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THEOBROMA CACAO SEED EXTRACT ATTENUATES RESERPINE-INDUCED HEMATOTOXICITY AND STOMACH TISSUE DISTORTIONS IN MALE WISTAR RATS

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Abstract

Alterations in blood cells and gastrointestinal (GIT) parameters are intertwined with various physiological, psychological, and environmental factors. This research elucidated the potential pharmacological roles of *Theobroma cacao* seed ethanolic extract on blood profile and stomach tissue aberration induced by reserpine. A total of thirty (30) adult male Wistar rats were used for this study for a period of 28 days and were divided into six groups as follows: Group A (control group), Group B (0.5mg/kg/ B.W of reserpine), Group C (20mg/kg/B.W fluoxetine), Group D (1000mg/kg/B.W of cocoa seed ethanolic extract), Group E (0.5mg/kg/B.W of reserpine + 1000mg/kg/B.W of cocoa seed ethanolic extract), and Group F

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(0.5mg/kg/B.W of reserpine + 20mg/kg/B.W of fluoxetine). The stomach tissue was harvested, placed in 10% neutral buffered formalin, and processed for histological analysis. A full blood count test was performed, and the results were analyzed using graph pad prism 9.0 software using one-way analysis of variance (ANOVA). The results showed a decrease in PCV (packed cell volume), hemoglobin, and neutrophils of the reserpine-induced rats. There was a significant increase in the treated groups. Histopathology assessments depicted that treatment with *Theobroma cacao* seed ethanolic extract groups restored mucosa thickness and curtailed glycogen accumulation in the stomach tissue. This study showed that the ameliorative effect of cocoa seed ethanolic extract is pronounced in reversing the harmful effects of reserpine on blood profiles and GIT.

Introduction

Reserpine is an indole alkaloid derived from the root of *Rauwolfia serpentina*, traditionally used to treat hypertension and certain psychiatric conditions (Cheung & Parmar, 2024). Unfortunately, its pharmacological significance is often limited because of its deleterious contraindications. These include gastrointestinal disturbances, central nervous system effects, and distortions of blood profiles (Slim *et al*., 2011). Reserpine-induced gastric mucosal lesions (GMLs) are a significant clinical concern because they result from increased cholinergic tone and excessive acid secretion, leading to life-threatening gastrointestinal disturbances (Pfeifer *et al*., 1976 & Ma *et al*., 2010). Recently, empirical evidence has widely elucidated the diverse potentials of naturally occurring bioactive compounds from plants in mitigating the adverse effects of conventional pharmacological agents (Adeoye *et al*., 2022a; Adeoye *et al*., 2022b; Adeoye *et al*., 2023; Adetunji *et al*., 2022). Theobroma cacao, commonly known as the cacao tree, is a tropical plant whose seeds are the primary source of cocoa and chocolate (Baharum *et al*., 2016). Worldwide intake of cocoa and its derivatives is common, largely because of its delicious taste and numerous health benefits. The seeds of *Theobroma cacao* are rich in bioactive compounds, including flavonoids, polyphenols, and other antioxidants, which have been extensively studied for their numerous health benefits (Llerena *et al*., 2023) and (Martínez-Pinilla *et al*., 2015). These compounds are well reputed to possess anti-inflammatory, cardioprotective, antiproliferative (Patil *et al*., 2022), neuroprotective, hematoprotective, and gastroprotective properties (Rusconi & Conti, 2010). *Theobroma cacao* seed extract, with its rich antioxidant profile, has shown promise in attenuating various forms of tissue damage and oxidative stress (Katz *et al*., 2011). Cocoa polyphenols employ similar methods to regulate microbial variety by stimulating the development of certain bacteria while inhibiting the growth of others, resulting in the production of prebiotic effects (Sorrenti *et al*., 2020). In this study, we aimed to elucidate the pharmacological roles of *Theobroma cacao* seed ethanolic extract in curtailing reserpine-induced hematotoxicity and stomach tissue distortions. By leveraging the protective properties of cacao's bioactive compounds, this research seeks to provide a natural adjunctive therapy to counteract the side effects of reserpine, thereby enhancing its therapeutic profile while minimizing its risks.

MATERIALS AND METHODS

Equipment

A plastic cage with iron mesh to allow for cross ventilation, pelletized food, bedding (wood shavings), feeding trough and water bottles, markers (for labeling), measuring scale, dissecting set, disinfectant, micro-hematocrit capillary tubes, oral cannula, reserpine, fluoxetine, cocoa powder, ethanol, cotton wool, sample bottles, syringes, needles, hand gloves and bowls.

Chemicals and Drugs

Normal Saline, Phosphate buffer, Formalin, Distilled water, Reserpine injection (0.5mg/kg/B. W), fluoxetine (20mg/kg/B. W), cocoa seed ethanolic extract (1000mg/kg/B. W)

ANIMAL CARE, CONDITIONING, AND MANAGEMENT

A total number of thirty (30) adult male Wistar rats were kept and cared for at the Babcock University Animal House, Ilisan Remo, Ogun state. They were kept in plastic cages with net covers to keep them cool. They were allowed to acclimatize in the animal house for 7 days (one week). The rats were allocated to six (6) groups with each cage containing five (5) rats. The animals were provided with distilled water and pelletized food daily. The animals' bedding was covered with wood shavings to avoid toxic ammonia accumulation and was replaced every three (3) days.

The feed of the rats was measured daily. This was done by measuring the weight of the leftover food per animal group in a cage.

