



Research article

Sub-chronic effects of ethanolic leaf extract of *Ocimum gratissimum* on glucose homeostasis, haematological indices, oxidative stress markers, and liver histology in adult Wistar rats

Chioma Akunnaya Ekenna-Ohanenye¹, Uloaku Akubueze Nto-Ezimah², Nto Johnson Nto*³, Faustina Chiamaka Irozulike¹, Peace Kelechi Godson¹

¹ Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Science, Rhema University, Aba, Nigeria

² Department of Chemical Pathology, Faculty of Medical Sciences, College of Medicine, University of Nigeria, Enugu Campus, Nigeria

³ Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria, Enugu Campus, Nigeria

Corresponding author: Nto Johnson Nto, ✉ johnson.nto@unn.edu.ng, **Orcid Id:** <https://orcid.org/0000-0001-9601-9870>

Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria, Enugu Campus, Nigeria

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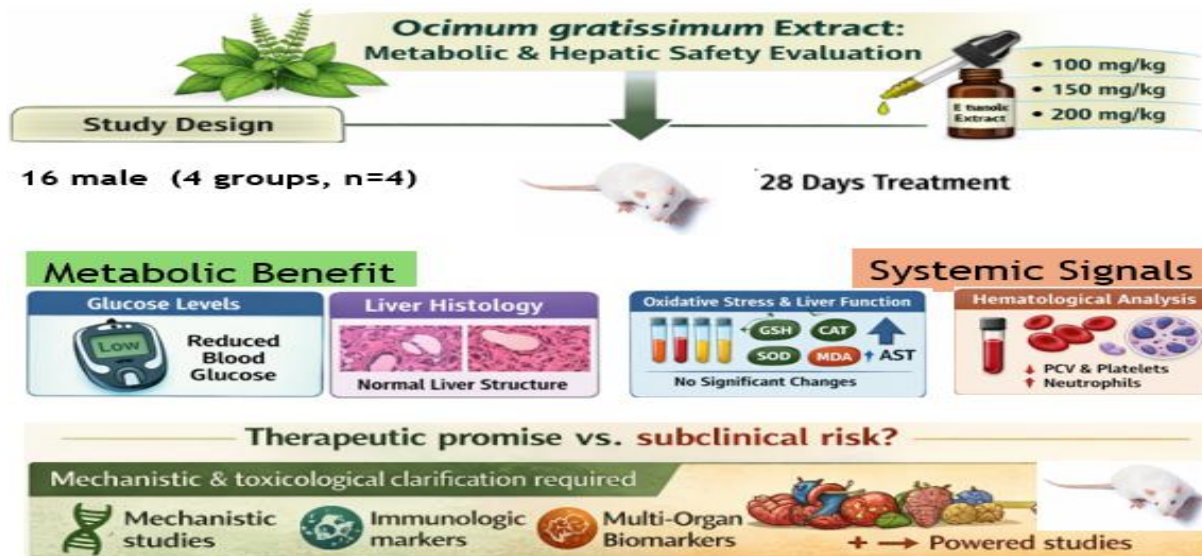
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ABSTRACT

Ocimum gratissimum is widely used in traditional medicine for metabolic disorders; however, systematic evaluation of its metabolic, haematological, and hepatic safety profile remains limited. This study investigated the effects of ethanolic leaf extract of *O. gratissimum* on glucose homeostasis, haematological indices, oxidative stress markers, liver enzymes, and hepatic histology in rats. Sixteen adult Wistar rats were randomly assigned to four groups (n = 4). The control group received standard feed and water, while treatment groups were administered ethanolic leaf extract of *O. gratissimum* at doses of 100, 150, and 200 mg/kg body weight daily for 28 days. Blood glucose level was measured, alongside comprehensive haematological profiling. Hepatic oxidative stress markers, reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were quantified. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities were assessed.



Liver histology was evaluated using hematoxylin and eosin staining. *O. gratissimum* extract administration reduced blood glucose level ($p < 0.05$). Most hematological parameters remained unchanged, although packed cell volume and platelet counts were reduced, while neutrophil percentages increased. Hepatic antioxidant enzyme activities and MDA levels showed no significant alterations. ALT and ALP activities remained comparable to controls, whereas AST levels increased dose-dependently ($p < 0.05$). Liver architecture remained intact across all treatment groups. Sub-chronic administration of ethanolic leaf extract of *O. gratissimum* resulted in antihyperglycaemic effects without histopathological evidence of hepatic injury, although mild dose-dependent AST elevation warrants further investigation.

Keywords: *Ocimum gratissimum*, Blood glucose, Oxidative stress, Liver enzymes, Haematology, Ethnopharmacology.

INTRODUCTION

Medicinal plants remain a major source of bioactive compounds with therapeutic relevance and continue to play a central role in primary healthcare, particularly in developing countries where access to conventional medicines may be limited [1–3]. The use of plant-based remedies underscores the need for systematic evaluation of their biological effects and safety profiles. *Ocimum gratissimum* L. (Lamiaceae), commonly known as African basil or scent leaf, is widely used in traditional medicine to manage metabolic disorders, infections, inflammatory conditions, and gastrointestinal disturbances [4–6].

The pharmacological activity of *O. gratissimum* has been linked to its rich phytochemical composition, including flavonoids, alkaloids, tannins, phenolic compounds, and essential oils [4,7,8]. These constituents have been reported to exhibit antioxidant, antimicrobial, anti-inflammatory, and hypoglycaemic activities [7–9]. Antioxidant properties are of particular interest due to their potential to mitigate oxidative stress-mediated tissue injury, especially in metabolically active organs such as the liver [10, 11]. However, bioactive compounds may exert dose- and duration-dependent effects, hence the need for toxicological evaluation.

The liver plays an important role in metabolism, detoxification, and systemic homeostasis, rendering it especially susceptible to xenobiotic-induced injury and oxidative stress [10–12]. Hepatic impairment is frequently accompanied by alterations in haematological parameters, including changes in packed cell volume, haemoglobin concentration, leukocyte distribution, and platelet count [13–15]. Consequently, assessment of haematological indices, together with liver histopathology and systemic oxidative stress markers such as superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), and malondialdehyde (MDA), provides a comprehensive approach for evaluating both the safety and biological effects of bioactive plant extracts.

Wistar rats are commonly utilised in experimental pharmacology and toxicology due to their well-characterised physiology and translational relevance [16]. Previous studies investigating *O. gratissimum* have reported variable haematological outcomes, ranging from enhanced erythropoietic activity to reductions in red blood cell indices and platelet counts, depending on

extract type, dosage, and duration of exposure [17–21]. Similarly, while some reports indicate preserved hepatic architecture following extract administration, others have documented mild histological alterations at higher doses [22, 23].

Despite its widespread ethnomedicinal use, there is limited data on the combined haematological and hepatic effects of sub-chronic administration of ethanolic leaf extract of *Ocimum gratissimum* on haematological parameters, systemic oxidative stress status, blood glucose levels, and liver histology. Therefore, this study aimed to evaluate the effects of ethanolic leaf extract of *O. gratissimum* on haematological indices, oxidative stress markers (SOD, GSH, CAT, and MDA), blood glucose levels, and liver histology in adult Wistar rats.

MATERIALS AND METHODS

Study design

This study was designed as an exploratory experimental investigation to evaluate the metabolic, hepatic, oxidative, and haematological effects of ethanolic leaf extract of *Ocimum gratissimum* in adult Wistar rats.

Four parallel groups were included ($n = 4$ animals per group). The experimental unit was an individual animal.

Primary outcomes were blood glucose concentration and serum liver enzyme activities (ALT, AST, ALP). Secondary outcomes were oxidative stress markers (SOD, CAT, GSH, MDA), haematological parameters, and histopathological changes in liver tissue

Ethical statement

All experimental procedures were conducted in accordance with the institutional guidelines for the care and use of laboratory animals and complied with national regulations governing animal research. Ethical approval was obtained from the College of Medicine Research Ethics Committee, University of Nigeria, Enugu Campus (UNN/FBS/REC). All efforts were made to minimise animal suffering and reduce animal numbers consistent with scientific objectives.

Sample size justification

Sixteen animals ($n = 4$ per group) were used. The study was conducted as a preliminary exploratory investigation to generate effect-size estimates for future adequately powered studies. No a

priori power calculation was performed due to the exploratory nature of the study.

Experimental animals

Species: Rat (*Rattus norvegicus*)

Strain: Wistar

Sex: Male

Age: 8–10 weeks

Weight range at baseline: 180 - 200 g

Animals were obtained from the Animal House, Department of Anatomy, University of Nigeria.

Housing and husbandry

The animals were acclimatised for two weeks under standard laboratory conditions (12-hour light/dark cycle, ambient temperature) and fed standard growers' mash with access to distilled water ad libitum. All experimental procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals.

Randomisation and blinding

Following acclimatisation, animals were randomly allocated to experimental groups using a simple randomisation method.

Outcome assessments (biochemical and histological analyses) were conducted by investigators blinded to group allocation.

Inclusion and exclusion criteria

Inclusion criteria: 1. Clinically healthy male Wistar rats. 2. No signs of infection or injury.

Exclusion criteria: 1. Animals showing illness during acclimatisation. 2. Samples with hemolysis affecting biochemical assays

No animals were excluded after allocation.

Plant material collection and authentication

Fresh leaves of *Ocimum gratissimum* L. were obtained from Ogbete Market, Enugu State, Nigeria. Botanical authentication was performed at the Department of Botany, University of Nigeria, Nsukka. A voucher specimen (UNH 360) was deposited in the University of Nigeria Herbarium. Leaves were washed, air-dried at room temperature for two weeks, and pulverised. Five hundred grams (500 g) of powdered material were used for extraction.

Preparation of ethanolic extract

Powdered leaves were extracted by maceration in ethanol at a defined solvent-to-plant ratio. The mixture was intermittently agitated over the extraction period, filtered, and concentrated under reduced pressure using controlled evaporation.

The crude extract yield (%) was calculated and recorded. Extracts were stored at 4°C in airtight containers until administration.

Treatment protocol

Following acclimatisation, rats were randomly assigned to four groups (n = 4 per group). Group I served as the control and received standard feed and water only. Groups II, III, and IV received ethanolic leaf extract of *O. gratissimum* at doses of 100, 150, and 200 mg/kg body weight, respectively. The extract was administered orally once daily via oral gavage for 28 consecutive days. Body weights were monitored throughout the experimental period. Dose selection was based on previous literature reporting the biological activity of *O. gratissimum* extracts within this range [4].

