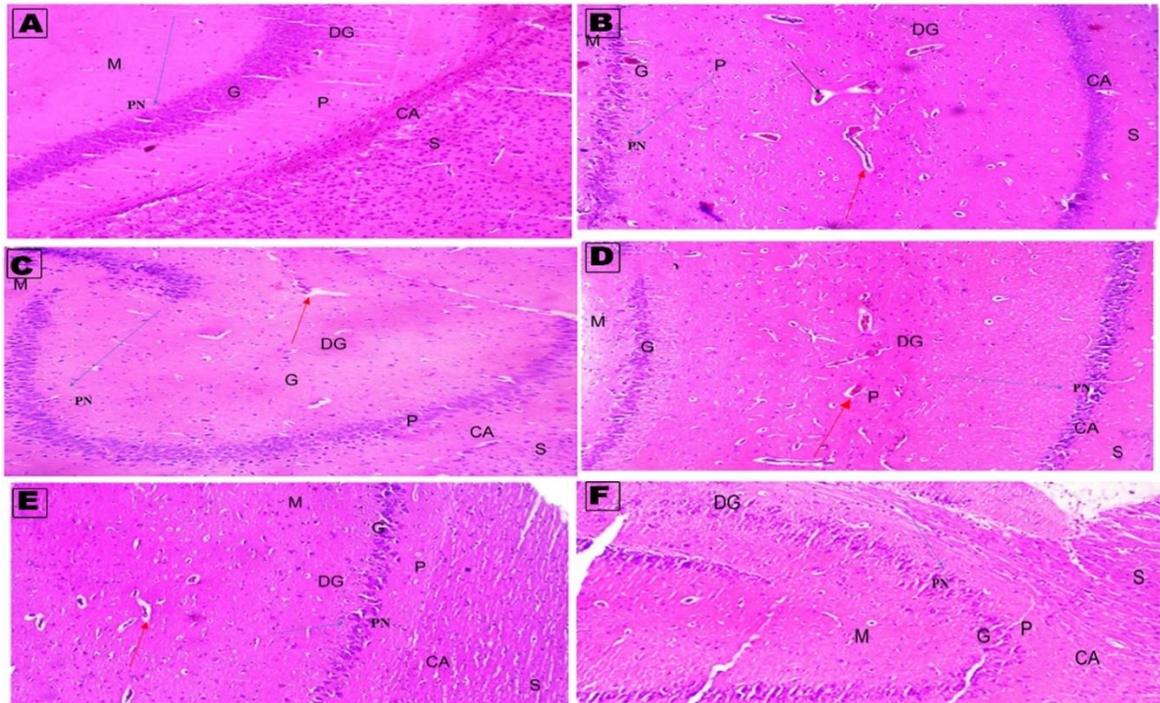
There was significant decrease in brain and total body weight in rats that were treated with DEX only (group B) when compared to the control group (group A). The group treated with combined administration of DEX and GCW showed significant decrease in brain and body weight when compared with the control (group A) and

GCW only group (group F) (Figure 6). Furthermore, there was significant increase in brain and body weight of the rats that were post-treated with GCW (groups C) when compared with rats treated with DEX only (group B) (Figure 6). There was no significant difference in brain and body weight of the rats that received GCW only (group F) when compared with the control (group A).

