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Research Article

Neuroprotective effects of *Theobroma cacao* against reserpine-induced depression in male Wistar rats

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Abstract

The current study investigates the potential antidepressant properties of *Theobroma cacao* ethanolic extract using a reserpine-induced depression model in male Wistar rats. The research aims to assess behavioral, neurochemical, and neuroanatomical parameters relevant to depression, with a focus on hippocampal structure and function. Before being randomly split into six groups—five rats apiece—thirty mature male Wistar rats were acclimatised for seven days. The treatment schedule included groups receiving fluoxetine (20 mg/kg/B.W), cocoa seed ethanolic extract (1000 mg/kg/B.W), or their combinations with reserpine, a negative control group treated with reserpine (0.5 mg/kg/B.W), a control group getting placebo water. While neurochemical tests gauged oxidative stress indicators (MDA and GSH) and dopamine levels, behavioural evaluations were performed utilising the open field test and forced swimming test. Hemotoxic and eosin and cresyl violet staining allowed histological assessments of the hippocampal tissue. Results showed that groups treated with fluoxetine and cocoa extract showed notably different body weights than control and reserpine groups. Consistent with depressed-like symptoms, neurobehavioral studies found reduced exploratory behaviour and increased immobility time in rats treated with reserpine. While oxidative stress indicators indicated a tendency towards lowered MDA levels, neurochemical studies revealed lowered dopamine levels in treatment groups. Histological study revealed changes in hippocampus cytoarchitecture, suggesting that therapies as well as reserpine affected hippocampal structure. In conclusion, *Theobroma cacao* ethanolic extract demonstrated potential antidepressant properties by mitigating depressive-like behaviors, reducing oxidative stress, and preserving hippocampal structure in reserpine-induced depressed male Wistar rats.

1. Introduction

Depression remains a significant global health issue, characterized by persistent sadness, cognitive impairments, and a lack of interest or pleasure, leading to substantial disability and a reduced quality of life [1]. According to the World Health Organization (WHO), approximately 264 million people worldwide are affected by depression, underscoring the urgent need for innovative therapeutic approaches [2]. Despite advancements in pharmacological and psychotherapeutic treatments, many individuals fail to achieve full remission or experience undesirable side effects, highlighting the importance of exploring alternative treatment strategies [3].

In recent years, growing attention has been directed toward the therapeutic potential of plant-derived natural compounds, particularly in the management of mental health disorders [4-6]. Among these, *Theobroma cacao*, commonly known as cocoa, has emerged as a promising candidate due to its rich phytochemical composition and neuroprotective effects [7]. Cocoa contains bioactive components such as flavonoids, alkaloids, and polyphenols, which have demonstrated antioxidant, anti-inflammatory, and neurotrophic properties in preclinical studies [8]. These properties position cocoa as a potential agent for modulating the neurological pathways implicated in depression.

Central to the pathophysiology of depression is the hippocampus, a critical region within the limbic system involved in memory, learning, and emotional regulation [9]. Impairments in hippocampal neurogenesis, synaptic plasticity, and neurotransmitter systems have been linked to the onset and progression of depression. Therefore, interventions targeting hippocampal structure and function hold significant promise for addressing the underlying mechanisms of depression and improving therapeutic outcomes [10].

The reserpine-induced depression model, a well-established preclinical tool, simulates depressive-like states by irreversibly inhibiting the vesicular monoamine transporter, leading to catecholamine depletion and reduced levels of dopamine, norepinephrine, and serotonin [11]. Animals treated with reserpine exhibit behavioral traits analogous to human depression, including anhedonia, social withdrawal, and helplessness, making this model invaluable for assessing novel antidepressant therapies [12].

Building on this foundation, the current study investigates the potential antidepressant properties of *Theobroma cacao* ethanolic extract using a reserpine-induced depression paradigm in male Wistar rats. By focusing on hippocampal structure and function, this research evaluates the effects of cocoa extract on behavioral, neurochemical, and neuroanatomical parameters relevant to depression. Addressing gaps in the understanding of cocoa's mechanisms of action, this work aims to contribute to the development of innovative therapeutic strategies for depression and related mood disorders.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Fluoxetine, reserpine, ethanol (70% alcohol), sucrose, phosphate buffer saline (PBS), 5-sulfosalicylic acid solution, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), glutathione reductase, NADPH, cresyl violet acetate, thiobarbituric acid (TBA), and other standard laboratory chemicals.

2.1.2. Equipment

Open field maze, digital camera, centrifuge, microplate reader, dounce homogenizer, multichannel pipette, mortar and pestle, glassware for staining procedures, and standard laboratory equipment.

2.2. Preparation of reagents

2.2.1. Cresyl violet acetate preparation

Cresyl violet acetate (0.1% w/v) was prepared by dissolving 0.1 g of the reagent in 100 mL of distilled water. The solution was filtered using Whatman No. 1 filter paper to remove particulate matter and stored at room temperature in an amber bottle to prevent light degradation.

2.3. Neurobehavioral study

2.3.1. Extraction of theobroma cacao

The cocoa seeds were air-dried and ground into a fine powder. The powder was soaked in 70% ethanol at a 1:5 (w/v) ratio for a minimum of 48 hours to ensure proper extraction of bioactive components. After

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Table 1. Experimental design

Group	Animals	Treatment Schedule	Rationale
Control Group	5	Placebo of water	Control
Group 1	5	Reserpine (0.5mg/kgB.W) for 14 days	Negative control for depression
Group 2	5	Fluoxetine (20 mg/kgB.W) for 14 days	Standard antidepressant
Group 3	5	Cocoa seed ethanolic extract (1000 mg/kgB.W) for 14 days	Control for cocoa
Group 4	5	Reserpine (0.5 mg/kgB.W) for 14 days + Cocoa seed ethanolic extract (1000 mg/kgB.W) for 7 days post-reserpine	To mitigate the effects of reserpine
Group 5	5	Reserpine for 14 days + Fluoxetine (20 mg/kgB.W) for 7 days post-reserpine	To mitigate the effects of reserpine

soaking, sieves of varying mesh sizes were used to remove any shafts and impurities.