Table 1: Study Design

	NO. OF RATS	TREATMENT SCHEDULE	RATIONALE
Group A	5	A placebo of water	Control group
Group B	5	Animals treated with (0.5mg/kg/B. W) of reserpine intraperitoneal for 14 days	Standard depressant
Group C	5	Animals treated with (0.5mg/kg/B. W) reserpine intraperitoneally for 14 days	Standard Anti-depressant
Group D	5	Animal treatment with 1000 mg/kg/B. W cocoa seed ethanol extract) for 14 days	Control for cocoa
Group E	5	Animal treatment intraperitoneally with reserpine for 14 days (0.5mg/kg/B. W) +oral with cocoa seed ethanoic extract for 7 days (1000mg/kg/B. W)	To mitigate the effects of reserpine
Group F	5	Animal treatment intraperitoneally with reserpine for 14 days +oral with fluoxetine for 7 days (20mg/kg/B. W)	To mitigate the effects of reserpine

ADMINISTRATION OF THE SOLUTION

Depression was induced in the rats through repeated intraperitoneal injection of Reserpine (depressant) for 14 days. The control group (Group A) was administered a placebo of water, group B was administered Reserpine (0.5mg/kg/B. W), group C was administered fluoxetine (20mg/kg/B. W), and group D was administered cocoa

seed ethanolic extract (1000mg/kg/B. W). Group A was left untreated, and groups B, C, and D were also left untreated to induce the effects of reserpine, fluoxetine, and cocoa ethanolic extracts, respectively.

After depression had been induced, group E was treated with 1000mg/kg/B. W of *Theobroma cacao* seed extract orally for seven (7) days using an oral cannula for administration, and group F was treated orally with 20mg/kg/B. W of fluoxetine, which is a standard antidepressant for seven (7) days.

MEASUREMENT OF BODY WEIGHTS AND FEED

The body weights of the animals were measured once every 3 days throughout the duration of administration using a weighing balance. This was done to access the weight gain or weight loss in each group. The feed of the rats was measured daily. This was done by measuring the weight of the leftover food per animal group in a cage.

FULL BLOOD COUNT TEST

After blood was collected from the rats in each group, a full blood count test was performed using an automated hematology analyzer. The full blood count (FBC) is a test that counts cells in the blood and collects information on their size and concentration. Any abnormalities in any of these types of cells indicate blood disorders. Blood cells include hemoglobin (HB), lymphocytes, neutrophils, basophils, eosinophils, and monocytes.

HISTOLOGY

The stomach was carefully excised, weighed, and fixed in 10% neutral buffered formalin. Thereafter, the tissues were embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin to demonstrate the general histology and morphology of the stomach.

ANIMAL PROCUREMENT AND ETHICAL CONSIDERATION

Animals were obtained from Babcock University Animal House for this research following all rules and regulations in animal research and education as approved by the National Research Council DHHS' Institute of Laboratory Animal Resources. Ethical clearance was obtained from the Babcock University Health Research Ethical Committee (BUHREC).

STATISTICAL ANALYSIS

The results obtained are expressed as mean ± SEM for each group. All data were evaluated statistically using GraphPad Prism 6.0 software and one-way analysis of variance (ANOVA). The student-Newman-Kleus post-hoc test was used to identify differences. A value of $p<0.05$ was considered significant.

RESULTS

MORPHOLOGICAL ANALYSIS

BODY WEIGHT

As shown below in figure1 there was statistical significance when Group C (fluoxetine), Group D (cocoa), Group E (reserpine + cocoa), and Group F (reserpine + fluoxetine) were compared with the control group. Group C (fluoxetine), Group D (cocoa), Group E (reserpine + cocoa), and Group F (reserpine + fluoxetine) were significantly different from Group B (reserpine). Group F (reserpine + fluoxetine) was statistically significant to Group C (fluoxetine), Group D (cocoa), and Group E (reserpine + cocoa). *, +, #, $, and @ show statistical significance when the experimental groups are compared at $p<0.05$.