Blood glucose determination

Blood glucose level was measured daily for 28 days. It was determined by tail-tip puncture, and measurements were obtained using an Accu-Chek® glucometer (Roche Diagnostics) in accordance with the manufacturer's protocol. Glucose concentrations were expressed in mg/dL.

Blood sample collection and haematological analysis

At study termination (Day 28), animals were anaesthetized. Blood samples were collected via cardiac puncture. Haematological parameters, including packed cell volume, haemoglobin concentration, red blood cell count, total white blood cell count, platelet count, and differential leukocyte counts, were analysed using an automated haematology analyser (Cell-Dyn, Abbott, USA) following standard laboratory procedures.

Assessment of oxidative stress and antioxidant enzyme activities

Liver tissues were excised immediately after sacrifice, rinsed in ice-cold normal saline, and homogenised in an appropriate cold phosphate buffer. The homogenates were centrifuged, and the resulting supernatants were used for biochemical analyses.

SOD activity in liver homogenates was determined spectrophotometrically based on the enzyme's ability to inhibit the autoxidation of epinephrine [24]. The reaction mixture consisted of carbonate buffer and tissue supernatant, and the change in absorbance was monitored at 500 nm over a defined time interval. SOD activity was expressed as the percentage inhibition of epinephrine autoxidation.

Reduced glutathione (GSH) levels in liver tissue were quantified using Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid; DTNB), based on the formation of a yellow-colored chromogen measured spectrophotometrically at 412 nm. Glutathione concentration was expressed relative to protein content [25, 26].

Catalase (CAT) activity in liver homogenates was assessed by measuring the rate of decomposition of hydrogen peroxide (H₂O₂) at 240 nm using a spectrophotometric method [25]. Catalase activity was expressed as units per milligram of protein.

Lipid peroxidation was evaluated by determining malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) assay [24, 25]. Serum samples were reacted with

thiobarbituric acid and incubated at high temperature to allow formation of the MDA–TBA complex. The resulting chromogen was measured spectrophotometrically, and MDA concentration was used as an index of lipid peroxidation.

Determination of serum liver enzyme activities

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities were determined using standard enzymatic colourimetric methods [27] with commercially available diagnostic assay kits, in accordance with the manufacturers' instructions. Briefly, blood samples were collected, allowed to clot, and centrifuged to obtain serum. Enzyme activities were measured spectrophotometrically at their respective wavelengths and expressed in international units per litre (IU/L).

Tissue collection and histological processing

Following blood collection, animals were sacrificed under anaesthesia, and liver tissues were carefully excised, rinsed in normal saline to remove blood, and fixed in 10% neutral buffered formalin for 24 hours. The tissues were dehydrated through ascending grades of ethanol, cleared in xylene, and embedded in molten paraffin wax at 60 °C [28].

Paraffin-embedded tissues were sectioned at approximately 5 µm thickness using a rotary microtome. Sections were mounted on glass slides, deparaffinized, rehydrated, and stained with haematoxylin and eosin (H&E) [29, 30]. Stained sections were examined under a light microscope for histopathological evaluation, and photomicrographs were captured to document histological features.

Statistical analysis

Data was analysed using the Statistical Package for Social Sciences (SPSS) Version 23 (IBM Computers USA). Results were expressed as mean ± standard deviation (SD). Normality assessed prior to analysis. Differences among groups were evaluated using one-way analysis of variance (ANOVA), and statistical significance was set at $p < 0.05$.

RESULTS

Effects of *Ocimum gratissimum* extract on blood glucose levels

Oral administration of ethanolic leaf extract of *Ocimum gratissimum* for 28 days resulted in a significant reduction in mean blood glucose levels ($p < 0.05$, one-way ANOVA) in the treated groups compared with the control group (Figure 1).

Effects of *Ocimum gratissimum* extract on haematological parameters

Erythrocyte indices

Mean PCV was significantly ($p < 0.05$; one-way ANOVA) reduced in rats treated with ethanolic leaf extract of *Ocimum gratissimum* compared with the control group, with this effect observed across all treatment doses (Figure 2). In contrast, no significant differences ($p > 0.05$; one-way ANOVA) were observed

in mean hemoglobin concentration (Figure 3) and RBC (Figure 4) count among the treatment groups compared with the control.

Mean MCV was significantly ($p < 0.05$; one-way ANOVA) elevated in all extract-treated groups compared with the control group (Figure 5). Mean MCHC did not differ significantly ($p > 0.05$ one-way ANOVA) between the low- and medium-dose groups and the control (Figure 6); however, a significant ($p < 0.05$; one-way ANOVA) increase in MCHC was observed in the high-dose group relative to both the control and other treatment groups (Figure 6). Mean MCH (showed no statistically significant difference ($p > 0.05$; one-way ANOVA) in the treatment groups compared to the control groups (Figure 7).

Platelet and leukocyte parameters

Platelet counts were significantly reduced ($p < 0.05$; one-way ANOVA) in rats administered *O. gratissimum* extract compared with the control group, with this effect evident at all treatment doses (Figure 8). Total white blood cell (WBC) counts did not differ significantly ($p > 0.05$; one-way ANOVA) among groups (Figure 9). Differential leukocyte analysis revealed a significant increase ($p < 0.05$; one-way ANOVA) in neutrophil percentage in all treatment groups compared with controls (Figure 10). Conversely, lymphocyte levels were significantly reduced ($p < 0.05$; one-way ANOVA) in extract-treated rats compared to the control group (Figure 11). No significant differences ($p > 0.05$; one-way ANOVA) were observed in eosinophil counts between treated and control animals (Figure 12).

Effects of *Ocimum gratissimum* extract on hepatic oxidative stress markers

The effects of ethanolic leaf extract of *Ocimum gratissimum* on hepatic antioxidant status and lipid peroxidation are presented in Figures 13-16. There were no statistically significant differences ($p > 0.05$; one-way ANOVA) in hepatic reduced glutathione (GSH) levels among the extract-treated groups compared with the control group (Figure 13). Similarly, hepatic superoxide dismutase (SOD) activity did not differ significantly ($p > 0.05$; one-way ANOVA) between treated animals and controls across all administered doses (Figure 14).

Catalase activity in liver tissue also remained comparable between the treatment groups and the control group, with no significant differences ($p > 0.05$; one-way ANOVA) observed following extract administration (Figure 15). In addition, hepatic malondialdehyde (MDA) levels, an index of lipid peroxidation, showed no significant differences ($p > 0.05$; one-way ANOVA) between extract-treated rats and controls (Figure 16).

Effects of *Ocimum gratissimum* extract on liver enzyme activities

Mean serum ALT activity did not differ significantly ($p > 0.05$; one-way ANOVA) between extract-treated rats and the control group following 28 days of oral administration (Figure 17).

Comparable ALT levels were observed across all treatment doses (100, 150, and 200 mg/kg) (Figure 17).

In contrast to ALT, serum AST activity was significantly elevated ($p < 0.05$; one-way ANOVA) in all extract-treated groups compared with the control group (Figure 18). This increase was dose dependent.

No statistically significant differences ($p > 0.05$; one-way ANOVA) were observed in serum ALP activity between extract-treated rats and the control group (Figure 19).

Effects of *Ocimum gratissimum* extract on liver tissue

Histological examination of liver sections stained with haematoxylin and eosin revealed preserved hepatic architecture

across all experimental groups (Figure 20). Liver sections from the control group demonstrated normal histological features, including well-organised hepatic cords, intact sinusoids, and a clearly defined portal triad comprising the portal vein, hepatic artery, and bile duct.

Similarly, liver sections obtained from rats treated with ethanolic leaf extract of *Ocimum gratissimum* at doses of 100, 150, and 200 mg/kg exhibited normal hepatic morphology. Hepatocytes appeared intact with preserved cytoplasmic and nuclear features, and no evidence of cellular degeneration, inflammatory infiltration, sinusoidal congestion, necrosis, or fibrosis was observed at any treatment dose. These findings indicate that oral administration of the extract did not induce overt histopathological alterations in liver tissue.

Figure 1: Effect of graded doses of ethanolic leaf extract of *Ocimum gratissimum* on mean blood glucose levels in adult Wistar rats following 28 days of oral administration

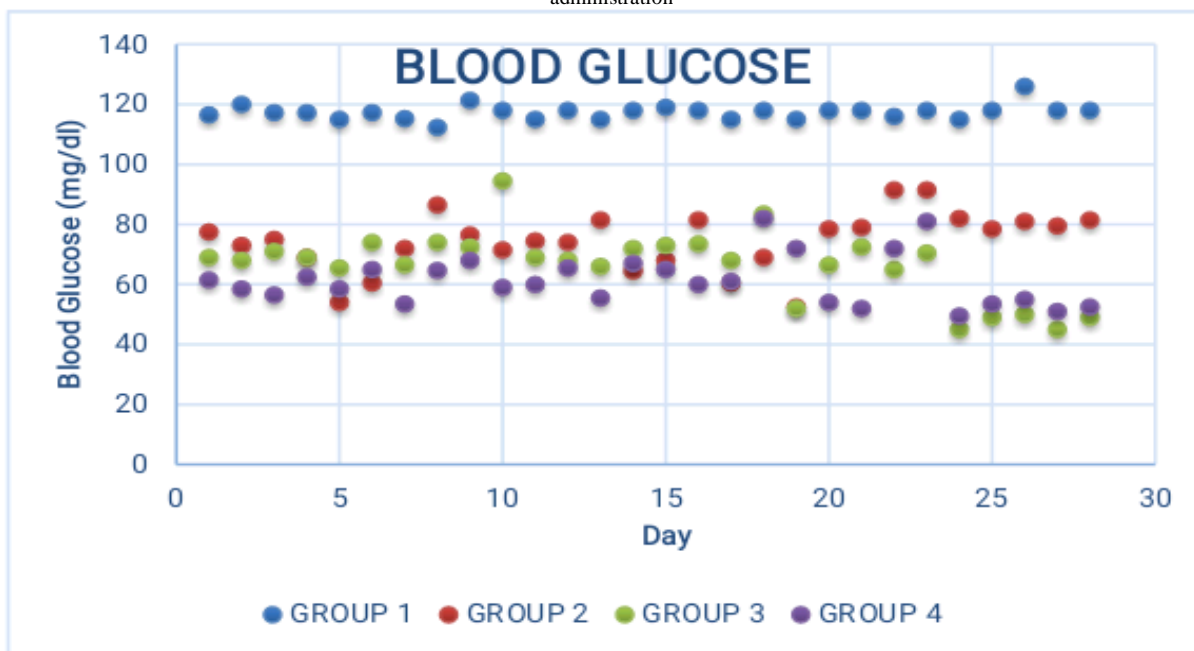


Figure 2: Effect of ethanolic leaf extract of *Ocimum gratissimum* on packed cell volume (PCV)