The filtrate was collected using Whatman No. 1 filter paper and concentrated using a water bath set at 40–50°C to evaporate the ethanol completely. The resulting sediment was dried to a powdery consistency, air-dried further to remove residual moisture, and sieved to ensure uniformity. Before administration to animals, the powdered cocoa extract was dissolved in distilled water at a concentration of 1000 mg/kg body weight (B.W).

2.3.2. Animal processing

Thirty adult male Wistar rats were obtained from the Babcock University animal house following. Rats were housed in plastic cages in a conducive animal house environment for seven days for acclimatization. All guidelines and regulations in animal research and education were approved by the National Research Council DHHS' Institute of Laboratory Animal Resources. Beddings were changed every two days, and rats were provided with food and water ad libitum. Rats were randomly divided into six groups (Table 1), each comprising five rats, to prevent overcrowding and facilitate identification during the experiment.

2.3.3. Open field test

The Open Field Test (OFT) is used to assess locomotor activity, anxiety, and exploratory behavior in animals. The test is conducted in a square arena divided into smaller sections, where parameters such as total distance traveled, number of lines crossed, time spent in the center square, grooming behavior, and rearing frequency are measured. The animal is placed in the center of the arena and observed for 5–10 minutes. Increased time spent in the center suggests reduced anxiety, while more time spent in the periphery indicates higher anxiety. Grooming and rearing behaviors also reflect stress levels, with heightened grooming often indicating anxiety. Data are collected either by direct observation or using video tracking software and analyzed to compare behavioral differences across groups, helping to assess the effects of treatments like antidepressants or extracts [13].

2.3.4. Forced swimming test

It was used to measure the immobility and struggling time of each rat. It involves placing rats in a cylinder filled with room temperature water and observing behavior for 5 minutes [14].

2.3.5. Sacrifice of animals and organ harvest

Animals were euthanized by cervical dislocation, and brains were carefully excised for further analysis. Carcasses were disposed of properly to prevent environmental pollution.

2.3.6. Histology and histochemical assay

Haematoxylin and eosin staining: Following a standard staining procedure.

Cresyl violet staining: Utilized for paraffin-embedded sections to highlight neuronal structures in brain tissue.

2.3.7. Neurotransmitter assay (Dopamine Assay)

Employed a competitive inhibition enzyme immunoassay technique to measure dopamine concentration in brain tissue samples [15].

2.4. Biochemical assay

2.4.1. Glutathione (GSH) assay

Involved in a kinetic assay using DTNB and glutathione reductase to measure GSH concentration [23].

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2.4.2. Malondialdehyde (MDA) assay

Utilized thiobarbituric acid to measure lipid peroxidation, particularly MDA concentration [24].

2.5. Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) to compare the means of different experimental groups. Post-hoc comparisons were performed using Tukey's HSD (Honestly Significant Difference) test to identify specific group differences following a significant ANOVA result. All statistical analyses were performed using GraphPad Prism, and p-values less than 0.05 were considered statistically significant.

3. Results

3.1. Body weight

There was a statistically significant difference in body weight between groups C (fluoxetine), D (cocoa), E (reserpine + cocoa), and F (reserpine + fluoxetine) compared to group A (control) (p = 0.04) (Fig. 1). Similarly, significant differences were observed between the same groups (C, D, E, and F) and group B (reserpine), further supporting the effects of the treatments. Additionally, a significant difference in body weight was observed between group F (reserpine + fluoxetine) and the other treatment groups (C (fluoxetine), D (cocoa), E (reserpine + cocoa)), suggesting a distinct effect of combining fluoxetine with reserpine.

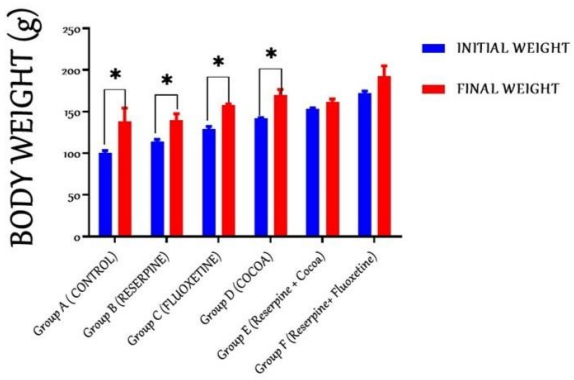

Figure 1. Bar chart showing comparison between initial and final body weights across all groups before amelioration. Values are expressed as Mean ± SEM; p values ($p < 0.05$)

3.2. Neurobehavioral study

3.2.1. Open field rearing

As shown in Fig. 2A below, the number of rears for Group A was 13 ± 4.0. The number of rears for group B was lower than group A at 5.8 ± 1.8. The number of rears for group C was lower than group A and group B at 4.2 ± 0.66. The number of rears for group D was lower than that of group A, B & C at 3.8 ± 0.66. The number of rears for group E was lower than that of group A, B, C & D at 2.4 ± 0.68. The number of rears for group F was higher than the other groups excluding group A at 8.4 ± 1.8. There was no statistically significance difference among all groups when compared to each other.

3.2.2. Centre square duration (CSD)

As shown in Fig. 2B below, before amelioration there is an observable decrease in the CSD of the groups administered reserpine when compared to the control group, this is because reserpine causes a sense of helplessness and hence the Wistar rats when observed tended to stay immobile. The CSD for group A was 2.2 ± 1.7. The CSD for group B was lower than group A at 0.60 ± 0.60. The CSD for group C was higher than group B but lower than group A at 0.80 ± 0.58. The CSD for group D was higher than group B & C but lower than group A at 1.2 ± 0.80. The CSD for group E was lower than all the groups at 0.00 ± 0.00. The CSD for group F was lower than all the groups but higher than group F at 0.20 ± 0.20. There is no statistical significance among all groups.

3.2.3. Grooming

As shown in Fig. 2C below, before amelioration, grooming for group A was 7.4 ± 1.2. The grooming of group B was lower than group A at 7.2 ± 1.8. The grooming of group C was higher than group A and group B at 8.4 ± 0.51. Group D grooming was lower than group A, B & C at 6.2 ± 0.86. Group E was lower than groups A, B, C & D at 4.6 ± 1.2. Group F was also lower than groups A, B, C & D at 4.6 ± 0.68. There was no statistical significance difference among all groups when compared with each other.