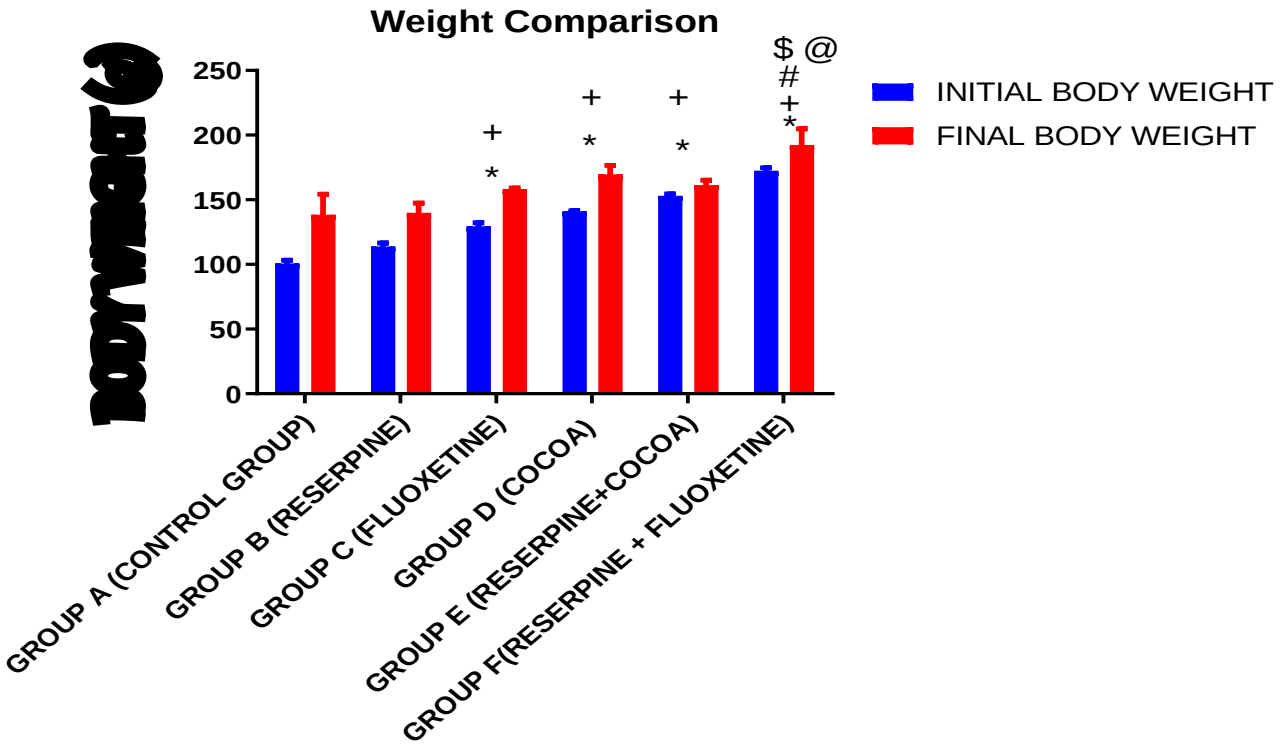

Figure 1: Graph showing the body weight change before and during treatment

FOOD INTAKE

As shown below in figure 2 the result indicates the rate of food intake before and during treatment. There was an observable increase in the rate of food consumption of groups E and F while they were receiving treatment. There was no significant difference across the groups compared with the control group.

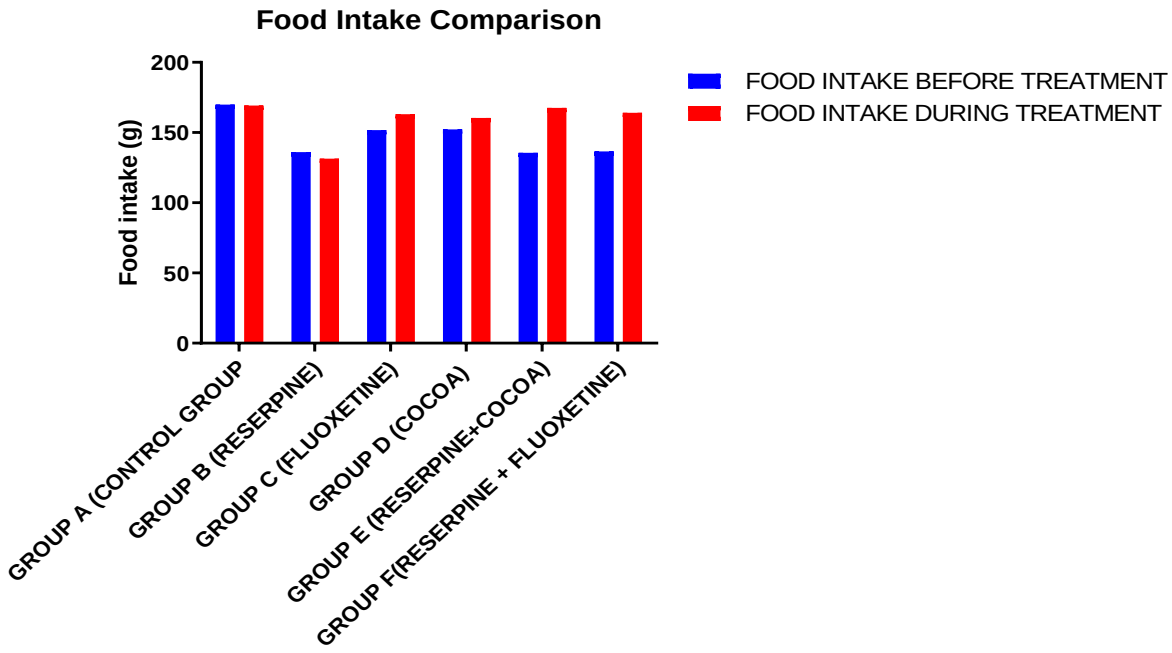

Figure 2: Graph showing the rate of food intake before and during treatment

FULL BLOOD COUNT ANALYSIS

PACKED CELL VOLUME (PCV)

As shown below in figure 3 the PCV of the control group was 44±1.4. The PCV of Group B (reserpine) was lower than that of the control group at 40±1.3. The PCV of Group C (fluoxetine) was higher than that of the control group at 45±1.2. The PCV of Group D (cocoa) was higher than that of the control group at 45±1.7. The PCV of Group E (reserpine + cocoa) was higher than that of the control group at 45±0.63. The PCV of Group F (reserpine + fluoxetine) was lower than that of the control group at 39±1.4. There was no statistical significance across the groups compared with the control group.