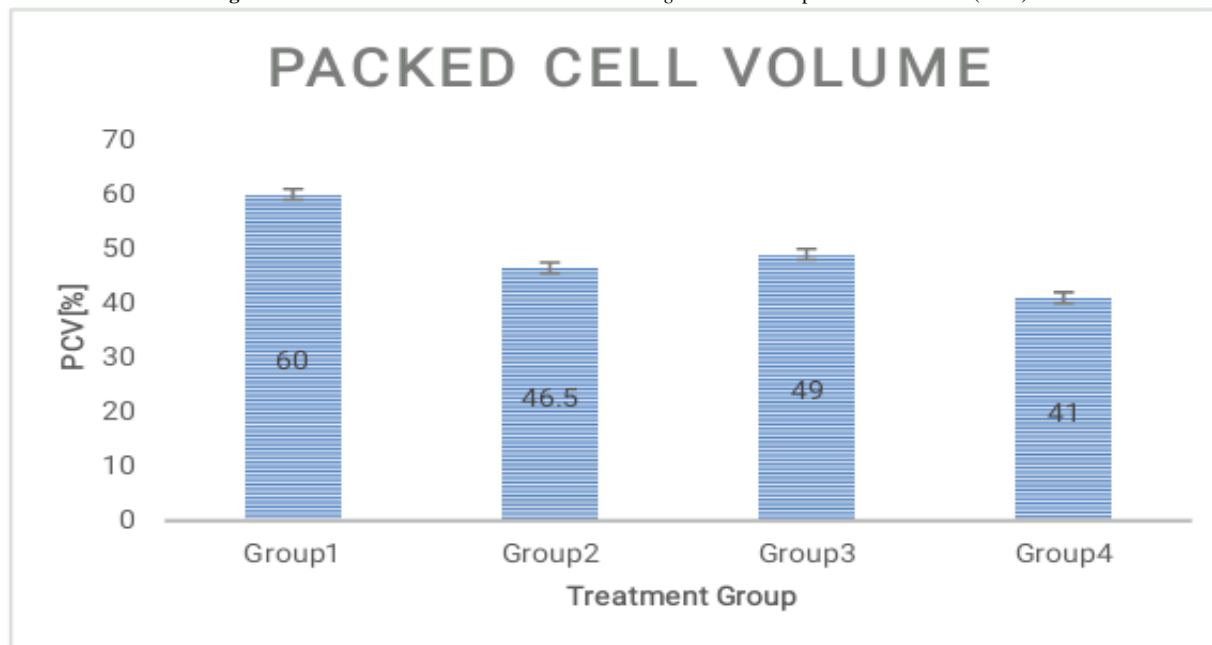


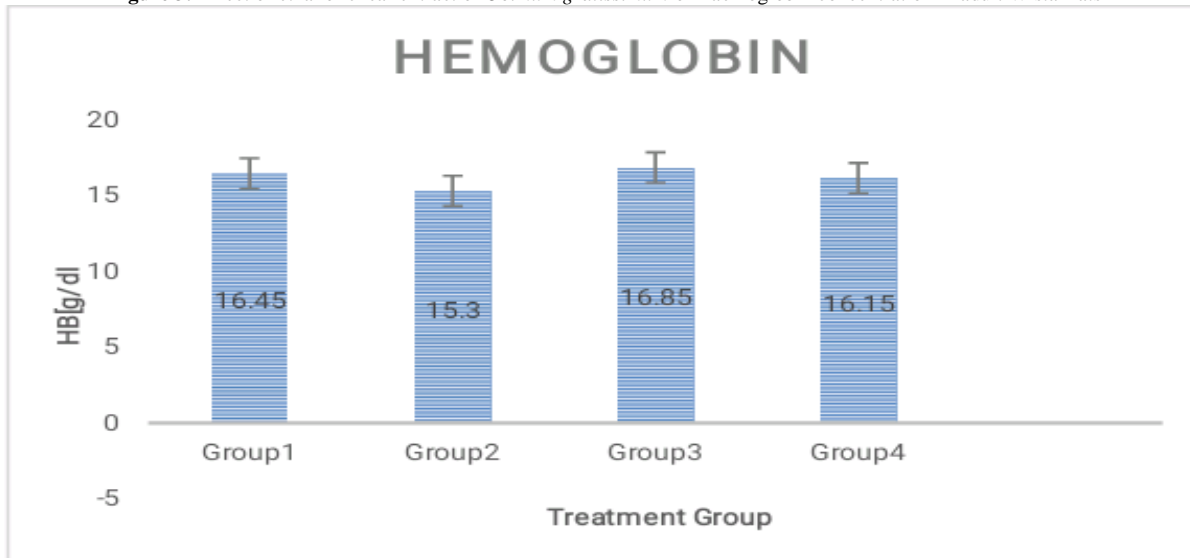
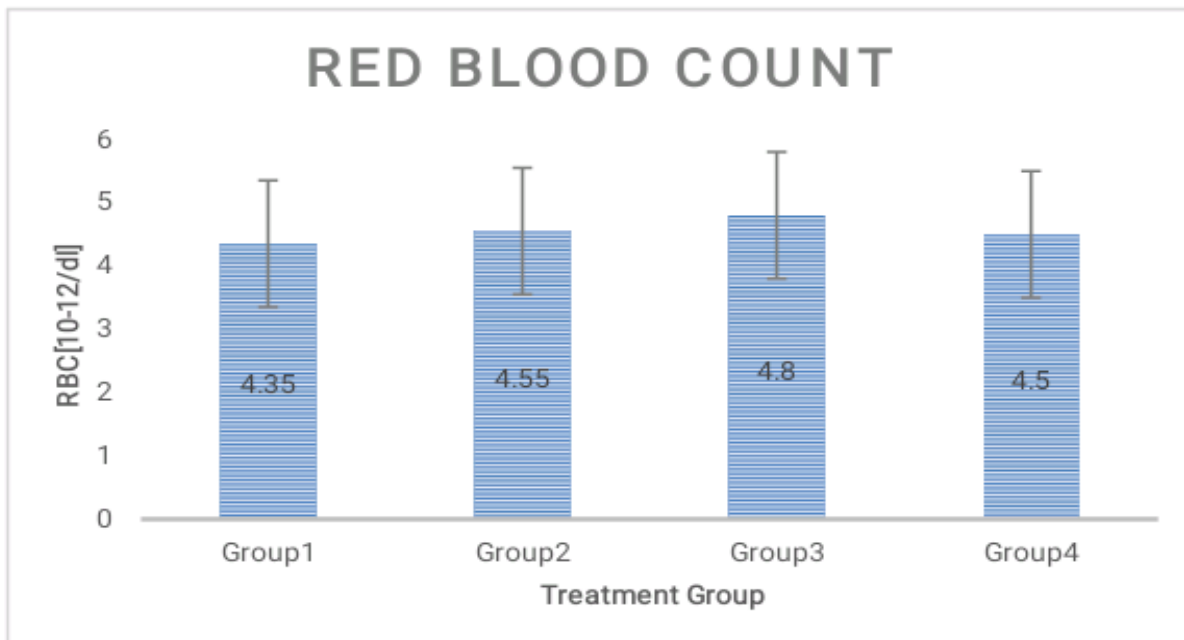
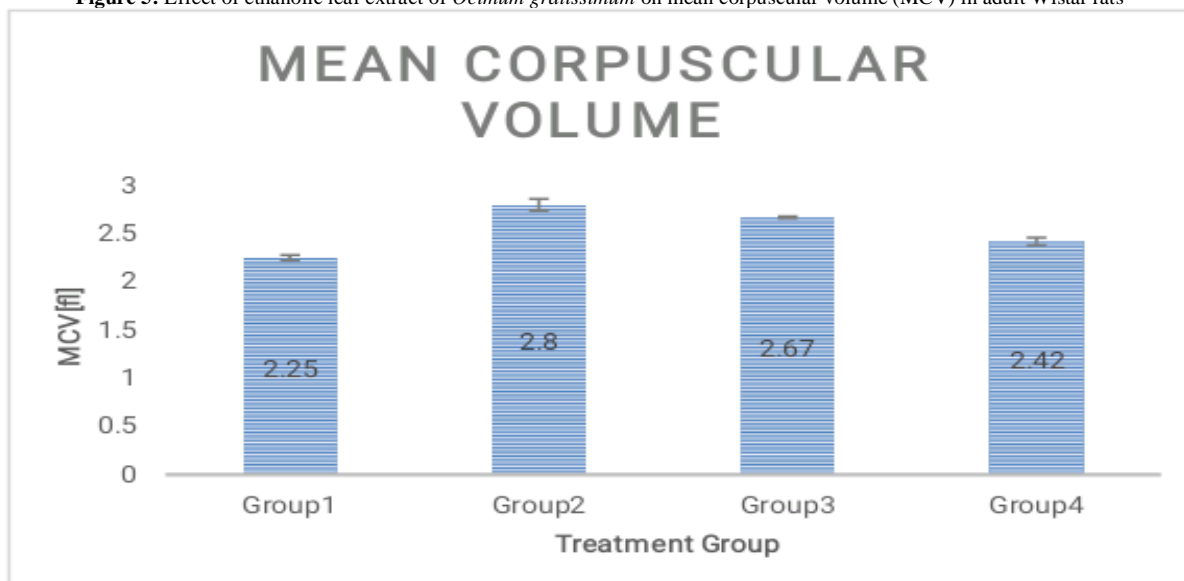
Figure 3: Effect of ethanolic leaf extract of *Ocimum gratissimum* on haemoglobin concentration in adult Wistar rats**Figure 4:** Effect of ethanolic leaf extract of *Ocimum gratissimum* on red blood cell count in adult Wistar rats**Figure 5:** Effect of ethanolic leaf extract of *Ocimum gratissimum* on mean corpuscular volume (MCV) in adult Wistar rats