3.2.4. Number of lines crossed (NOLC)

Due to tendencies of reserpine causing depression, there is reduced mobility and exploration during depression and this is shown in Fig. 2D below. Before amelioration, the number of lines crossed for group A was at 47 ± 13. NOLC for group B was lower than group A at 28 ± 8.2. NOLC for group C was lower than group B& A at 24± 8.4. NOLC for group D was higher than group C & B but lower than group A at 37 ± 18.

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NOLC for group E was lower than all the groups at 12 ± 3.9. NOLC for group F was higher than all the groups excluding group A at 39 ± 4.7. There was no statistical significance difference among all groups when compared with each other.

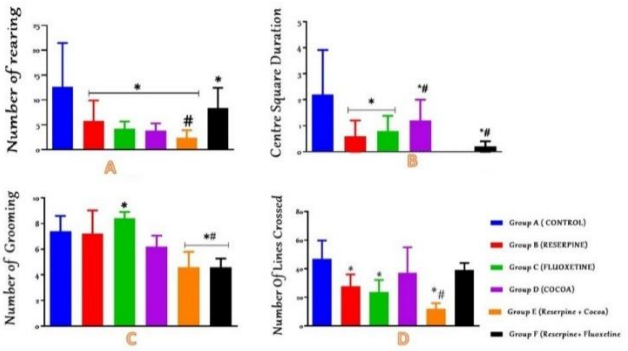

Figure 2. Showing the Effects of *Theobroma cocoa* ethanolic extract on reserpine induced behavioral distortions.

3.2.5. Centre square entries (CSE)

As shown in Fig. 3A below, before amelioration there is an observable decrease in the CSE of the groups administered reserpine when compared to the control group, this is because reserpine causes a sense of helplessness and hence the Wistar rats when observed tended to stay immobile. There was no statistical significance difference among all groups when compared with each other.

3.2.6. Freezing

There was no statistically significance difference among all groups when compared with each other but there is an observable increase in the freezing duration of the reserpine induced rats compared to the control group as seen in Fig. 3B below. This is as a result of the sense of helplessness caused by reserpine, hence the Wistar rats when observed tended to freeze.

3.2.7. Defecation

There was no statistically significance difference among all groups when compared with each other (Fig. 3C). Although, compared to the control group there is an observable increase in the level of defecation of the groups by the reserpine administered. This is because increased defecation shows increased levels of depression and anxiety.

3.2.8. Total locomotion activities (TLA)

There was no statistical significance difference among all groups when compared with each other. However, in Fig. 3D is an observable decrease in the TLA of the reserpine induced rats compared to the control group. As a result of the sense of helplessness caused by reserpine, hence the Wistar rats, when observed tended to have low locomotion activities.

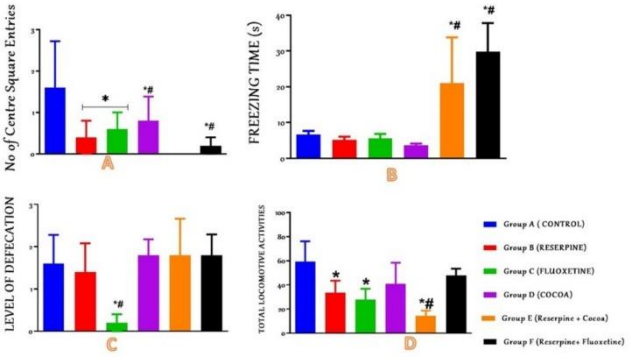

Figure 3. Showing the effects of reserpine, fluoxetine, *Theobroma cacao* ethanolic extract on number of centre square durations, freezing time, defecation and locomotory activities.

3.3. Forced swimming test

3.3.1. Immobility

There was no statistical significance difference among all groups when compared with each other. However, in Fig. 4A, there is an observable increase in the immobility of reserpine induced groups compared to the control group. As a result of the sense of helplessness caused by reserpine, hence the Wistar rats when observed tended to be immobile.

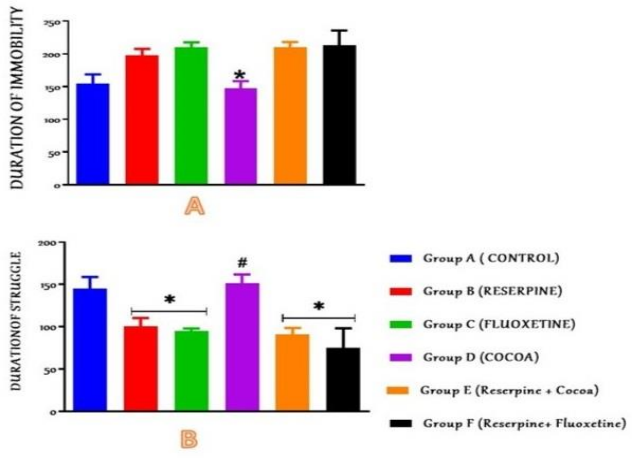

Figure 4. Showing the effects of reserpine, fluoxetine, *Theobroma cacao* ethanolic extract on the parameters of forced swimming test.

3.3.2. Struggling

In Fig. 4B, there was no statistical significance

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difference among all groups when compared with each other. However, there is an observable decrease in the duration of struggling in the groups induced with reserpine compared to the control group.

3.4. Biochemical assay

3.4.1. MDA

In Fig. 5A, the MDA level for group A was at 54 ± 13. The MDA level for group B was lower compared to group A at 52 ± 7.1. The figure shows an observable increase in the MDA level of group C compared to groups A & B. The MDA level of group C was higher than group A & B at 96 ± 31. The MDA level of group D was higher than groups A, B & C at 110 ± 35. The MDA levels of group E were lower compared to groups C & D but were higher compared to groups A & B at 69 ± 12. The MDA levels of group F were lower compared to C, D & E but higher than groups A & B at 64 ± 33. Therefore, the MDA levels of this research decreased in depression. There was no statistical significance difference among all groups when compared with each other.