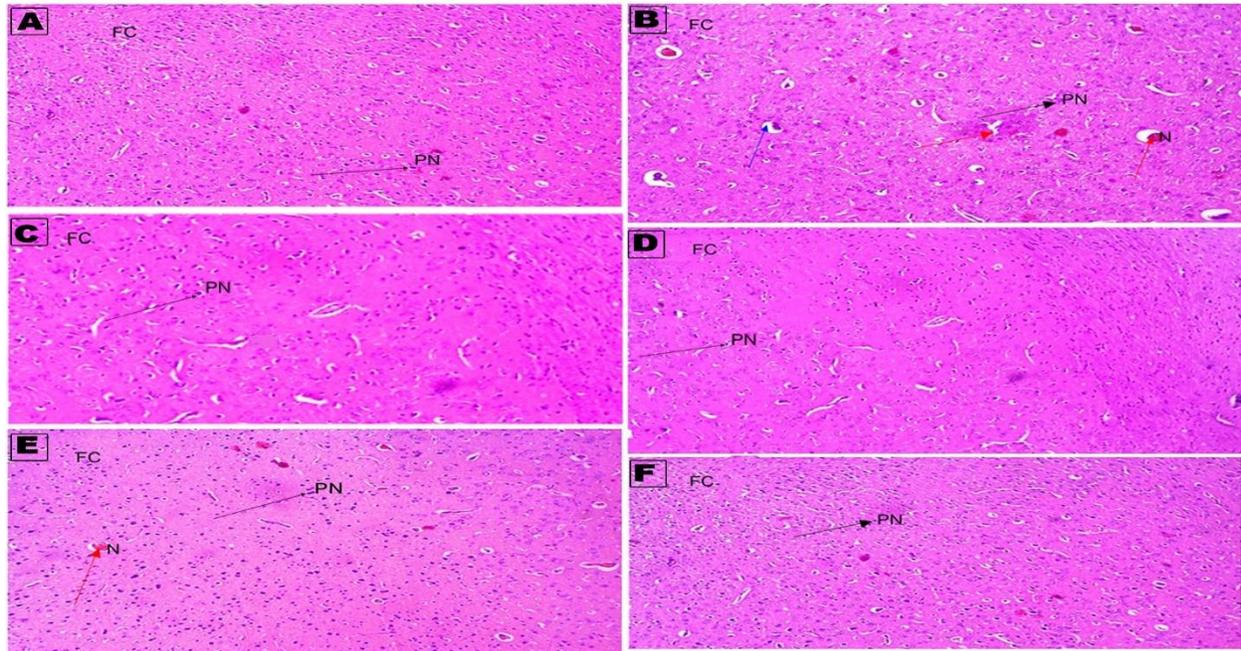


**FIGURE 1: The effects of GCW on FST and TST in DEX-treated rats**

**A:** Control (Placebo only), **B:** Dexamethasone 21 days (0.75mg/kgbw Dexamethasone for 21 days), **C:** Dexamethasone 21 days + GCW 21 days (0.75mg/kgbw Dexamethasone for 21 days then treated with 10ml/kg Green coconut water for 21 days), **D:** Dexamethasone 21 days + Recovery 21 days (0.75mg/kgbw Dexamethasone for 21 days then left for recovery for 21 days), **E:** (Dexamethasone & GCW)<sub>21 days</sub> (0.75mg/kgbw Dexamethasone for 21 and 10ml/kg Green coconut water for 21 days), **F:** Green Coconut water only 21 days (10ml/kg Green Coconut water for 21 days). Graphs represent mean values ± SEM. (<sup>b</sup> p < 0.05 vs (A, C, D), <sup>c,d</sup> p < 0.05 vs B, <sup>e</sup> p < 0.05 vs (A, F))