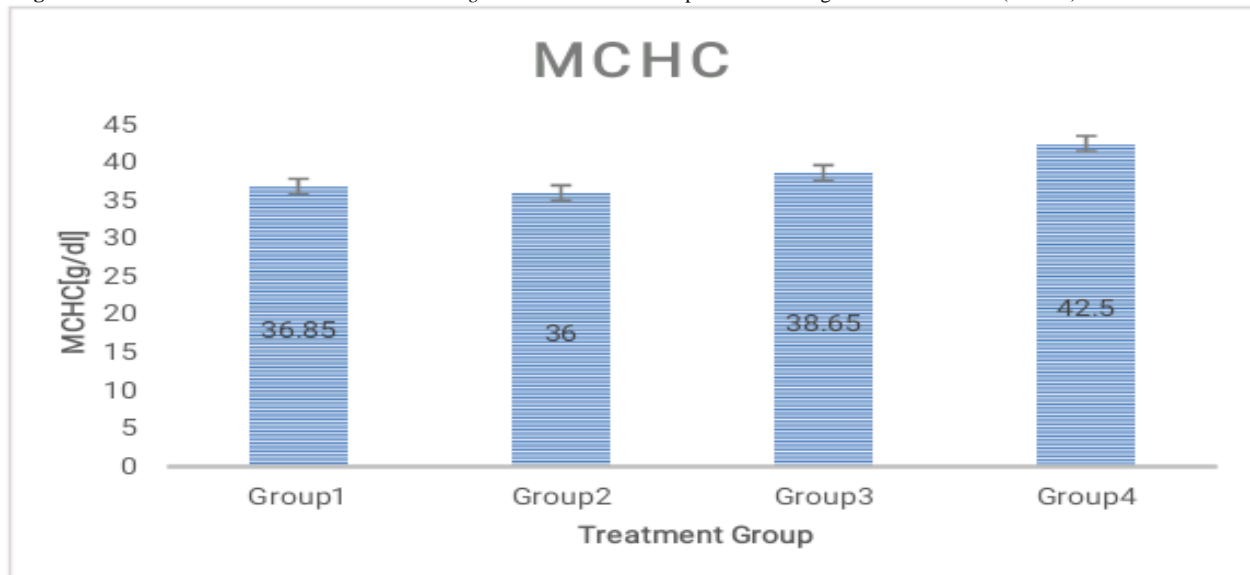
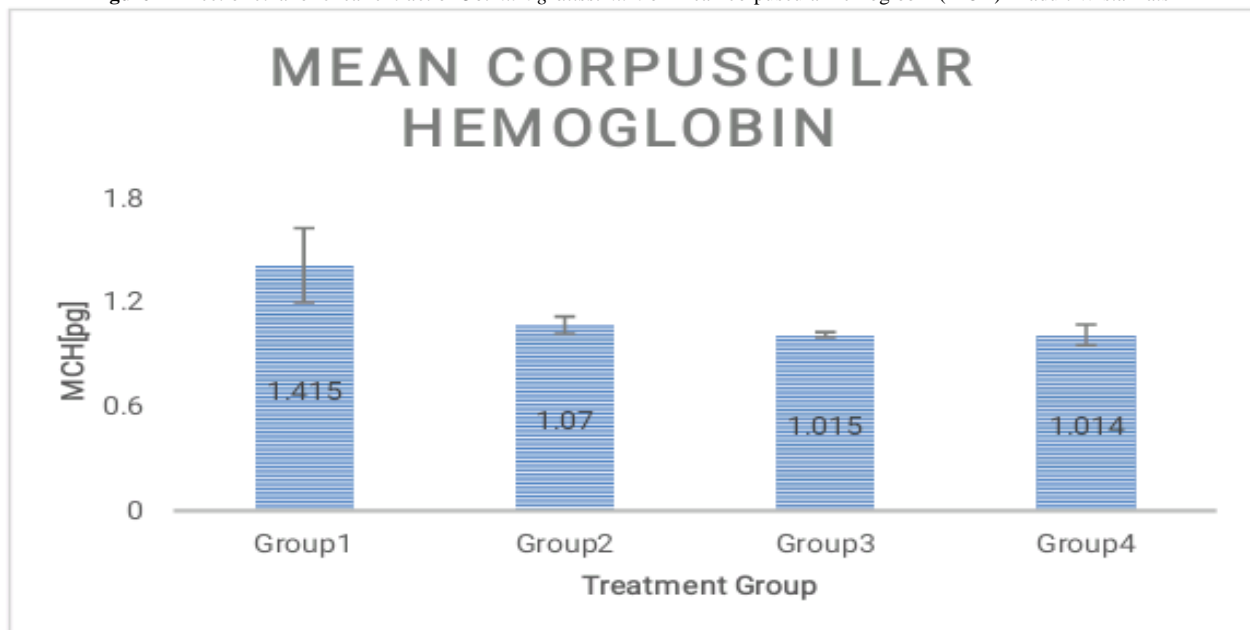
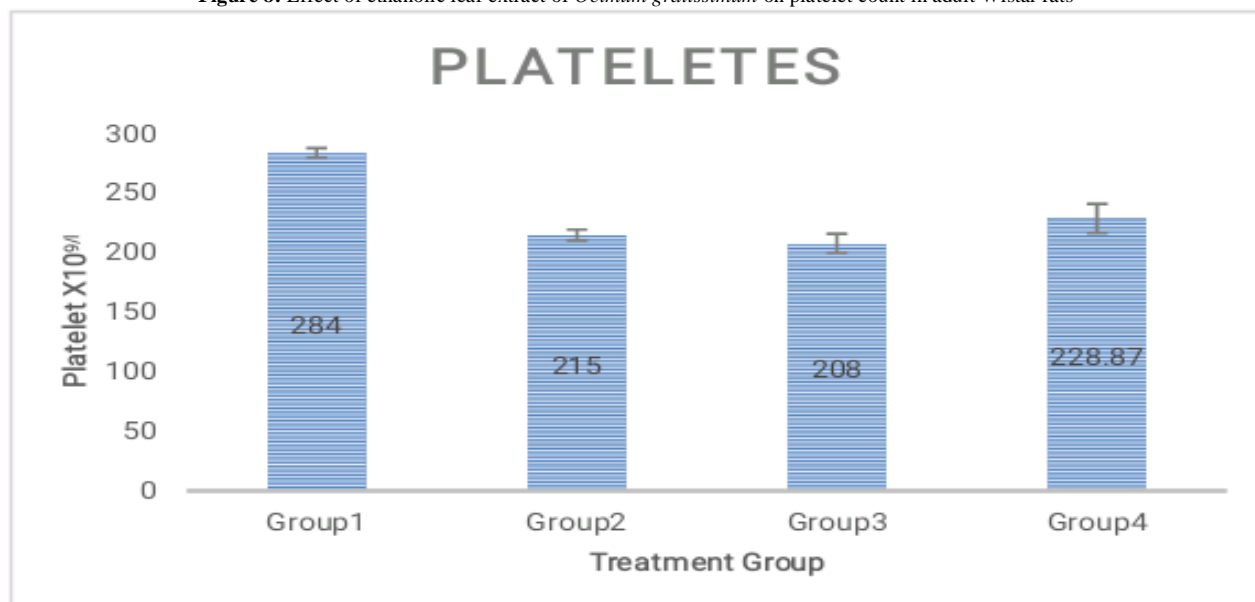
Figure 6: Effect of ethanolic leaf extract of *Ocimum gratissimum* on mean corpuscular haemoglobin concentration (MCHC) in adult Wistar rats**Figure 7** Effect of ethanolic leaf extract of *Ocimum gratissimum* on mean corpuscular hemoglobin (MCH) in adult Wistar rats**Figure 8:** Effect of ethanolic leaf extract of *Ocimum gratissimum* on platelet count in adult Wistar rats