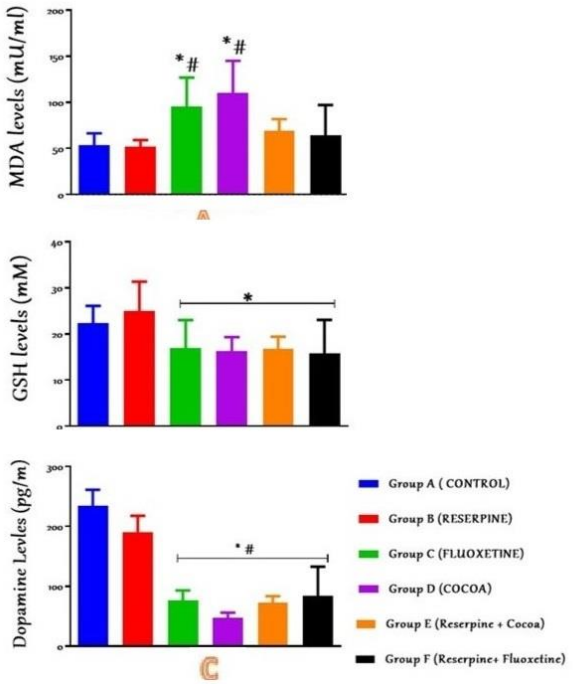

Figure 5. Showing the effects of reserpine, fluoxetine, *Theobroma cacao* ethanolic extract on antioxidant and neurotransmission parameters.

3.4.2. GSH

In Fig. 5B, the GSH level for group A was at 22 ± 3.8. GSH level of group B was higher compared to group A at 25 ± 6.3. GSH level of group C was higher compared to groups A & B at 17 ± 6.0. GSH level of group D was lower compared to groups A, B & C at 16 ± 3.0. GSH level of group E was higher compared to group D and was lower compared to groups A & B at 17 ± 2.6. GSH level of group F is lower compared to A, B, C & E at 16 ± 7.2. There was no statistical significance difference among all groups when compared with each other.

3.4.3. Dopamine

When the levels of dopamine in the groups were compared with each other using multiple comparisons, the dopamine levels of groups C (76 ± 17), D (48 ± 8.5), E (73 ± 11) & F (84 ± 49) showed a significant decrease when compared to group A (235 ± 26). There was also a statistically significant decrease in groups C (76 ± 17), D (48 ± 8.5), E (73 ± 11) & F (84 ± 49) compared to group B (190 ± 28) (Fig. 5C).

3.4.5. Histolopathological assessment

Photomicrographs show the hippocampal formation in the brains of rats for each group stained with Hematoxylin and Eosin to view the cytoarchitecture (Plates 1-10). This confirms the area of interest to be the hippocampus having a sea horse shape with several layers.

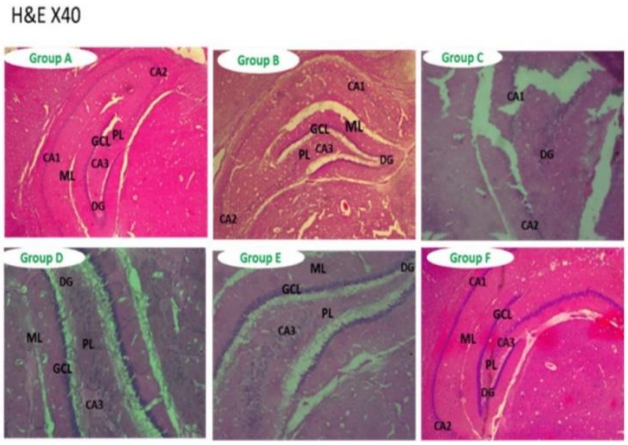

Plate 1. Photomicrographs of sections showing the general overview of hippocampal formation of the brains stained with Hematoxylin and Eosin (×40).
(DG: Dentate Gyrus; GL: Granule cell layer; PL: Polymorphic layer; CA 1, 2, 3: Cornu Ammonis 1, 2 & 3; ML: Molecular Layer).

4. Discussion

This study aimed to investigate the potential antidepressant effects of *Theobroma cacao* ethanolic extract in a reserpine-induced depression model using male Wistar rats, with particular emphasis on

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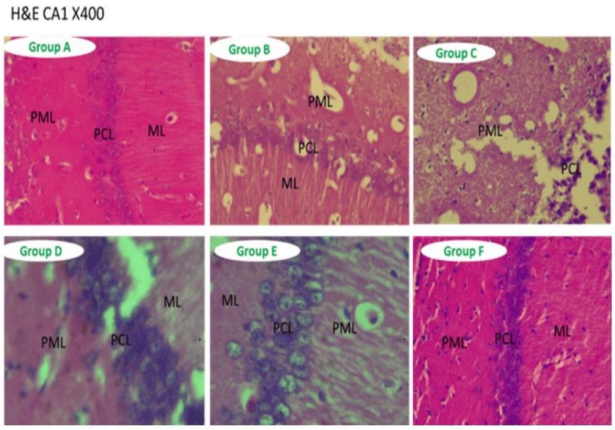

Plate 2. Photomicrographs of sections showing the cytoarchitecture of Cornu Ammonis 1 in the hippocampus of the brains stained with Hematoxylin and Eosin (×400).
(DG: Dentate Gyrus; GL: Granule cell layer; PL: Polymorphic layer; CA 1, 2, 3: Cornu Ammonis 1, 2 & 3; ML: Molecular Layer).

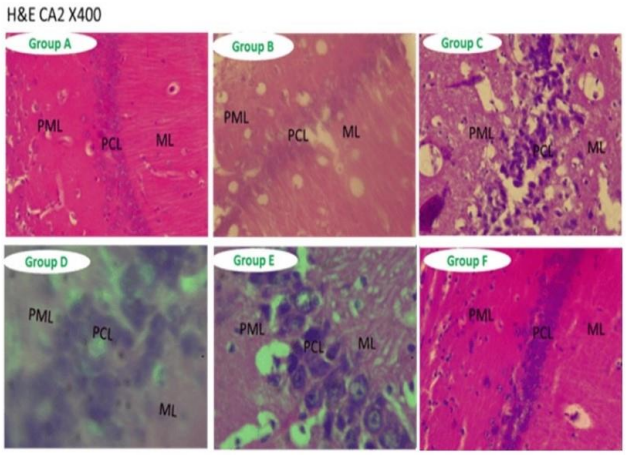

Plate 3. Photomicrographs of sections showing the cytoarchitecture of Cornu Ammonis 2 in the hippocampus of the brains stained with Hematoxylin and Eosin (×400).
(DG: Dentate Gyrus; GL: Granule cell layer; PL: Polymorphic layer; CA 1, 2, 3: Cornu Ammonis 1, 2 & 3; ML: Molecular Layer).