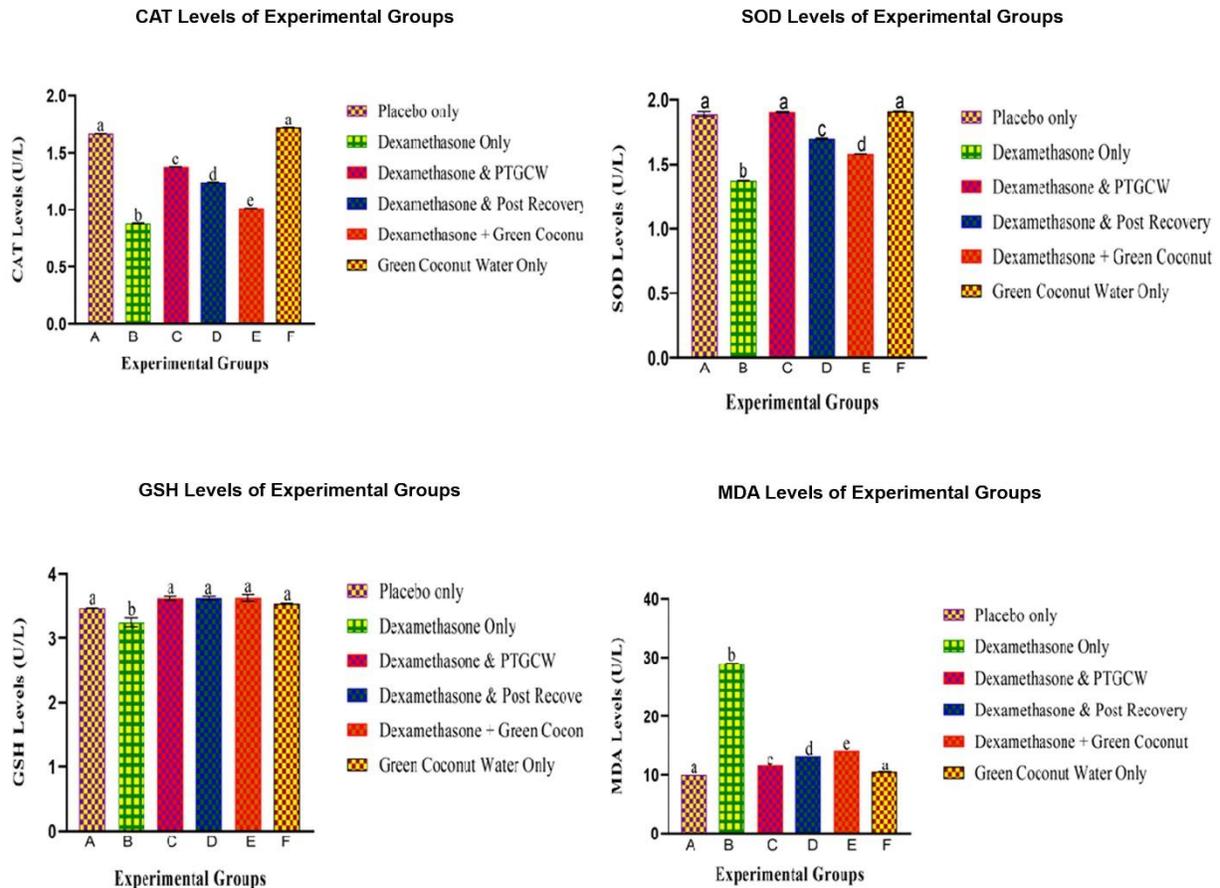


**FIGURE 2: The effects of GCW on the histology of hippocampal tissue of DEX-treated rats.** A. Normal Control rats showing the normal histological structure of pyramidal neurons No evidence of neuronal loss or abnormalities seen; B. Dexamethasone-induced rats showing pyknotic, atrophy, shrinkage and degeneration of pyramidal neurons (Reduced hippocampal Volume) (Blue arrow); C. Post treated rats with Green Coconut water showing reduced shrinkage and degeneration of pyramidal neurons. Little evidence of neuronal loss; D. Post Recovery rats showing reduced shrinkage and degeneration of pyramidal neurons. Little evidence of neuronal loss; E. Dexamethasone and Green Coconut water co treated rats showing sporadic necrosis of pyramidal neurons; F. Green Coconut only treated rats showing the normal histological structure of pyramidal neurons No evidence of neuronal loss or abnormalities seen (Blue arrow; pyramidal neuron, Red arrow; blood vessel, Neuron vacuolization; Black)(PN (Pyramidal Neuron), DG (Dentate Gyrus), M (Molecular layer of dentate gyrus), P (Polymorphic layer of dentate gyrus), G (Granule layer of dentate gyrus) CA (*Cornu Ammonis*), S(Subiculum) H & E stain. X100.