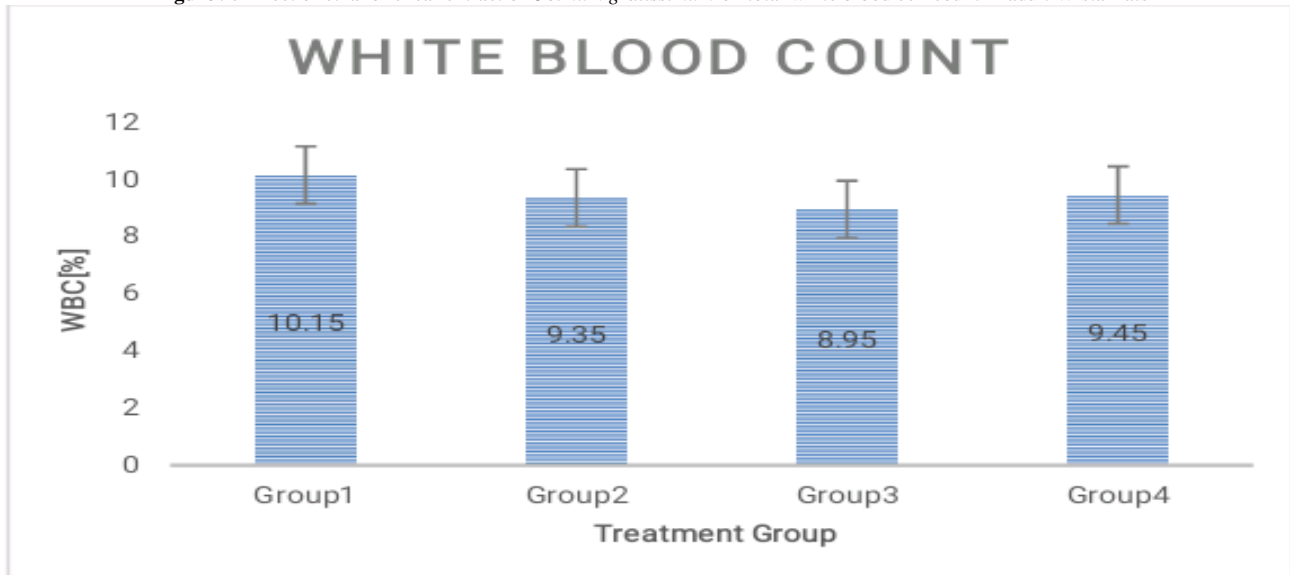
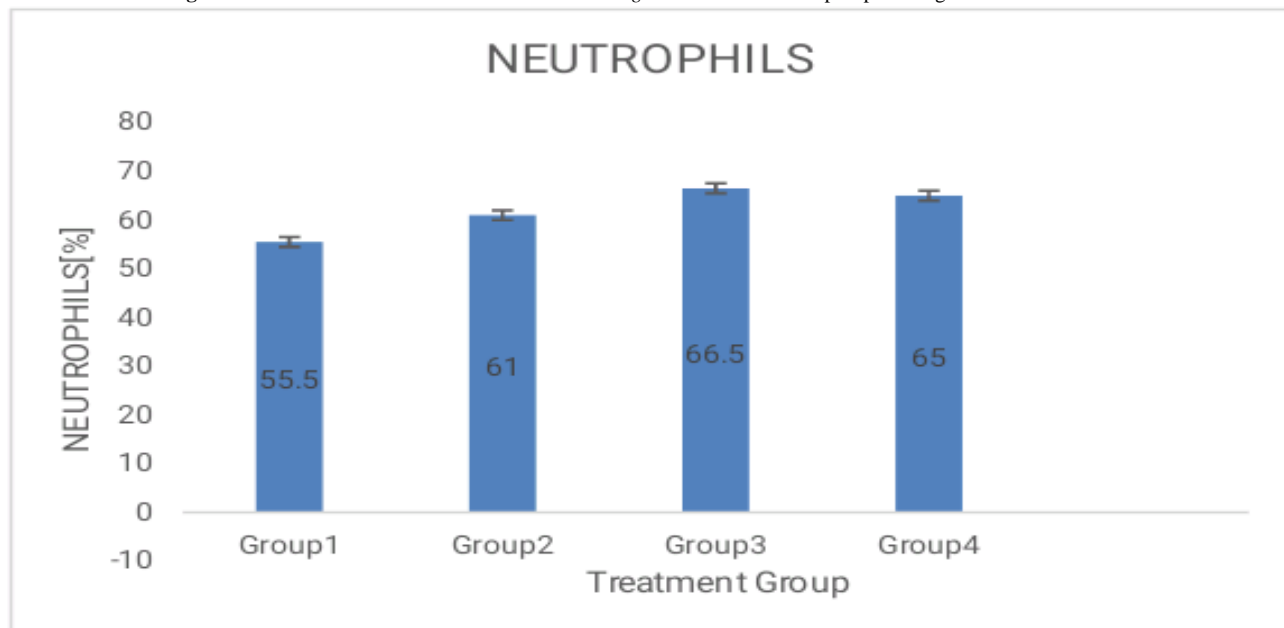
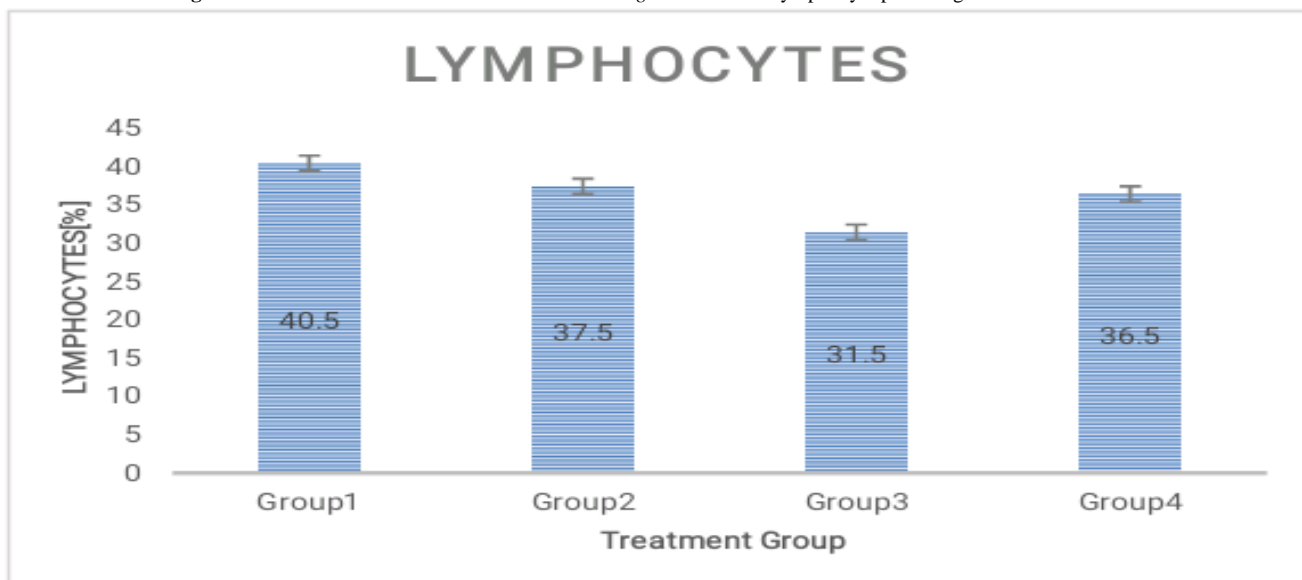
Figure 9: Effect of ethanolic leaf extract of *Ocimum gratissimum* on total white blood cell count in adult Wistar rats**Figure 10:** Effect of ethanolic leaf extract of *Ocimum gratissimum* on neutrophil percentage in adult Wistar rats**Figure 11:** Effect of ethanolic leaf extract of *Ocimum gratissimum* on lymphocyte percentage in adult Wistar rats

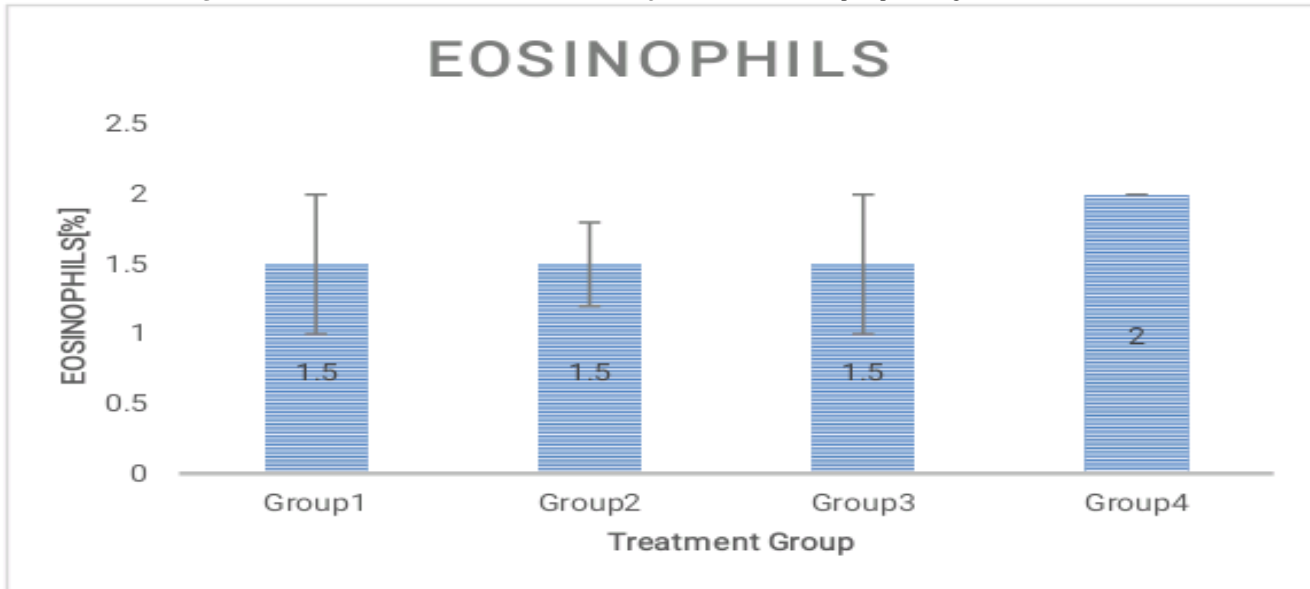
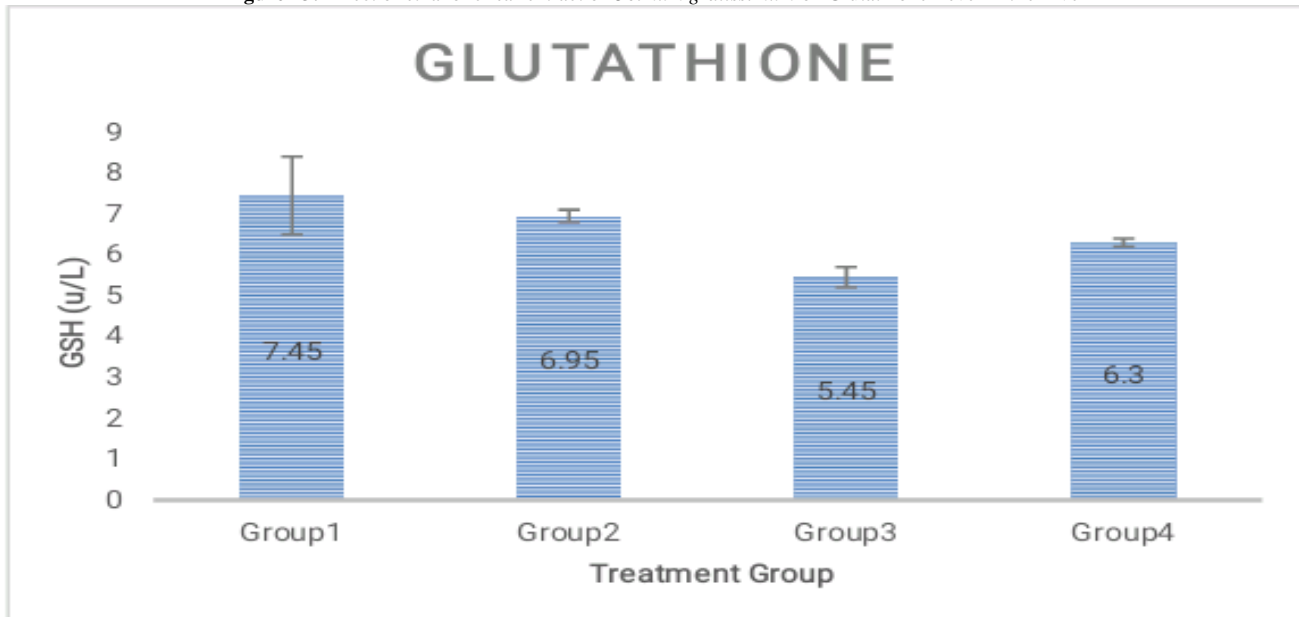
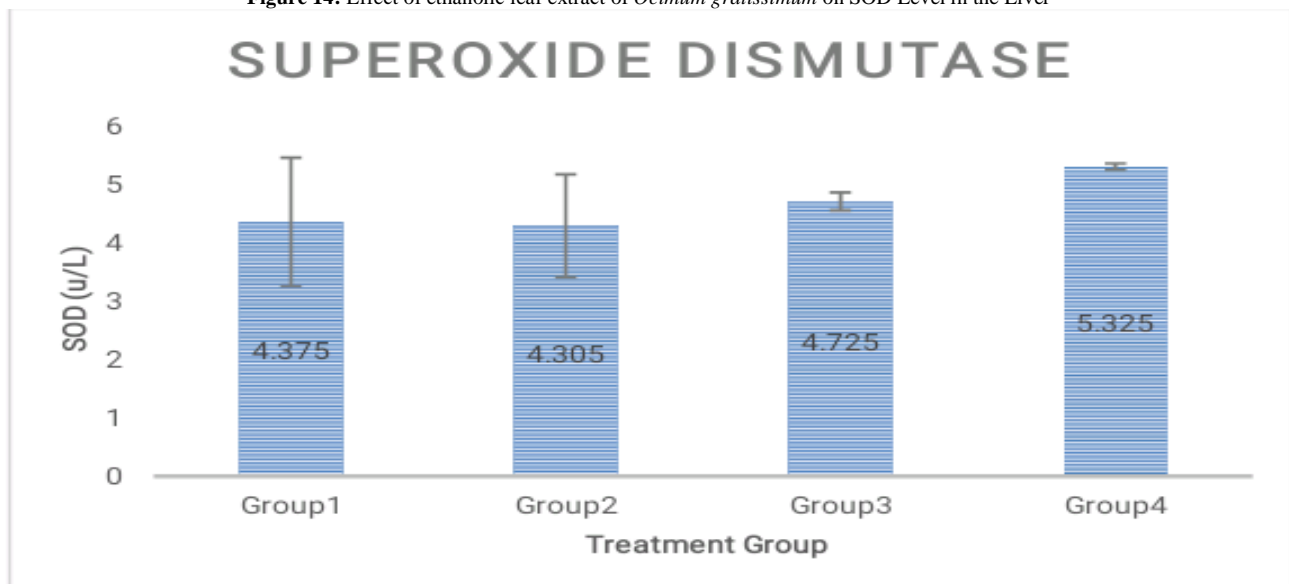
Figure 12: Effect of ethanolic leaf extract of *Ocimum gratissimum* on eosinophil percentage in adult Wistar rats**Figure 13:** Effect of ethanolic leaf extract of *Ocimum gratissimum* on Glutathione Level in the Liver**Figure 14:** Effect of ethanolic leaf extract of *Ocimum gratissimum* on SOD Level in the Liver