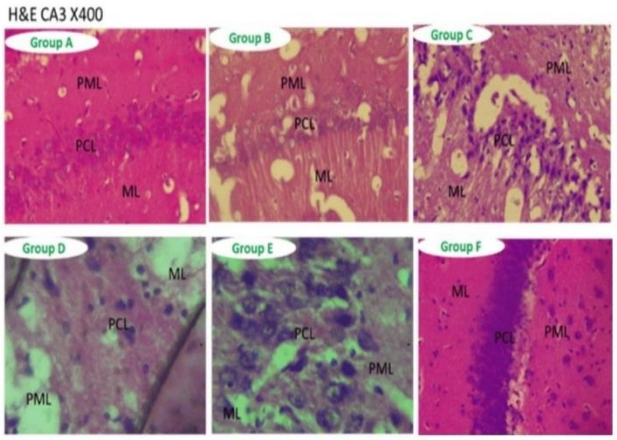

Plate 4. Photomicrographs of sections showing the cytoarchitecture of Cornu Ammonis 3 in the hippocampus of the brains stained with Hematoxylin and Eosin (×400).
(DG: Dentate Gyrus; GL: Granule cell layer; PL: Polymorphic layer; CA 1, 2, 3: Cornu Ammonis 1, 2 & 3; ML: Molecular Layer).

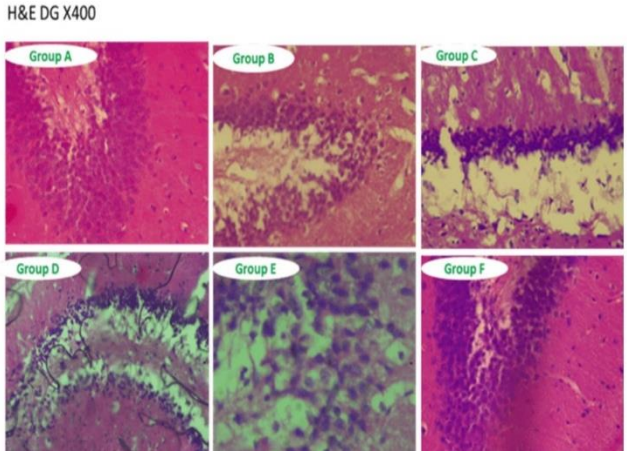

Plate 5. Photomicrographs of sections showing the cytoarchitecture of Dentate Gyrus in the hippocampus of the brains stained with Hematoxylin and Eosin (×400).
(DG: Dentate Gyrus; GL: Granule cell layer; PL: Polymorphic layer; CA 1, 2, 3: Cornu Ammonis 1, 2 & 3; ML: Molecular Layer).

hippocampal structure and function. Our findings revealed significant differences in body weight between groups receiving fluoxetine, cocoa, or their combinations with reserpine, compared to both the control and reserpine-only groups. Notably, the combination of reserpine and fluoxetine showed the most pronounced effect on body weight, significantly differing from the other treatment groups. These results align with previous studies that have reported morphological and biochemical effects of *Theobroma cacao* on body weight in animal models. For instance, research conducted by [16] demonstrated significant increases in body weight in the control group when compared to the initial and final weights of experimental animals. The body weight findings in this study provide further evidence that *Theobroma cacao* and fluoxetine may have a positive influence on metabolic functions and could potentially mitigate the weight loss observed in depression models.

The behavioral testing, specifically the open field test, revealed a general trend of decreased exploratory behavior in reserpine-treated rats compared to the control group. This was evidenced by reductions in rearing, center square duration, the number of lines crossed, and center square entries. Although these differences did not reach statistical significance, they

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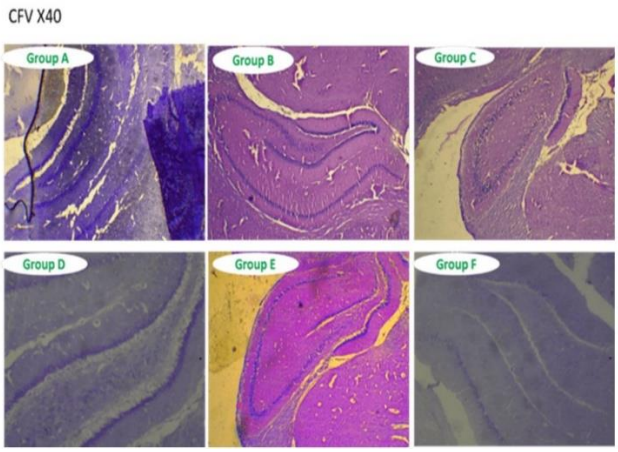

Plate 6. Photomicrographs of sections showing the general overview of hippocampal formaton of the brains stained with cresyl fast violet (×40)
(DG: Dentate Gyrus; GL: Granule cell layer; PL: Polymorphic layer; CA 1, 2, 3: Cornu Ammonis 1, 2 & 3; ML: Molecular Layer).