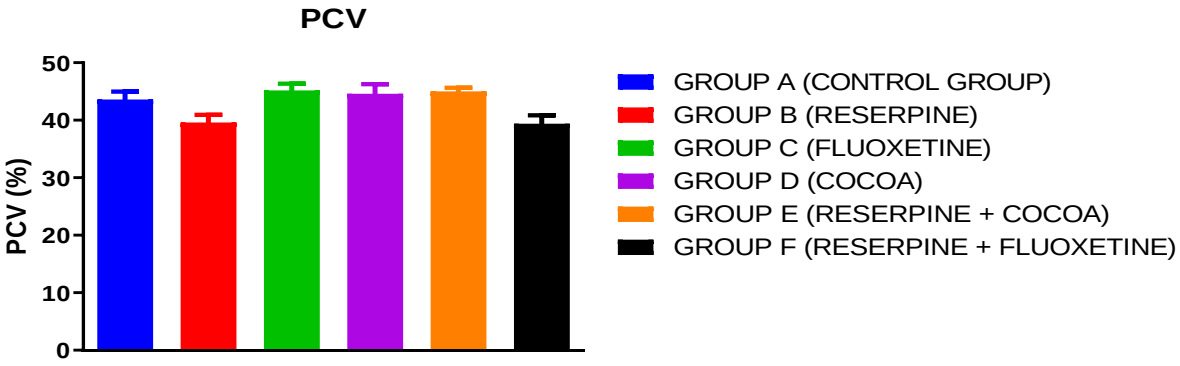

Figure 3: Graph showing PCV levels across the experimental groups.

HEMOGLOBIN (HB)

As shown below in figure 4 the HB of the control group was 11±2.8. The HB of Group B (reserpine) was higher than that of the control group at 13±0.45. The HB of Group C (fluoxetine) was higher than that of the control group at 15±0.37. The HB of Group D (cocoa) was higher than that of the control group at 15±0.57. The HB of Group E (reserpine + cocoa) was higher than that of the control group at 15±0.18. The HB of Group F (reserpine + fluoxetine) was higher than that of the control group at 13±0.45. There was no statistical significance across all groups compared with the control group.

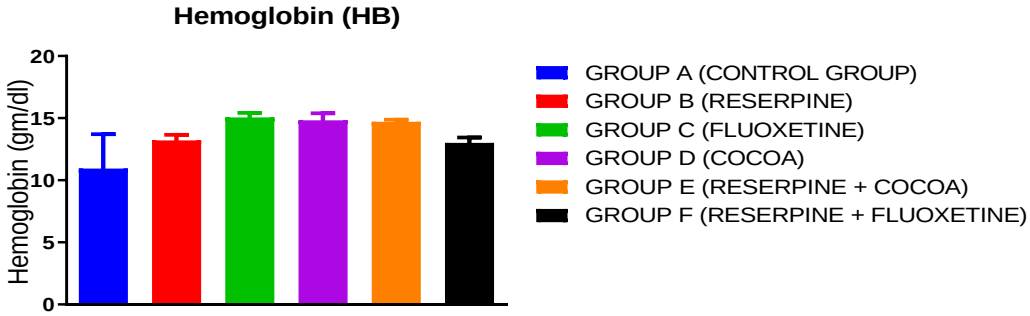

Figure 4: Graph showing HB levels across the experimental groups.

Leukocytes (WBCs)

As shown below in figure 5, the WBC count of the control group was 1.0760±1.736. The WBC count of Group B (reserpine) was higher than that of the control group at 1.3840±1.942. The WBC count of Group C (fluoxetine) was higher than that of the control group at 1.3120±1.539. The WBC count of Group D (cocoa) was higher than that of the control group at 1.4300±2.074. The WBC count of Group E was lower than that of the control group at 9.460±2.56. The WBC count of Group F was lower than that of the control group at 7.800±8.50. There was no statistical significance across all groups compared with the control group.

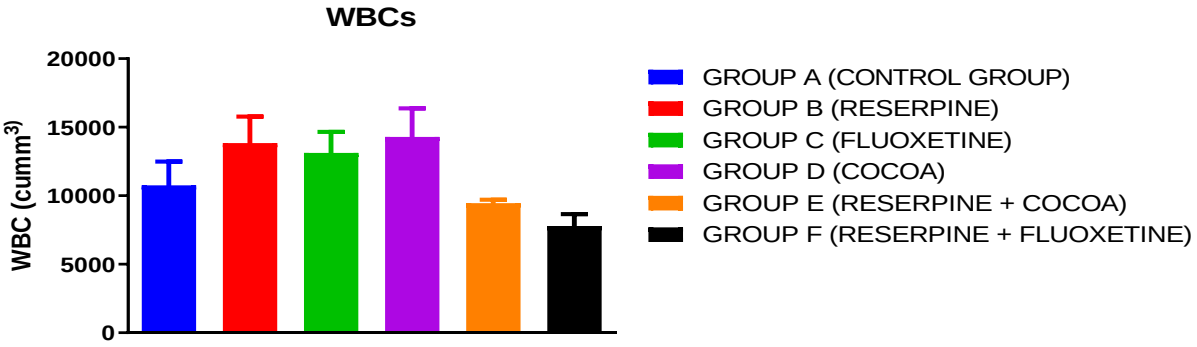

Figure 5: Graph showing the WBC levels across the experimental groups

LYMPHOCYTES

As shown below in figure 6, the lymphocyte count of the control group was 57±4.1. The lymphocyte count in Group B (reserpine) was higher than that in the control group at 70±4.1. The lymphocyte count in Group C (fluoxetine) was lower than that in the control group at 59±3.0. The lymphocyte of Group D (cocoa) was higher than the control group at 62±3.7. The lymphocyte of Group E (reserpine + cocoa) was higher than the control group at 61±2.1. The lymphocyte of Group F (reserpine + fluoxetine) was higher than the control group at 63±3.1. There was statistical significance across Groups B and C compared with the control group. * and + show statistical significance when the experimental groups are compared with the control group at $p<0.05$.