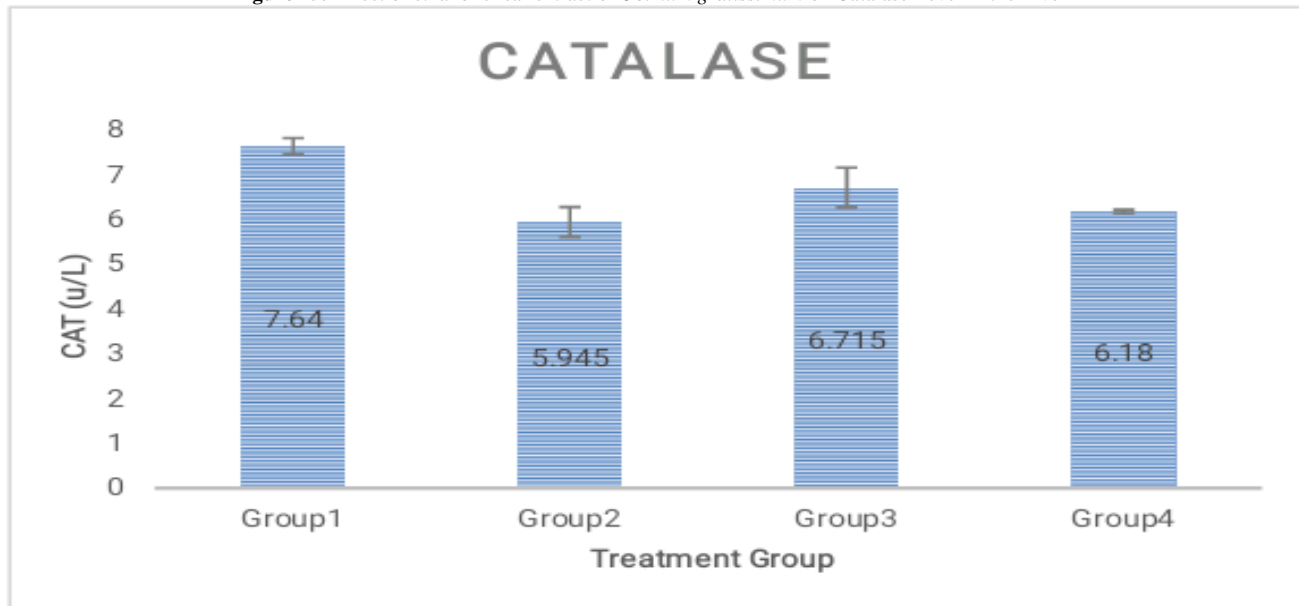
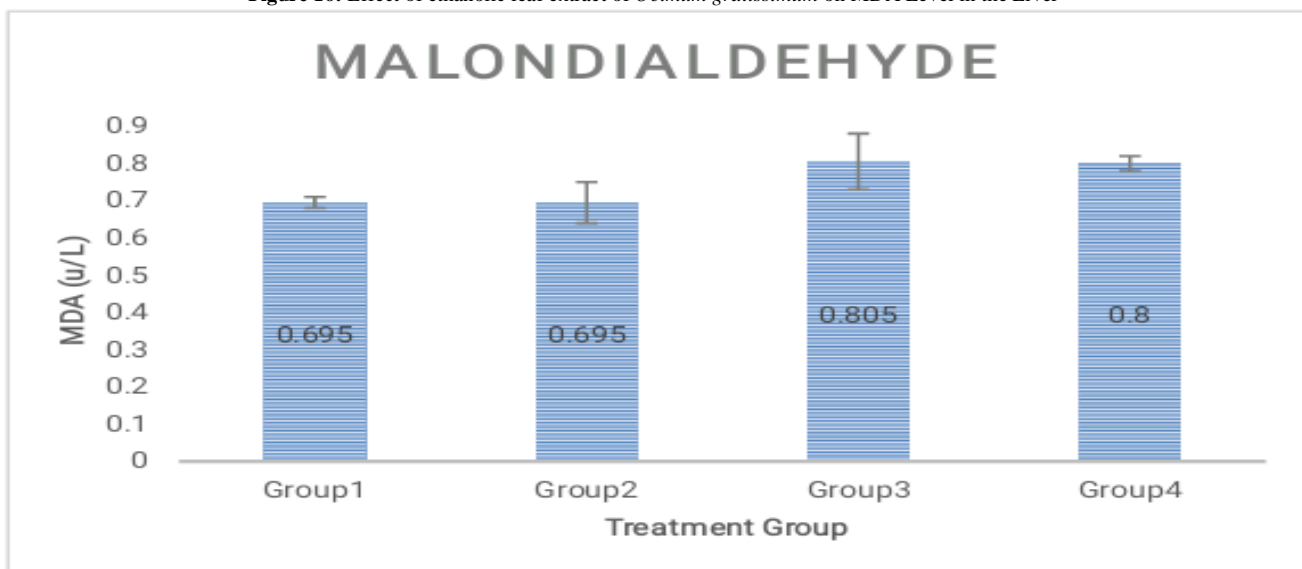
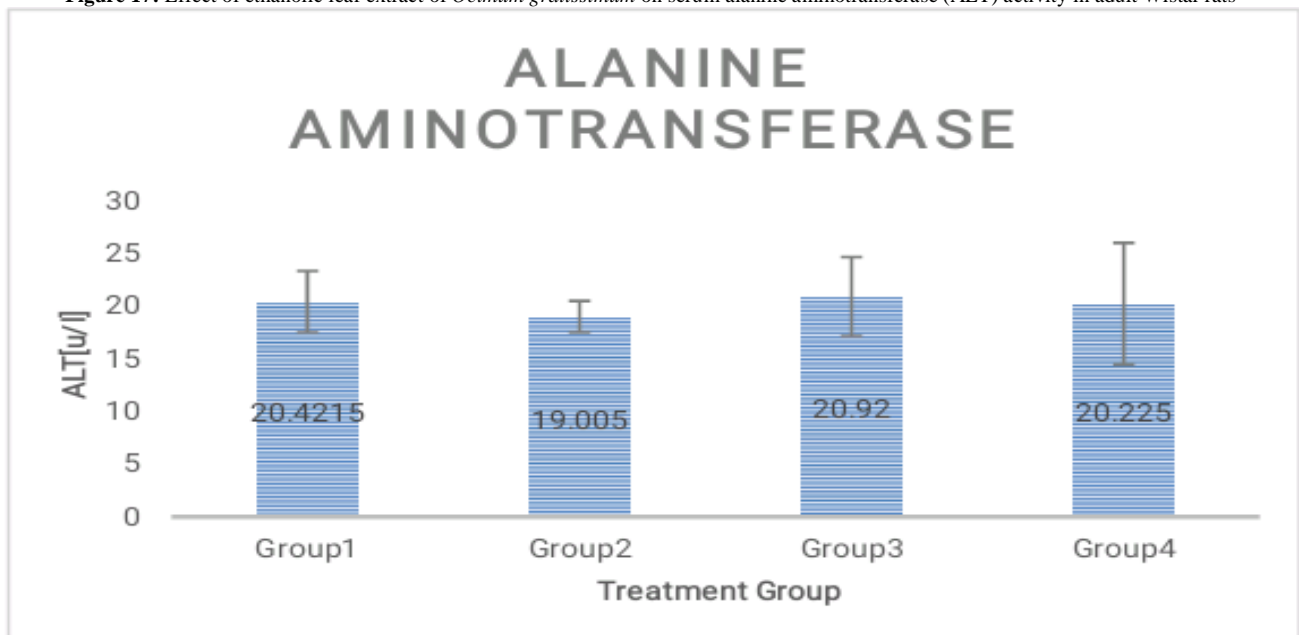
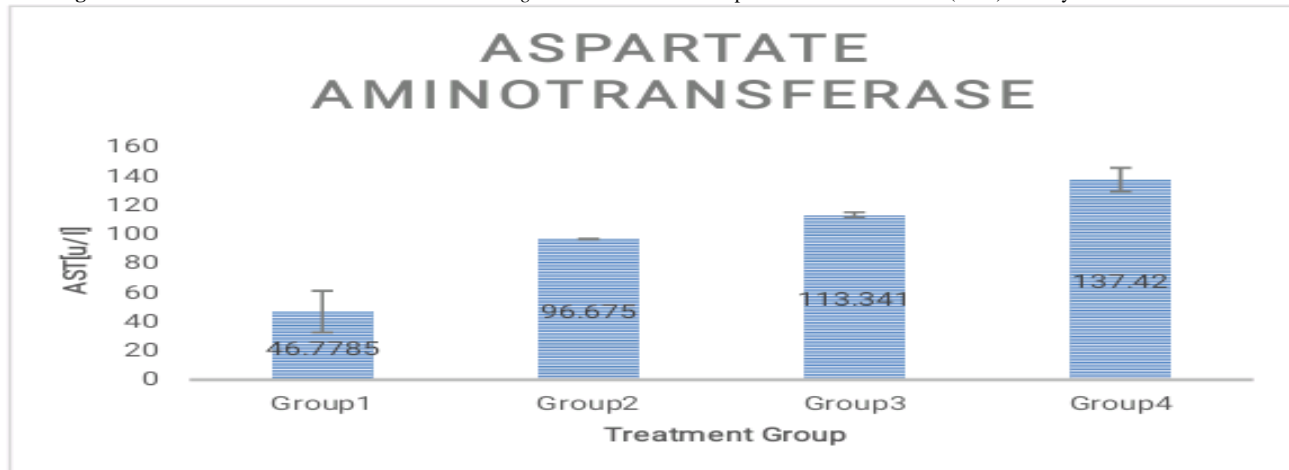
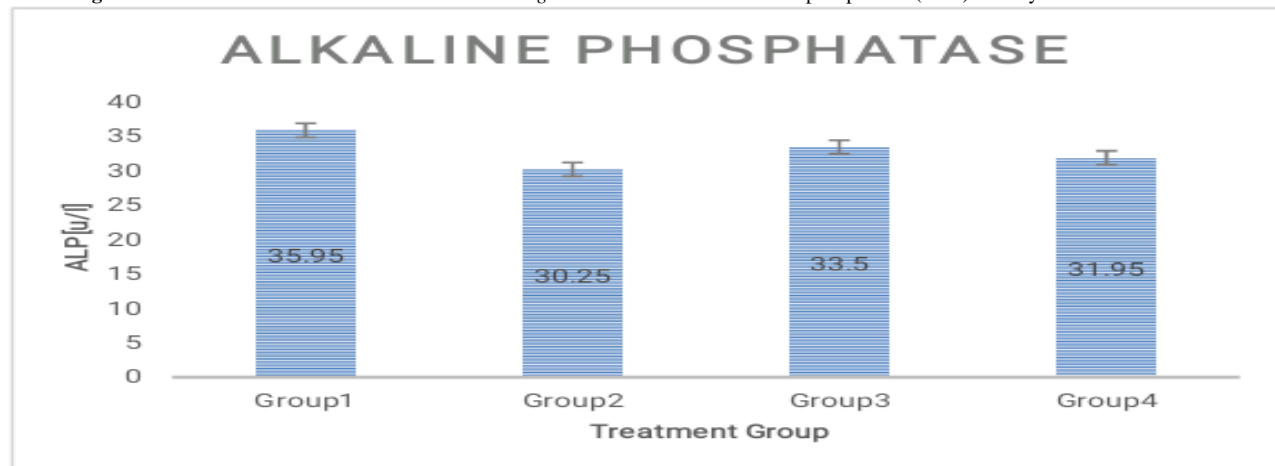
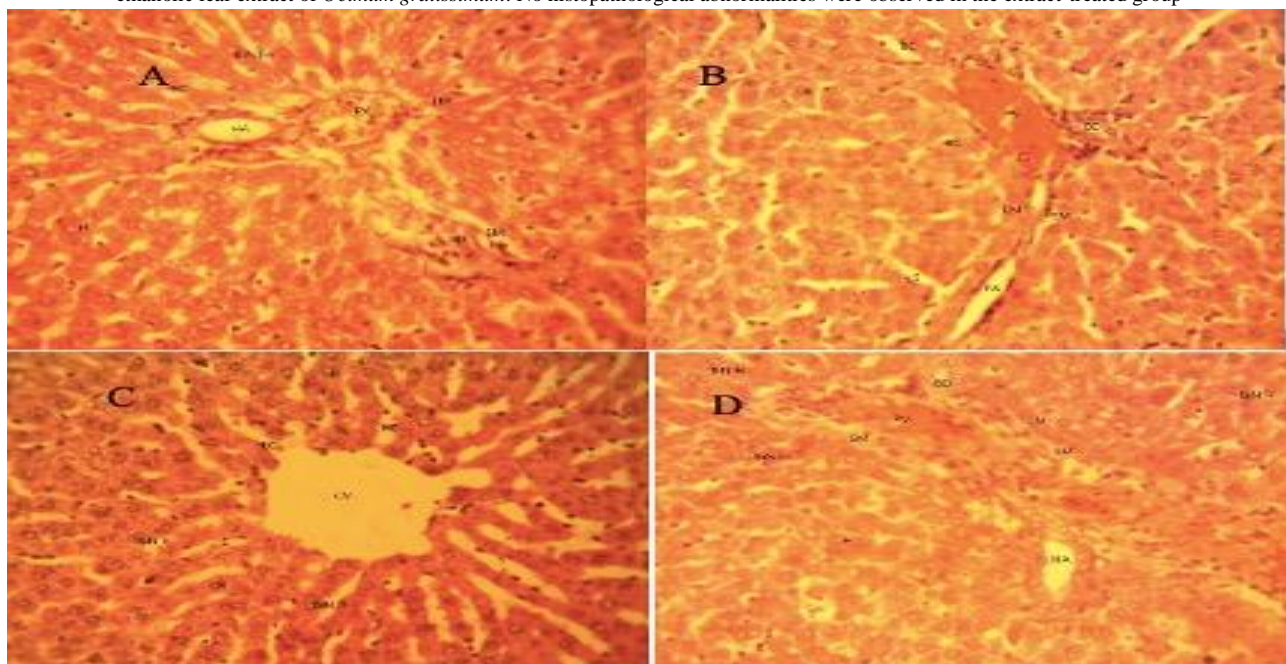
Figure 15: Effect of ethanolic leaf extract of *Ocimum gratissimum* on Catalase Level in the Liver**Figure 16:** Effect of ethanolic leaf extract of *Ocimum gratissimum* on MDA Level in the Liver**Figure 17:** Effect of ethanolic leaf extract of *Ocimum gratissimum* on serum alanine aminotransferase (ALT) activity in adult Wistar rats