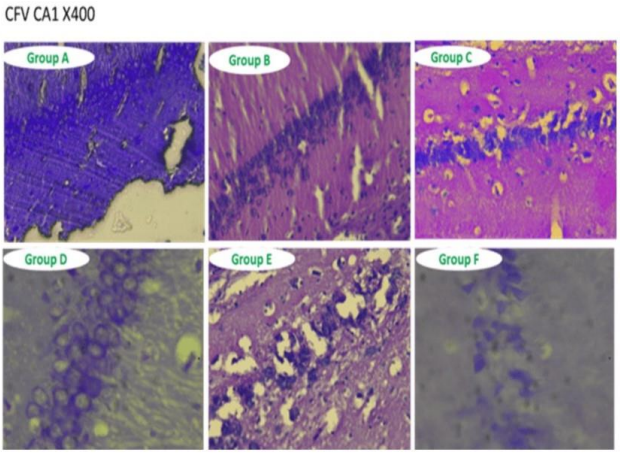

Plate 7. Photomicrographs of section showing the nissl bodies of neurons in Cornu Ammonis 1 in the hippocampus of the brains stained with cresyl fast violet (×400)
(DG: Dentate Gyrus; GL: Granule cell layer; PL: Polymorphic layer; CA 1, 2, 3: Cornu Ammonis 1, 2 & 3; ML: Molecular Layer).

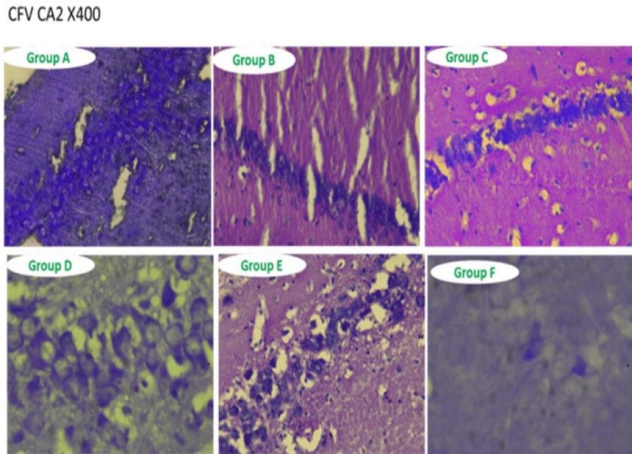

Plate 8. Photomicrographs of section showing the nissl bodies of neurons in Cornu Ammonis 2 in the hippocampus of the brains stained with cresyl fast violet (×400)
(DG: Dentate Gyrus; GL: Granule cell layer; PL: Polymorphic layer; CA 1, 2, 3: Cornu Ammonis 1, 2 & 3; ML: Molecular Layer).

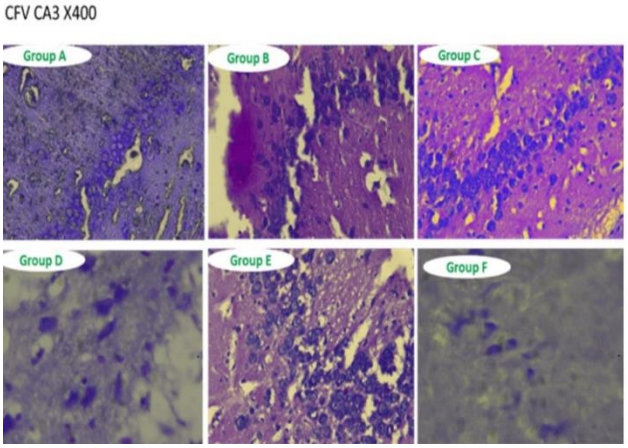

Plate 9. Photomicrographs of section showing the nissl bodies of neurons in Cornu Ammonis 3 in the hippocampus of the brains stained with cresyl fast violet (×400)
(DG: Dentate Gyrus; GL: Granule cell layer; PL: Polymorphic layer; CA 1, 2, 3: Cornu Ammonis 1, 2 & 3; ML: Molecular Layer).

are consistent with the expected depressive-like behavior induced by reserpine. Reserpine induces catecholamine depletion, which is associated with reduced motivation and energy, leading to lower activity levels and increased immobility. This trend in reduced exploratory activity mirrors findings from other studies on reserpine-induced depression models, where decreased exploratory behavior is often observed as part of the depressive-like phenotype [17]. The lack of statistical significance in the open field test may be attributed to the variability of the data, highlighting the need for larger sample sizes in future studies to detect more subtle differences between treatment groups.

In the forced swimming test, we observed a significant increase in immobility time and a significant decrease in struggling duration for the reserpine-induced groups compared to the control group. This is consistent with the characteristic behavioral despair associated with depression models, where increased immobility time reflects a lack of coping behavior and a state of learned helplessness [18]. The results from this test further support the establishment of a depressive-like state induced by reserpine, which is widely accepted as a model for studying antidepressant efficacy. Interestingly, the groups

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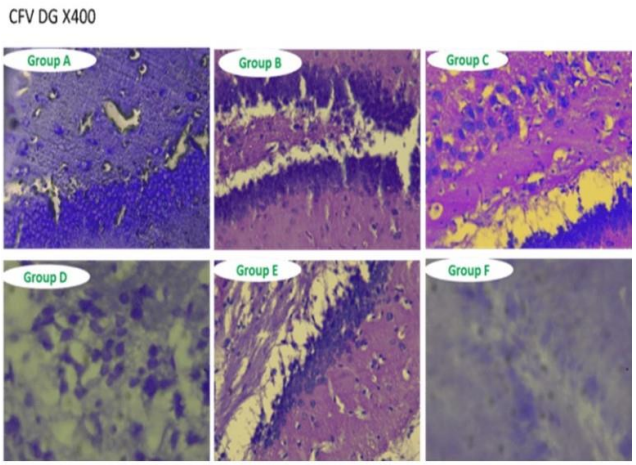

Plate 10. Photomicrographs of section showing the nissl bodies of neurons in Dentate Gyrus in the hippocampus of the brains stained with cresyl fast violet (×400).

treated with *Theobroma cacao* extract or fluoxetine in combination with reserpine exhibited reduced immobility time compared to the reserpine-only group, suggesting that both interventions may offer therapeutic effects by attenuating depressive-like behavior in rats. These behavioral changes are in line with previous studies on the antidepressant activities of *Theobroma cacao* in various animal models.

The study also examined markers of oxidative stress, specifically malondialdehyde (MDA) and glutathione (GSH) levels, to assess the impact of depression and antidepressant treatments on oxidative damage. MDA, a byproduct of lipid peroxidation, is often used as a biomarker for oxidative stress. Interestingly, while a trend toward a decrease in MDA levels was observed in the depression groups, no significant differences were detected across the treatment groups. This finding contradicts some previous studies that have associated increased oxidative stress with depression [19]. For instance, the research observed significant increases in MDA concentration in the reserpine-treated group compared to the control and other experimental groups in a similar depression model, suggesting a potential role of oxidative stress in the pathophysiology of depression [25]. Despite the lack of statistical significance in our MDA results, these observations may suggest a possible antioxidant effect of *Theobroma cacao* extract, which warrants further investigation.