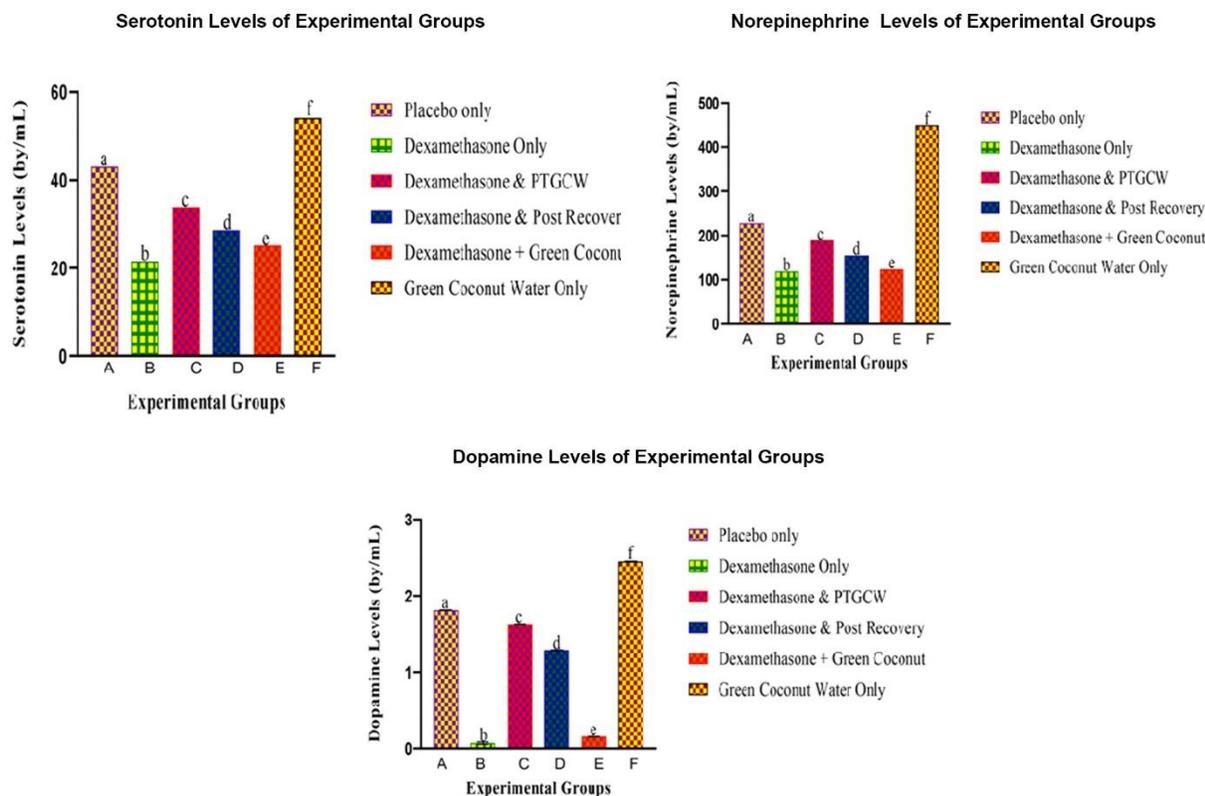


**FIGURE 3: The effects of GCW on the histology of prefrontal cortex of DEX-treated rats.**

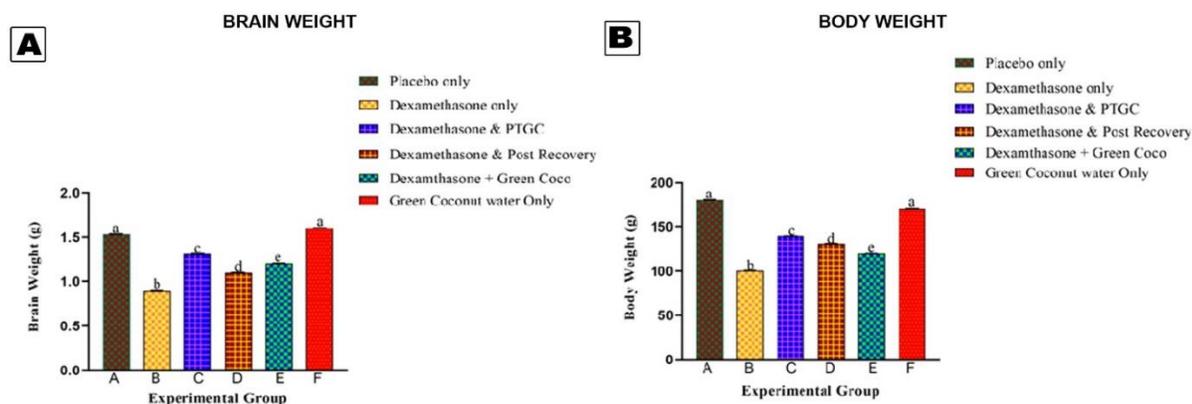
A. Normal Control rats showing the normal histological structure of prefrontal cortex. No evidence of neuronal loss or abnormalities seen and No frontal cortices induced neuro-degeneration or other abnormalities are seen; B. Dexamethasone induced rats showing frontal cortices induced nucleolemma invaginations implying the shrinkage of the nuclei and/or nuclear lysis, vesicular open face nuclei and numerous degenerated neurons characterized by alterations in their shape and staining, shrinkage, and dark-stained neurons eosinophilic cytoplasm with prominent Nissl's granules are also seen (Reduced Prefrontal cortex volume) (Black arrow); C. Post treated rats with Green Coconut water showing reduced shrinkage of the nuclei and cytoplasm in frontal cortices with little disappearance of the prominent Nissl granules ; D. Post Recovery rats showing reduced shrinkage of the nuclei and cytoplasm in frontal cortices with little disappearance of the prominent Nissl granules; E. Dexamethasone and Green Coconut water co treated rats showing little nuclei and cytoplasm shrinkage. Little reduced prefrontal volume observed: F. Green Coconut only treated rats showing the normal histological structure of prefrontal cortex neurons. No evidence of neuronal loss or abnormalities seen and no frontal cortices induced neuro-degeneration or other abnormalities are seen. (Black arrow; Pyramidal neuron, Blue arrow; vesicular open face nuclei, Red arrow; Nuclei vacuolization and Nissl granules). PN (Pyramidal Neuron), FC (Frontal Cortices) H & E stain. X100.