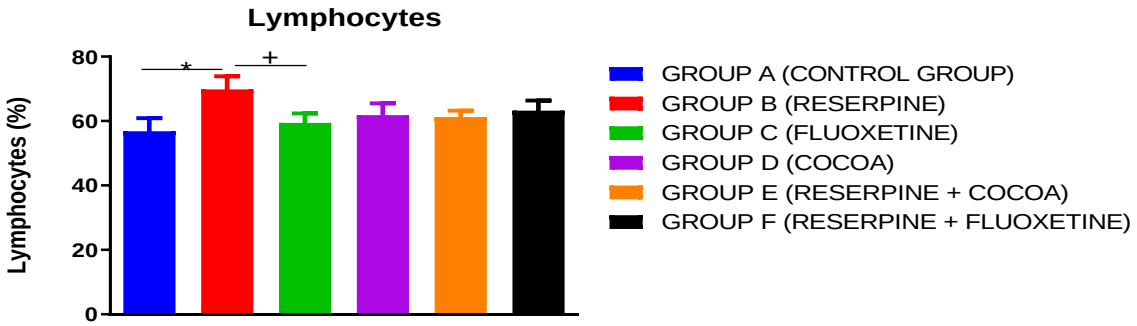

Figure 6: Graph showing lymphocyte levels across the experimental groups

NEUTROPHIL

As shown below in figure 7, the neutrophil count of the control group was 41±3.4. The neutrophil count in Group B (reserpine) was lower than that in the control group at 30±3.9. The neutrophil count in Group C (fluoxetine) was lower than that in the control group at 40±3.0. The neutrophil of Group D (cocoa) was lower than the control group at 38±3.7. The neutrophil count of Group E (reserpine+ cocoa) was lower than that of the control group at 38±2.4. The neutrophil count in Group F (reserpine+ fluoxetine) was lower than that in the control group at 35±3.4. There was statistical significance across Groups B and C compared with the control group. * and + show statistical significance when the experimental groups are compared with the control group at $p<0.05$.

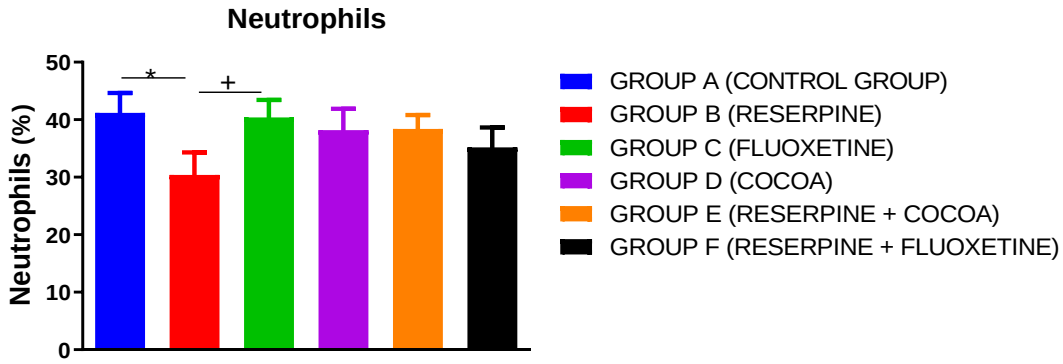

Figure 7: Graph showing neutrophil levels across the experimental groups

HISTOLOGICAL ANALYSIS

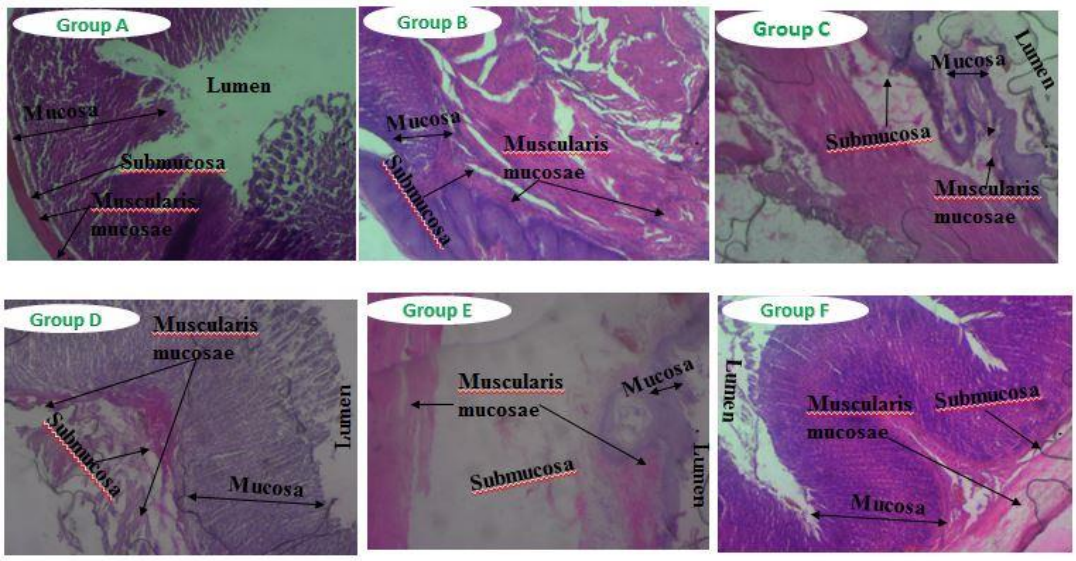

PLATE 1: Groups A and F show sections of normal Gastric tissue consisting of the lumen, mucosa, blood vessels, and muscularis. H and E 40: Groups B, C, D, and E sections reveal the lumen, mucosa, blood vessels, and muscularis with no remarkable epithelial damage or fibrosis.