Figure 18: Effect of ethanolic leaf extract of *Ocimum gratissimum* on serum aspartate aminotransferase (AST) activity in adult Wistar rats**Figure 19:** Effect of ethanolic leaf extract of *Ocimum gratissimum* on serum alkaline phosphatase (ALP) activity in adult Wistar rats**Figure 20:** Representative photomicrographs of liver sections from adult Wistar rats stained with hematoxylin and eosin (H&E; $\times 100$). (A) Control group showing normal hepatic architecture with a well-defined portal triad comprising the portal vein (PV), hepatic artery (HA), and bile duct (BD). (B) Rats treated with 100 mg/kg ethanolic leaf extract of *Ocimum gratissimum*. (C) Rats treated with 150 mg/kg ethanolic leaf extract of *Ocimum gratissimum*. (D) Rats treated with 200 mg/kg ethanolic leaf extract of *Ocimum gratissimum*. No histopathological abnormalities were observed in the extract-treated group6 μm Scale: 0.240 μm / pixel

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Microscope model: Olympus BH-2 CCD Scion

DISCUSSION

In this exploratory in vivo study, 28-day oral administration of ethanolic leaf extract of *Ocimum gratissimum* produced a reduction in blood glucose levels, accompanied by selective haematological alterations, preserved hepatic oxidative balance, and a discordant biochemical–histological hepatic profile characterised by dose-dependent elevation of AST in the absence of structural liver injury. Collectively, these findings suggest metabolic bioactivity with a nuanced systemic response that calls for a careful interpretation.

Antihyperglycaemic effects

The observed reduction in blood glucose across treatment groups (Figure 1) supports prior reports describing antidiabetic properties of *O. gratissimum*, often attributed to its phytochemical constituents, including flavonoids, phenolics, and eugenol-rich fractions [4, 7-9, 31, 32]. The reduction in glucose level over 28 days suggests a biologically relevant effect rather than a transient glycaemic fluctuation [31, 32]. Although insulin sensitivity indices were not mechanistically interrogated, concurrent glucose lowering without evidence of hepatic oxidative stress argues against overt metabolic toxicity. Instead, the extract may enhance peripheral glucose utilisation, modulate intestinal glucose absorption, or influence hepatic gluconeogenesis. Definitive mechanistic delineation, however, will require insulin profiling, HOMA-IR estimation, glucose tolerance testing, and molecular interrogation of insulin signalling pathways [33].

Hematological modulation

Haematological analysis (Figures 2-12) revealed selective alterations in erythrocyte indices following extract administration. A notable finding was a reduction in PCV and platelet counts across treatment groups, alongside elevated MCV and neutrophil predominance (Figure 10), with reciprocal reductions in lymphocytes. Importantly, haemoglobin concentration and RBC count remained unchanged, suggesting that the reduced PCV may reflect altered erythrocyte morphology or plasma volume shifts rather than frank anaemia [34].

The increase in MCV across doses may indicate macrocytic modulation of erythrocytes, potentially reflecting effects on erythropoiesis or membrane dynamics. Meanwhile, dose-independent thrombocytopenia raises questions regarding platelet turnover or bone marrow responsiveness. Although these alterations were not accompanied by overt clinical instability, they signal systemic biological activity beyond glucose regulation [35].

The neutrophil–lymphocyte shift may represent mild immunomodulatory stimulation. *O. gratissimum* may possess immunoregulatory properties; however, the pattern observed here could alternatively reflect low-grade inflammatory signalling. Without cytokine profiling or bone marrow histology, the

mechanistic basis remains speculative. These haematological findings, therefore, temper any simplistic interpretation of metabolic benefit without systemic impact.

Hepatic oxidative status

Despite biochemical and haematological changes, hepatic antioxidant defence parameters (SOD, CAT, GSH) and lipid peroxidation (MDA) remained unchanged (Figures 13–16). This preservation of redox homeostasis suggests that, within the administered dose range and exposure duration, the extract did not induce measurable oxidative stress in hepatic tissue. These findings align with reports attributing antioxidant capacity to *O. gratissimum* [10-12]. The absence of antioxidant upregulation may reflect a lack of oxidative challenge rather than inefficacy of the *O. gratissimum* extract. This strengthens the argument that the glucose-lowering effect was not secondary to hepatic injury. However, longer exposure periods or higher doses may reveal delayed redox effects not captured in the present timeframe.

AST elevation without histological injury

A central interpretative challenge lies in the dose-dependent elevation of AST (Figure 18) in the absence of ALT or ALP (Figures 16 & 17) changes and without histopathological evidence of hepatic injury. This biochemical–histological discordance calls for cautious interpretation.

AST is not liver-specific and is also expressed in skeletal muscle, cardiac muscle, and erythrocytes [36]. The isolated rise in AST, particularly with preserved ALT and intact hepatic architecture, suggests the possibility of extrahepatic contribution or early, subclinical cellular stress insufficient to produce structural injury detectable by routine H&E staining. The concurrent haematological shifts further support the possibility of systemic biological modulation rather than isolated hepatotoxicity.