**FIGURE 4: The effects of GCW on oxidative stress markers in DEX-treated rats. A:** Control (Placebo only), **B:** Dexamethasone 21 days (0.75mg/kgbw Dexamethasone for 21 days), **C:** Dexamethasone 21 days + GCW 21 days (0.75mg/kgbw Dexamethasone for 21 days then treated with 10ml/kg Green coconut water for 21 days), **D:** Dexamethasone 21 days + Recovery 21 days (0.75mg/kgbw Dexamethasone for 21 days then left for recovery for 21 days), **E:** (Dexamethasone & GCW)21 days (0.75mg/kgbw Dexamethasone for 21 and 10ml/kg Green coconut water for 21 days), **F:** Green Coconut water only 21 days (10ml/kg Green Coconut water for 21 days). Graphs represent mean values ± SEM. (<sup>b</sup> p < 0.05 vs (A, C, D), <sup>c</sup> p < 0.05 vs B, <sup>e</sup> p < 0.05 vs (A, F)



**FIGURE 5: The effects of GCW on norepinephrine, dopamine and serotonin in DEX-treated rats.** A: Control (Placebo only), B: Dexamethasone 21 days (0.75mg/kgbw Dexamethasone for 21 days), C: Dexamethasone 21 days + GCW 21 days (0.75mg/kgbw Dexamethasone for 21 days then treated with 10ml/kg Green coconut water for 21 days), D: Dexamethasone 21 days + Recovery 21 days (0.75mg/kgbw Dexamethasone for 21 days then left for recovery for 21 days), E: (Dexamethasone & GCW)21 days (0.75mg/kgbw Dexamethasone for 21 and 10ml/kg Green coconut water for 21 days), F: Green Coconut water only 21 days (10ml/kg Green Coconut water for 21 days). Graphs represent mean values  $\pm$  SEM. (<sup>b</sup>  $p < 0.05$  vs (A, C, D), <sup>c,d</sup>  $p < 0.05$  vs B, <sup>e</sup>  $p < 0.05$  vs (A, F), <sup>f</sup>  $p < 0.05$  vs A)