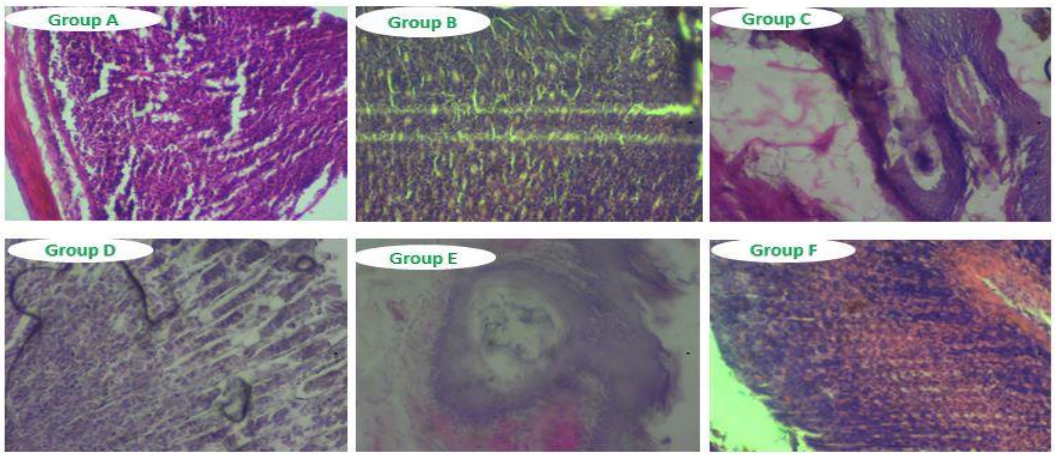

PLATE 2: Groups A and F show sections of normal Gastric tissue consisting of the lumen, mucosa, blood vessels, and muscularis. Group B, C, D, and E sections revealed the lumen, mucosa, blood vessel, and muscularis with no remarkable epithelial damage and fibrosis: H & E 100.

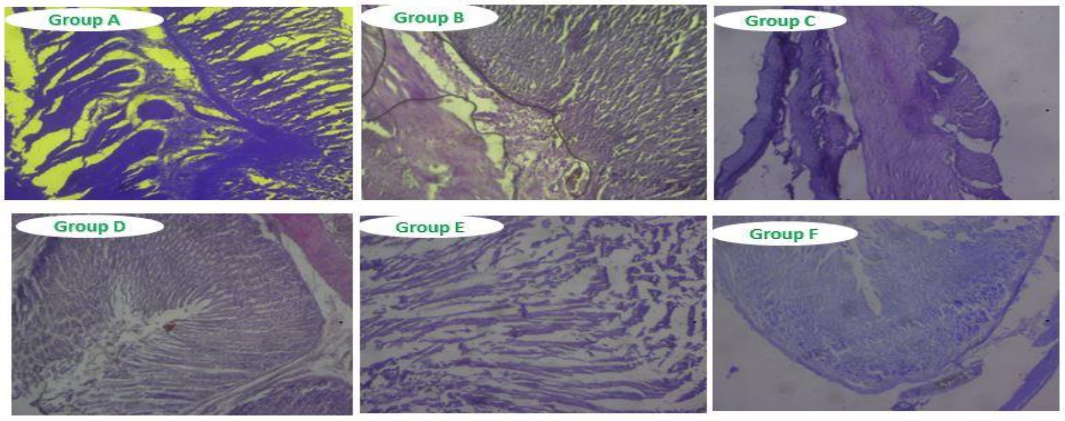

PLATE 3: photomicrograph of the stomach following exposure to reserpine, stained with Periodic Acid Schiff's to demonstrate glycogen. Group B shows the presence of glycogen compared with other groups. PAS ×40.

DISCUSSION

This study examined the effects of administering reserpine and the possible therapeutic advantages of *Theobroma cacao* seed ethanolic extract and fluoxetine on different physiological parameters, such as body weight, food consumption, and hematological indices. The results demonstrated substantial changes in these variables among several experimental groups, providing insight into the pharmacological effects of the treatments and their possible consequences for health and well-being.

The examination of body weight fluctuations among the experimental groups revealed notable disparities, underscoring the possible influence of distinct interventions on weight control (Fig 1). Groups administered fluoxetine (Group C), cocoa extract (Group D), and the combination of reserpine with either fluoxetine (Group F) or cocoa extract (Group E) showed notable distinctions in comparison to both the control group (Group A) and the reserpine-only group (Group B). Group F had notable disparities in comparison with Groups C, D, and E. These data indicate that the therapies had different impacts on the regulation of body weight, which were impacted by their biochemical and physiological actions. Group B, which was administered reserpine as the sole treatment, exhibited weight reduction, which aligns with the established effects of reserpine in diminishing hunger and augmenting energy expenditure through the depletion of monoamines such as serotonin and dopamine (Strawbridge *et al*., 2023). On the other hand, Group C, which received fluoxetine treatment, exhibited notable enhancement in body weight in comparison to the reserpine group. Fluoxetine, a selective serotonin reuptake inhibitor (SSRI), increases serotonin levels, thus reversing the weight loss caused by reserpine by boosting mood and appetite (Arisha, 2022; Fava *et al*., 2000; Hillhouse & Porter, 2015).

The group treated with cocoa extract, known as Group D, also showed a notable ability to maintain their weight compared with the group that received reserpine. The bioactive components found in cocoa, including flavonoids, have antioxidant and anti-inflammatory characteristics that can help reduce weight loss caused by reserpine (Edo *et al*., 2023; Fideles *et al*., 2023; Scapagnini *et al*., 2014; Sorrenti *et al*., 2020). Cocoa extract has been reported to can elevate serotonin levels, comparable to fluoxetine (Adebola *et al*., 2020), which can further support the maintenance of weight. Groups E and F, which were administered combinations of reserpine with cocoa extract or fluoxetine, respectively, exhibited noteworthy enhancements in body weight compared with Group B. This suggests that the combination treatments successfully countered the weight loss caused by reserpine, indicating a synergistic impact.