GSH, a key antioxidant, plays a crucial role in maintaining the redox balance and mitigating oxidative damage in the brain. The GSH levels in this study varied across the treatment groups, and although no statistical significance was observed, these fluctuations highlight the potential role of GSH alterations in psychiatric disorders like depression. Previous research has indicated that alterations in GSH levels are implicated in oxidative stress and neuroinflammation associated with depression [13]. The potential therapeutic effects of *Theobroma cacao* on GSH levels and its implications for oxidative stress and neuroinflammation should be explored in greater detail in future studies.

Regarding dopaminergic signaling, we observed significant decreases in dopamine levels in groups treated with fluoxetine, *Theobroma cacao* extract, and their combinations with reserpine, compared to both the control and reserpine-only groups. This suggests that both fluoxetine and cocoa extract may modulate dopaminergic pathways in a way that differs from the acute effects of reserpine alone. Dopamine is a neurotransmitter that plays a central role in mood regulation and reward processing, and its dysregulation is often associated with depressive disorders. Our findings indicate that the administration of fluoxetine and *Theobroma cacao* extract may affect dopaminergic signaling pathways, which could contribute to their antidepressant effects. Further research into the specific mechanisms by which *Theobroma cacao* modulates dopamine levels could provide valuable insights into the neurochemical basis of its antidepressant properties [20].

Histological analysis using hematoxylin and eosin (H&E) staining revealed alterations in the cytoarchitecture of the hippocampus, particularly in the dentate gyrus, across treatment groups. The changes in cellular organization and density observed in the hippocampus suggest that both reserpine and the interventions (fluoxetine and *Theobroma cacao* extract) may influence hippocampal structure. These findings align with the known importance of hippocampal plasticity in depression and antidepressant responses [21]. Moreover, cresyl violet staining of Nissl bodies provided further insights into the neuronal integrity across hippocampal subregions. The alterations in Nissl body distribution and intensity could reflect changes in protein synthesis

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machinery, which may be relevant to the neurotrophic hypothesis of depression. This hypothesis proposes that impaired neurogenesis and synaptic plasticity in regions such as the hippocampus are central to the pathophysiology of depression and the mechanism of action of antidepressant treatments [22].

5. Conclusions

In conclusion, this study offers promising preliminary evidence supporting the antidepressant potential of *Theobroma cacao* ethanolic extract in a reserpine-induced depression model. The observed improvements in behavioral, neurochemical, and histological parameters suggest that cocoa extract may have a multifaceted therapeutic effect on depression, potentially modulating oxidative stress, dopaminergic signaling, and hippocampal function. These findings contribute to the expanding body of research on natural compounds as viable alternatives or adjuncts to traditional antidepressant therapies. However, further studies are needed to fully elucidate the mechanisms of action, optimal dosage, and long-term efficacy of *Theobroma cacao* in treating depression, emphasizing the need for continued exploration of plant-based compounds in the search for novel, effective interventions for mood disorders.

Ethical statement

This study was conducted following ethical guidelines and approved by the Babcock University Health Research Ethics Committee (BUHREC), with the approval number BUHREC/078/22. All procedures involving animals adhered to international standards for the care and use of laboratory animals.

Authors' contributions

Supervised, drafted the manuscript and approved the final version, O.P.A.; Conceptualized and designed the study, O.A.A.; Neurochemical analyses and interpreted the data, B.O.A.; Performed histological processing and tissue analysis, A.E.A.; Animal handling and behavioral assessments, A.D.A., O.E.K.; Carried out statistical analysis, S.T.O.; Analyzed oxidative stress markers, N.L.N., O.M.O.; Assisted in biochemical assays, C.J.N.; S.O.O.; Conducted histological staining and imaging, contributed to manuscript review and editing, K.A.A., A.T.B.; Assisted in animal model preparation and dosing, O.A.E., H.C.A.; Supported literature review and manuscript formatting, S.O.A.

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Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

1. Adetunji, O.A.; Adegunle, A.O.; Adeoye, B.O.; Nwobi, J.C.; Fabiyi, O.S.; Akano, O.P.; Ogunsanya, S.T.; Oyewumi, S.O.; Nwobi, N.L.; Adeoye, A.D.; Olayinka, O.O. Chronic stress-induced neurodegenerative changes in Wistar rats: Hippocampal evidence and the mitigating effects of *Allium Sativum*. Babcock Univ. Med. J. 2024, 7(1), 117–131. https://doi.org/10.38029/babcockuniv.med.j.v7i1.423
2. Chand, S.P.; Arif, H. Depression. In Stat Pearls; StatPearls Publishing: 2022. http://www.ncbi.nlm.nih.gov/books/NBK430847/
3. Balanzá-Martínez, V.; Cervera-Martínez, J. Lifestyle prescription for depression with a focus on nature exposure and screen time: A narrative review. Int. J. Environ. Res. Public Health. 2022, 19(9), 5094. https://doi.org/10.3390/ijerph19095094
4. Adeoye, O.; Ayobola, I.; Daniyan, M.; Victor, O.E.; David, A.; Abijo, A.; Bimbola, A.A. Ameliorative effects of nigerian bitter honey on streptozotocin-induced hepatorenal damage in Wistar rats. J. Krishna Inst. Med. Sci. Univ. 2022, 11, 65–76.
5. Adeoye, B.; Iyanda, A.; Michael, O.; Oyebimpe, O.; Fadeyi, B. Inhibitory effects of nigerian sweet and bitter