**FIGURE 6: The effects of GCW on brain and total body weight in DEX-treated rats.** A: Control (Placebo only), B: Dexamethasone 21 days (0.75mg/kgbw Dexamethasone for 21 days), C: Dexamethasone 21 days + GCW 21 days (0.75mg/kgbw Dexamethasone for 21 days then treated with 10ml/kg Green coconut water for 21 days), D: Dexamethasone 21 days + Recovery 21 days (0.75mg/kgbw Dexamethasone for 21 days then left for recovery for 21 days), E: (Dexamethasone & GCW)21 days (0.75mg/kgbw Dexamethasone for 21 and 10ml/kg Green coconut water for 21 days), F: Green Coconut water only 21 days (10ml/kg Green Coconut water for 21 days). Graphs represent mean values  $\pm$  SEM. (<sup>b</sup>  $p < 0.05$  vs (A, C, D), <sup>c,d</sup>  $p < 0.05$  vs B, <sup>e</sup>  $p < 0.05$  vs (A, F))

## DISCUSSION

Clinical and experimental animal studies have indicated that changes with increase in the ratio of the free radicals to antioxidant levels in the body which is an indicator of oxidative stress can affect the functional pathway of the hypothalamus pituitary adrenal (HPA) axis. This can lead to the hyperactivation of the HPA-axis characterized by increased production of corticotropin releasing hormone (CRH). This invariably causes further stimulation of the adrenocorticotropin hormone (ACTH) leading to the overproduction of cortisol hormones particularly glucocorticoids. This increased glucocorticoid triggers series of events underlined in the pathogenesis of depression. As a result of global rise in the adverse effects of several pharmacological therapies employed in the management of neuropsychological disorders, therefore by virtue of fewer side effects, the use of natural medicinal products has gained momentum as a complementary and alternative therapy in the global health sector. This is due to its availability and affordability; natural products are regarded as a viable source of treatment. Additionally, statistical evidence has shown that some natural products and supplements have suppressive effects on depression-like behaviors. In a study by Anushiravani *et al.*, (2019), a comparison between a combined herbal drug (Echium Amoenum) with citalopram which is a selective serotonin reuptake inhibitor was carried out. The result in the study revealed that both were able to reduce depression with 52% of patients who received citalopram suffering from various complications while 12% of the patients who received combined Echium Amoenum and citalopram showed few complications. This result showed that herbal products have higher clinical efficacy in the treatment of depression (Anushiravani *et al.*, 2019). In recent studies, some medicinal plants have also been used in the management of depression. A study reported that a plant called hypericum perforatum was effective in long term prevention of recurrent depression. Hypericum perforatum also known as St. John's wort contains hypericin, hyperforin and flavoids which are responsible for its antidepressant activity (Nobakht *et al.*, 2022). Stojcheva and Quintela, (2022) also reported that a compound Salidroside in a particular plant called rhodiola rosea has antidepressant activity. Additionally, Lopez *et al.*, (2017) reported that depression in a high dose corticosterone-induced rats was significant reduced with essential oil from the lavender plant (Karabagias *et al.*, 2019).

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In this present study, the group post-treated with GCW demonstrated significant reversal of all parameters evaluated in depressed rats. It was substantiated by a decrease in immobility during FST and TST when compared to the induced group that was treated with DEX only. Increased concentrations of antioxidant enzyme (SOD, CAT and GSH) and reduced MDA concentration confirmed the antioxidant property of GCW in the management of oxidative stress induced by DEX administration. In the induced group, reduced concentrations of antioxidant enzyme (SOD, CAT and GSH) and increased MDA concentration was reported which indicates oxidative stress damage induced by DEX which may lead to the hyperactivation of HPA-axis (Trifunovic *et al.*, 2021). In previous study by Mori *et al.*, (2022) it was stated that DEX administration induces oxidative stress shown to trigger depression is in line with the result of this present study which reported a decrease in the level of antioxidant enzymes (SOD, CAT and GSH) and reduced MDA concentrations after DEX administration. Similarly, Nova *et al.*, (2020) investigated the effect of GCW on MDA and result showed that GCW lowered MDA level in diabetic rats.