The observed effects are attributed to the modification of the serotonin pathway, the antioxidative and anti-inflammatory properties of cocoa flavonoids, and the normalization of metabolic rates. Elevated serotonin levels

enhance both mood and appetite, thereby reducing the anorexic effects caused by reserpine. The flavonoids in cocoa provide protection against cellular damage and inflammation caused by reserpine (Bellavite, 2023), which in turn helps to maintain or increase body weight (Lamuela-Raventós *et al*., 2005). In addition, these treatments can help restore normal metabolic rates, which can lead to an increase in weight. The notable variations in body weight across the different groups in the trial highlight the potential of *Theobroma cacao* seed extract and fluoxetine in reducing the weight loss caused by reserpine. These findings highlight the significance of investigating natural substances such as cocoa extract as prospective therapeutic agents for regulating the negative effects of drugs on body weight regulation.

The examination of food consumption among the experimental groups provides insight into the effects of various therapies on hunger and eating behavior (Fig 2). Significantly, Groups E (reserpine + cocoa) and F (reserpine + fluoxetine) showed a rise in food intake throughout the treatment period, indicating potential appetite-enhancing effects. Nevertheless, when the control group (Group A) was compared, there were no notable disparities in food consumption among the groups. The alterations in food consumption can be ascribed to the physiological and biochemical impacts of the provided medications (Cheng & Wong, 2020; D'Alessandro *et al*., 2022). Group B, which was administered reserpine alone, often exhibited decreased food consumption because of the anorexic properties of the medication. Reserpine interferes with central monoamines such as serotonin and dopamine, which play vital roles in controlling hunger. This disruption may result in hypophagia. Group F, which received a combination of reserpine and fluoxetine, had a notable increase in food consumption. This suggests that fluoxetine effectively counteracts the anorexic effects of reserpine (Kaye *et al*., 2001; Sohel *et al*., 2024). Fluoxetine increases serotonin levels by blocking its reuptake, leading to improved mood and increased hunger, thus restoring normal eating patterns that have been interrupted by reserpine (Sohel *et al*., 2024). Similarly, Group E, which was administered a combination of reserpine and cocoa extract, also demonstrated an elevated level of food intake. Theobromine and flavonoids are present in cocoa extract and can affect hunger regulation and mood. Moreover, the antioxidative characteristics of cocoa may help alleviate oxidative stress caused by reserpine, indirectly promoting regular feeding behavior (Scapagnini *et al*., 2014).

The absence of notable disparities in food consumption among all groups compared with the control group indicates that the therapies did not elicit excessive overeating or appetite loss beyond typical physiological limits. Instead, they assisted in establishing a standard level of food consumption, demonstrating their capacity to manage the reduction in appetite caused by reserpine. The observed processes responsible for these effects involve the regulation of the serotonin pathway, leading to an improvement in mood and a reduction in stress (Hopkins *et al*., 2000; Unick *et al*., 2021). Fluoxetine and cocoa extract both counteract the appetite-suppressing effects of reserpine by increasing serotonin levels and improving mood. In addition, the presence of healthier gastrointestinal tissues indicated in histopathological examinations may lead to improved digestion and absorption, thereby boosting overall appetite and food consumption.

The full blood count (FBC) investigation examined the hematological effects of reserpine and the possible therapeutic benefits of *Theobroma cacao* seed ethanolic extract and fluoxetine. Multiple indices, such as packed cell volume (PCV), hemoglobin (Hb), white blood cells (WBCs), lymphocytes, and neutrophils, were analyzed (Fig 3-7). The control group had a PCV (packed cell volume) of 44±1.4, which represents the fraction of blood volume occupied by erythrocytes (Fig 3). Group B, which was administered reserpine, showed a decreased packed cell volume (PCV) of 40±1.3, suggesting the presence of anemia or hematological suppression induced by reserpine. In contrast, Groups C (fluoxetine), D (cocoa), and E (reserpine + cocoa) exhibited elevated PCV values, indicating that fluoxetine and cocoa extract may potentially promote erythropoiesis or enhance the stability of red

blood cells (Katz *et al*., 2011; Sorrenti *et al*., 2020). However, there was no statistical significance compared with the control group, indicating subtle variations that may not be clinically significant under these conditions.

Hb levels, crucial for assessing oxygen-carrying capacity, the control group showed a level of 11±2.8 (Fig 4). Surprisingly, Group B (reserpine) exhibited higher Hb levels (13±0.45), possibly due to compensatory mechanisms. Groups C, D, and E showed further increases in Hb levels, indicating the potential benefits of fluoxetine and cocoa extract on Hb levels (Becker *et al*., 2013; Sorrenti *et al*., 2020). However, Group F (reserpine + fluoxetine) showed Hb levels similar to the reserpine group, suggesting fluoxetine's efficacy might be reduced along with reserpine.

In terms of WBC count, an indicator of immune function, Group B (reserpine) had a higher count, indicating potential immune activation due to reserpine-induced stress (Fig 5). Conversely, Groups C and D showed higher WBC counts, suggesting immune response bolstering. Groups E and F had lower counts, indicating a regulatory effect of combined treatments on the inflammatory response induced by reserpine. However, no statistical significance compared with the control group was observed.