https://doi.org/10.58985/jpam.2025.v03i01.27

honey on pancreatic alpha amylase activity. Niger. J. Nutr. Sci. 2022, 43, 19–24.
6. Adeoye, A.A.; Amadike, K.; Adeoye, B.; Oyebanjo, O.; Adelakin, L.; David, A. Effect of ethanol extract of *Annona muricata* on lipopolysaccharide induced neuroinflammation in Wistar rats. Sch. Int. J. Anat. Physiol. 2023, 6, 153–161. https://doi.org/10.36348/sijap.2023.v06i10.002
7. Adetunji, O.A.; Marvellous, L.C.; Adeoye, B.O.; Akano, O. P.; Ogunsanya, S. T.; Oyewumi, S. O.; Adeoye, A. D.; Olayinka, O. O.; Adeogun, A.E. Chronic lead exposure impairment of hypothalamic-pituitary-gonadal axis in adult male Wistar rats: Biochemical and histomorphological evidence. East Afr. Sch. J. Med. Sci. 2024, 7(5). https://doi.org/10.36349/easms.2024.v07i05.006
8. Llerena, W.; Samaniego, I.; Vallejo, C.; Arreaga, A.; Zhunio, B.; Coronel, Z.; Quiroz, J.; Angós, I.; Carrillo, W. Profile of bioactive components of cocoa (*Theobroma cacao* L.) by-products from ecuador and evaluation of their antioxidant activity. Foods. 2023, 12(13), 2583. https://doi.org/10.3390/foods12132583
9. Baharum, Z.; Akim, A.M.; Hin, T.Y.Y.; Hamid, R.A.; Kasran, R. Theobroma cacao: Review of the extraction, isolation, and bioassay of its potential anti-cancer compounds. Trop. Life Sci. Res. 2016, 27(1), 21. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4807961/
10. Patil, P.P.; Khanal, P.; Patil, V. S.; Charla, R.; Harish, D.R.; Patil, B.M.; Roy, S. Effect of *Theobroma cacao* L. on the efficacy and toxicity of doxorubicin in mice bearing ehrlich ascites carcinoma. Antioxidants. 2022, 11(6), 1094. https://doi.org/10.3390/antiox11061094
11. Weir, M.R. Reserpine: A new consideration of an old drug for refractory hypertension. Am. J. Hypertens. 2020, 33(8), 708–710. https://doi.org/10.1093/ajh/hpaa069
12. Ma, X.; Lu, G.; Song, S.; Liu, W.; Wen, Z.; Zheng, X.; Lü, Q.; Su, D. The features of reserpine-induced gastric mucosal lesions. Acta Pharmacol. Sin. 2010, 31(8), 938–943. https://doi.org/10.1038/aps.2010.74
13. Adebola, A.O.; Ayodeji, A.O.; Adetayo, L.I.; Adeboye, E.C.; Toyin, A.I. Anti-depressant activities of *Theobroma cacao* extract on reserpine-induced depression in female Wistar rats. J. Krishna Inst. Med. Sci. 2020, 9(1).
14. Adeoye, A.D.; Oladele, A.A.; Akano, O.P.; Abijo, A.Z.; Adeoye, B.O.; Oyebanjo, O.T.; Adetunji, O.A. Neuroprotective effects of garcinia kola ethanol seed extract on haloperidol-induced catalepsy in mice. Trop. J. Nat. Prod. Res. 2022, 6(2), 281–286. https://doi.org/10.26538/tjnpr/v6i2.18
15. Opoggen, T.; Daniyan, M.; Asiyanbola, I.; Olufunso, A.; Oyemitan, I. Effects of the essential oil of dried fruits of *Piper guineense* (Piperaceae) on neurological syndromes associated with cerebral malaria in mice. Univ. J. Pharm. Res. 2023, 8(1). https://doi.org/10.22270/ujpr.v8i1.902
16. Kebe, E.O. Morphological and biochemical effect of *Theobroma cacao* on liver of Wistar rats. EC Pharmacol. Toxicol. 2019, 7, 01–09.
17. Antkiewicz-Michaluk, L.; Wąsik, A.; Możdżeń, E.; Romańska, I.; Michaluk, J. Antidepressant-like effect of tetrahydroisoquinoline amines in the animal model of depressive disorder induced by repeated administration of a low dose of reserpine: Behavioral and neurochemical studies in the rat. Neurotox. Res. 2014, 26(1), 85–98. https://doi.org/10.1007/s12640-013-9454-8
18. Porsolt, R.D.; Bertin, A.; Jalfre, M. Behavioral despair in mice: A primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 1979, 229(2), 327–336.
19. Maes, M.; Galecki, P.; Chang, Y.S.; Berk, M. A review on the oxidative and nitrosative stress (O & NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. Prog. Neuropsychopharmacol. Biol. Psychiatry. 2011, 35(3), 676–692. https://doi.org/10.1016/j.pnpbp.2010.05.004
20. Belujon, P.; Grace, A.A. Dopamine system dysregulation in major depressive disorders. Int. J. Neuropsychopharmacol. 2017, 20(12), 1036–1046. https://doi.org/10.1093/ijnp/pyx056
21. Duman, R.S.; Aghajanian, G.K.; Sanacora, G.; Krystal, J.H. Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. Nat. Med. 2016, 22(3), 238–249. https://doi.org/10.1038/nm.4050
22. Duman, R.S.; Monteggia, L. M. A Neurotrophic Model for Stress-related Mood Disorders. Biol. Psychiatry. 2006, 59(12), 1116–1127. https://doi.org/10.1016/j.biopsych.2006.02.013
23. Ellman, G.L. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 1959, 82(1), 70–77. https://doi.org/10.1016/0003-9861(59)90090-6
24. Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 1979, 95(2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3
25. Adetunji, O.A.; Adeoye, B.O.; Akano, O.P.; Adeoye, A.D.; Adeogun, A.E.; Adebayo, I.O.; Fabiyi, O.S.; Ogunsanya, S.T.; Nwobi, N.L.; Nwobi, J.C.; Osundina, O.B.; Achor, C.B.; Olatinwo, G.O.; Kolawole, O.E.; Lawal, I.A.; Adebayo, B.T.; Ademoye, K.A. *Theobroma cacao* seed extract attenuates reserpine-induced hematotoxicity and stomach tissue distortions in male Wistar rats. Int. Res. J. Med. Pharm. Sci. 2024, 9(2), 23–37. https://doi.org/10.5281/zenodo.11402037