Zulaikhah and Wahyuwibowo, (2020) also demonstrated that GCW prevented lipid peroxidation by lowering MDA levels and increasing antioxidant enzymes such as; CAT, SOD and GSH in lead-induced rats. The hyperactivation of HPA-axis causes events highlighted by the overproduction of glucocorticoids hormone by the adrenal gland (Hinda *et al.*, 2022). The prolonged exposure to excess glucocorticoids results into synaptic loss, neuronal death and changes in neuronal dendrites which may be responsible for the reduction in volume of the hippocampus and prefrontal cortex (Belleau *et al.*, 2019). In this study photomicrograph of the histological section of the induced group treated DEX showed pyramidal neuron degeneration and death with reduced volume of the hippocampus and prefrontal cortex which may be initiated by exposure to excess glucocorticoids.

Furthermore, a study by Nova *et al.*, (2020) reported the effects of GCW in the mitigation of oxidative stress by inhibiting the process of lipid peroxidation in pregnant diabetic models which further supports the antioxidant property of GCW reported in this study. In a previous investigation by Rao and Najam, (2016) GCW administration reduced immobility time during FST and TST

evaluations which supported the result in this study where the post-treated group with GCW following DEX administration showed reduced immobility time during FST and TST. It was established that DEX administration resulted in reduced body weight in mice and this is consistent with the present study which reported a reduction in body weight after DEX administration (Filippopoulou *et al.*, 2021). There were reduced levels of serotonin, norepinephrine and dopamine during depression state which correlates with the results of this study stating a reduction in levels of serotonin, norepinephrine and dopamine in the induced group treated with DEX only (Gbadamosi *et al.*, 2022). A previous study by Rao and Najam, (2016) established the ability of GCW in the amelioration of depression via homeostasis of monoamine synthesis, it can be explained by the non-specific inhibition of the MAO activity by GCW administration which prevent the enzymatic breakdown of monoamine resulting in increased levels monoamine is in accordance with this study result. It is evident that DEX administration induces the production of reactive oxygen species or free radicals (Liu *et al.*, 2018). This increased production of ROS causes the hyperactivation of the HPA-axis (Trifunovic *et al.*, 2021). It was investigated and reported in a study by Anylam and Opara, (2023) that GCW contains phenolic compounds (catechin, salicylic acid and epicatechin). A study also investigated and confirmed the presence of ascorbic acid and caffeic acid in GCW. These compounds have been reported to exhibit antioxidant properties (Shayanthavi *et al.*, 2024). Free radicals in body are scavenged by these phenolic chemicals, shielding them from oxidative damage. Therefore, hypothetically these phenolic compounds present in GCW may help to clear free radicals or reactive oxygen species produced as a result of DEX administration to stop the pathway of events leading to depression.

## CONCLUSION

Overall, this study substantiated the antidepressant effects of GCW in rat models. The results from the findings showed reversal in the oxidative stress parameters induced during DEX administration, increased levels of monoamine, reduced immobility time during FST and TST, increased hippocampal and prefrontal cortex tissue volume. Indeed, this current study provided evidence that GCW offers protective function on HPA-axis by eradicating free radical production as a result of its anti-oxidant property and this *Bio-Research Vol.21 No.1 pp.2369-2382 (2024)*

prevents cascade of events leading to depression. This gives further lead into further research work on GCW exhibiting anti-depressant function in this regard. More importantly, advanced investigations in this line of study at the molecular level will further unravel the basis behind these effects and finally provide the drive for the discovery of GCW as a potential therapeutic agent in the treatment of depression.

## Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

The department of Anatomy, College of Medicine, University of Lagos for providing the research space and facility for this study

## Author contribution

ETT was involved in conceptualization, project administration, data analysis, result interpretation and manuscript writing. ADB was involved conceptualization, project administration, data analysis, result interpretation and manuscript writing. SAM participated in conceptualization, data analysis and result interpretation.

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