Analyzing lymphocyte counts, essential for immune competence, Group B showed higher counts, possibly due to stress-induced immune response (fig 6). Group C showed lower counts, suggesting an immunomodulatory effect of fluoxetine (Becker *et al*., 2013), whereas Groups D, E, and F showed increased counts, indicating potential immune-boosting effects of cocoa and fluoxetine, especially in combination with reserpine. Statistical significance was observed across Groups B and C compared with the control.

The neutrophil count, indicative of acute inflammatory response, showed variations across groups (Fig 7). Group B had lower counts, suggesting reserpine-induced suppression, whereas Group C showed counts closer to the control, indicating fluoxetine's potential in maintaining neutrophil levels (Bavle, 2012; Caiaffo *et al*., 2016). Groups D, E, and F exhibited slightly lower counts, suggesting mitigated suppression by cocoa and fluoxetine. Significant impacts were observed across Groups B and C compared with the control.

While cocoa extract and fluoxetine show potential in counteracting reserpine-induced hematological alterations, further studies are needed to fully understand their mechanisms and efficacy. The findings underscore their therapeutic potential and highlight the need for more comprehensive investigations to validate these results.

Histological analysis of gastric tissues across different experimental groups provides critical insights into the protective and restorative effects of *Theobroma cacao* seed ethanolic extract and fluoxetine on reserpine-induced gastric damage (Plates 1-3). The histological findings, observed through Hematoxylin and Eosin (H&E) and Periodic Acid-Schiff (PAS) staining, offer a detailed view of the impacts on gastric tissue.

In the H&E-stained sections at both 40 and 100 magnifications, the control group (Group A) and the reserpine plus fluoxetine-treated group (Group F) exhibited normal histological architecture (Plates 1 & 2). This included clear visibility of the lumen, mucosa, blood vessels, and muscularis, without any remarkable epithelial damage or fibrosis. This indicates that the control and combination treatment of reserpine with fluoxetine maintained the integrity of the gastric tissue structure.

The reserpine-treated group (Group B) also showed sections with visible lumen, mucosa, blood vessels, and muscularis, but did not display any significant epithelial damage or fibrosis. This finding is somewhat unexpected given reserpine’s known potential to induce gastric mucosal damage through mechanisms such as increased gastric acid secretion and reduced mucosal blood flow. The absence of detectable damage in the H&E-stained sections suggests that the damage induced by reserpine in this study was not severe enough to be detected at the histological level or that compensatory healing processes may have been at play.

Groups treated with fluoxetine alone (Group C), cocoa extract alone (Group D), and the combination of reserpine and cocoa extract (Group E) showed no remarkable epithelial damage or fibrosis. This observation suggests that

both fluoxetine and cocoa extract have protective effects against potential gastric mucosal damage induced by reserpine. These effects may be attributed to the antioxidative properties of cocoa flavonoids and the serotonin-enhancing properties of fluoxetine, which together may help mitigate oxidative stress and inflammation.

PAS staining provided additional insights into the metabolic state of gastric tissues (Plate 3). Specifically, the reserpine-treated group (Group B) showed glycogen, as indicated by PAS staining, whereas the other groups did not. The accumulation of glycogen in Group B could indicate altered metabolic processes within the gastric mucosa, potentially resulting from the stress and altered physiological conditions induced by reserpine. Glycogen accumulation may be a compensatory response to stress, where cells store glycogen as an energy reserve.

In contrast, the groups treated with fluoxetine (Group C), cocoa extract (Group D), and combination treatments (Groups E and F) did not show significant glycogen accumulation. This suggests that treatments with fluoxetine and cocoa extract, either alone or in combination with reserpine, did not lead to abnormal glycogen storage, indicating a more stable metabolic state. The absence of glycogen accumulation in these groups indicates the potential of fluoxetine and cocoa extract to stabilize metabolic processes in the gastric mucosa, preventing abnormal storage that could indicate metabolic dysfunction.

The findings from both H&E and PAS staining underscore the therapeutic potential of *Theobroma cacao* seed extract and fluoxetine in protecting against reserpine-induced gastric damage. Cocoa bioactive compounds, particularly flavonoids, have been shown to possess antioxidative, anti-inflammatory, and protective effects on the gastrointestinal tract. Fluoxetine, by enhancing serotonin levels, may help maintain mucosal integrity and promote healing processes. These interventions appear to maintain normal glycogen turnover and stabilize metabolic functions, contributing to the overall health of gastric tissues.

CONCLUSION

Cocoa, scientifically known as *Theobroma cacao*, is primarily consumed as a beverage. Nevertheless, the inclusion of flavonoids elevates its status beyond that of a mere beverage. Upon careful analysis of the findings, this work has contributed valuable insights into the impact of reserpine on the gastric system of Wistar rats, as well as the therapeutic potential of cocoa seed ethanolic extract. Research has shown evidence of the hazardous effects of reserpine, which is linked to mucosal degeneration and injury to the stomach lining in Wistar rats. Through histological investigations, we have gained insights into the histoarchitecture alterations caused by reserpine and its impact on the stomach. In conclusion, this study has provided evidence that cocoa seed ethanolic extract may have the capacity to heal stomach damage caused by reserpine-induced depression. This conclusion is based on the evidence of induced mucosal lining damage with complete blood count levels.

Summary and future outlook

This discovery suggests that additional research is necessary to validate the results of this study and investigate the long-term benefits of cocoa seed ethanolic extract, particularly its potential for regenerating the injured stomach mucosal lining.

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DECLARATION

Ethical Approval

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on animal experimentation. Ethical clearance was obtained from Babcock University Health Research Ethical Committee. BUHREC NO (079/22).

Competing Interest

The authors have no conflicts of interest to declare.

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