Nevertheless, the dose-dependent nature of AST elevation precludes dismissal as incidental variation. Future studies incorporating creatine kinase assays, histological scoring systems, ultrastructural analysis, and inflammatory biomarkers would be necessary to localize the source and clarify clinical significance.

Safety interpretation and translational context

Taken together, the extract demonstrated antihyperglycaemic activity without overt histopathological liver injury or measurable oxidative stress under the conditions tested. However, the hematologic alterations and AST elevation indicate that systemic biological effects may occur alongside glycaemic modulation. Therefore, the findings should be interpreted as evidence of metabolic activity with accompanying physiological perturbations rather than unequivocal safety.

From a translational standpoint, these results underscore the importance of comprehensive toxicological profiling of

phototherapeutics frequently regarded as benign due to traditional use. The study highlights that botanical extracts may exert multi-system effects even when structural organ damage is absent.

Limitations

Several limitations must be acknowledged. The small sample size restricts statistical power and generalizability. The absence of insulin measurements, inflammatory cytokine profiling, creatine kinase assessment, and mechanistic molecular analyses limits causal inference. Additionally, the 28-day exposure period may not capture longer-term toxicological consequences. These constraints define the present work as hypothesis-generating rather than definitive.

CONCLUSION

Ethanol leaf extract of *Ocimum gratissimum* reduced blood glucose levels in Wistar rats over 28 days and preserved hepatic oxidative status and architecture. However, concomitant haematological modulation and dose-dependent AST elevation indicate systemic biological effects that require further mechanistic and toxicological clarification. Larger, adequately powered studies incorporating metabolic, immunologic, and organ-specific biomarkers are essential to delineate therapeutic potential from subclinical risk.

Authors' contribution

CAO, UAN-E and NJN conceptualized and designed the study. NJN performed the statistical analysis and drafted the original manuscript. CAO, UAN-E, NJN, CI, and PKG contributed to data collection and acquisition. CAO, UAN-E, NJN, and CI revised the manuscript for intellectual content. All authors (CAO, UAN-E, NJN, CI, and PKG) read and approved the final manuscript and agreed to be accountable for all aspects of the work.

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REFERENCES

- Najmi A, Javed SA, Al Bratty M, et al, 2022. Modern approaches in the discovery and development of plant-based natural products and their analogues as potential therapeutic agents. *Molecules*. 27(2), Page 349. Doi: 10.3390/molecules27020349.
- Sun W, Shahrajabian MH, 2023. Therapeutic potential of phenolic compounds in medicinal plants—Natural health products for human health. *Molecules*. 28(4), Page 1845. Doi: 10.3390/molecules28041845.
- Chaachouay N, Zidane L, 2024. Plant-derived natural products: a source for drug discovery and development. *Drugs and Drug Candidates*. 13(1), 184-207. Doi: <https://doi.org/10.3390/ddc3010011>.
- Edo GI, Samuel PO, Ossai S, et al, 2023. Phytochemistry and pharmacological compounds present in scent leaf: A review. *Food Chemistry Advances*. 3, Pages 100300. Doi: <https://doi.org/10.1016/j.focha.2023.100300>.
- Ugbogu OC, Emmanuel O, Agi GO, et al, 2021. A review on the traditional uses, phytochemistry, and pharmacological activities of clove basil (*Ocimum gratissimum* L.). *Heliyon*. 7(11). Page e08404. Doi: <https://doi.org/10.1016/j.heliyon.2021.e08404>.
- Ikeotuonye C, Uronnachi E, Nwakile C, et al. 2023. *Ocimum gratissimum* essential oil: A review of extraction methods, phytochemical constituents, pharmacological uses and formulation approaches. *Journal of Current Biomedical Research*. 3(5), Pages 1178-1196. Doi: 10.54117/jcbr.v3i5.1.
- Conde de la Rosa L, Goicoechea L, Torres S, et al, 2021. Role of oxidative stress in liver disorders. *Livers*. 2(4), Pages 283-314. Doi: <https://doi.org/10.3390/livers2040023>.
- Mooli RG, Mukhi D, Ramakrishnan SK, 2022. Oxidative stress and redox signaling in the pathophysiology of liver diseases. *Comprehensive Physiology*. 12(2), Pages 3167-3192. Doi: 10.1002/cphy.c200021.
- Singh D, Cho WC, Upadhyay G, 2016. Drug-induced liver toxicity and prevention by herbal antioxidants: an overview. *Frontiers in Physiology*. 6, Page 363. Doi: <https://doi.org/10.3389/fphys.2015.00363>.
- Owoade AO, Adetutu A, Olorunnisola OS, 2019. Hematological and biochemical changes in blood, liver and kidney tissues under the effect of tramadol treatment. *J Alcohol Drug Depend*. 7(327), Doi: 10.35248/2329-6488.19.7.326.
- Ahmed I, Zakiya A, Fazio F, 2022. Effects of aquatic heavy metal intoxication on the level of hematocrit and hemoglobin in fishes: a review. *Frontiers in Environmental Science*. 10, Page 919204. Doi: <https://doi.org/10.3389/fenvs.2022.919204>.
- Seferli M, Kotanidou C, Lefkaki M, et al, 2024. Bioactives of the freshwater aquatic plants, *Nelumbo nucifera* and *Lemna minor*, for functional foods, cosmetics and pharmaceutical applications. *Applied Sciences*. 14(15), Page 6634, Doi: <https://doi.org/10.3390/app14156634>.
- Bishop ML, Fody EP, Schoeff LE. *Clinical Chemistry: Principles, Procedures, Correlation*. 2005;3(4).
- Etim NN, Williams ME, Akpabio U, et al, 2014. Haematological parameters and factors affecting their values. *Agricultural Science*. 2(1), Pages 37-47. Doi:10.12735/as.v2i1p37.
- Shittu ST, Oyeyemi WA, Lasisi TJ, et al, 2016. Aqueous leaf extract of *Ocimum gratissimum* improves hematological parameters in alloxan-induced diabetic rats. *International Journal of Applied and Basic Medical Research*. 6(2), Pages 96-100. Doi: 10.4103/2229-516X.179016.
- Ofem OE, Ani EJ, Eno AE, 2012. Effect of aqueous leaves extract of *Ocimum gratissimum* on hematological parameters in rats. *International Journal of Applied and Basic Medical Research*. 2(1), Pages 38-42. Doi: 10.4103/2229-516X.96807.

17. Aribo EO, Ofem OE, Moses ME, 2019. Prolonged administration of leaf extract of *Ocimum gratissimum* reduces RBC, PCV and platelet count but increases total WBC and lymphocyte counts in rats. *European J Med Plants*. Doi: 10.9734/ejmp/2019/v27i230111.
18. Ebeye OA, Ekundina OV, Wilkie IE, 2014. Histological and biochemical effects of aqueous extract of *Ocimum gratissimum* on the liver and kidney of adult Wistar rats. *African Journal of Cellular Pathology*. 2(4), Pages 59-64. Doi: 10.5897/AJCPATH14.008.
19. Nto NJ, Paul SD, Mba C, et al, 2025. Anti-seizure effects of *Datura stramonium*: Impact on neurobehavior, antioxidant enzymes, and inflammatory markers. *JKIMSU*. 14(1).
20. Anyanwu GE, Ovosun A, Ovosun EC, et al, 2023. Zingerone improves memory impairment in Wistar rats exposed to cadmium via modulation of redox imbalance. *JKIMSU*. 12(1).
21. Odoh CU, Nto-Ezimah UA, Nto NJ, et al, 2025. Peroxidase activity in crude extract and fractions of *Solanum nigrum* berries. *PriMera Scientific Surgical Research and Practice*. 6(5), Pages 20-22.
22. Anyanwu GE, Kalu EC, Nto NJ, et al, 2020. Protective effect of *Anacardium occidentale* aqueous leaf extract on lead-induced hepatotoxicity in male Wistar rats. *EJPMR*. 7(7), Pages 813-818.
23. Hassan LA, Anyanwu GE, Nto JN, et al, 2018. Protective effect of aqueous extract of *Cyperus esculentus* on flutamide-induced testicular defect. *Sch J App Med Sci*. 6(6), Pages 2391-2395. Doi: 10.36347/sjams.2018.v06i06.015
24. Akpobasaha S, Ezugworie JO, Nto NJ, et al, 2020. Curative effect of aqueous extract of the bark of *Boswellia dalzielii* on flutamide-induced testicular toxicity. *Int J Med Health Dev*. 25, Pages 11-5. Doi:10.4103/ijmh.IJMH_22_19.
25. Effraim KD, Jacks TW, Sadipo OA, 2003. Histopathological studies on the toxicity of *Ocimum gratissimum* leaf extract on some organs of the rabbit. *African Journal of Biomedical Research*. 6(1), Doi: <https://doi.org/10.4314/>.
26. Okoduwa SIR, Umar IA, James DB, et al, 2017. Anti-diabetic potential of *Ocimum gratissimum* leaf fractions in streptozotocin-treated rat model. *Medicines (Basel)*. 4(4), Page 73. Doi: 10.3390/medicines4040073.
27. Eddouks M, Bidi A, El Bouhali B, et al, 2014. Antidiabetic plants improving insulin sensitivity. *J Pharm Pharmacol*. 66(9), Pages 1197-214. Doi: 10.1111/jphp.12243.
28. Restivo I, Attanzio A, Tesoriere L, et al, 2022. Anti-eryptotic activity of food-derived phytochemicals and natural compounds. *Int J Mol Sci*. 23(6), Pages 3019. Doi: 10.3390/ijms23063019.
29. Sharifi-Rad J, Quispe C, Zam W, et al, 2021. Phenolic bioactives as antiplatelet aggregation factors. *Oxid Med Cell Longev*. Page 2195902. Doi: 10.1155/2021/2195902
30. Giannini EG, Testa R, Savarino V, 2005. Liver enzyme alteration: a guide for clinicians. *CMAJ*. 172(3), Pages 367-79. Doi: 10.1503/cmaj.1040752.31.
31. Effraim KD, Jacks TW, Sadipo OA, 2003. Histopathological studies on the toxicity of *Ocimum gratissimum* leaf extract on some organs of the rabbit. *African Journal of Biomedical Research*. 6(1).
32. Okoduwa SIR, Umar IA, James DB, et al, 2017. Anti-diabetic potential of *Ocimum gratissimum* leaf fractions in streptozotocin-treated rat model. *Medicines (Basel)*. 4(4), Pages 73-83. Doi: 10.3390/medicines4040073.
33. Eddouks M, Bidi A, El Bouhali B, et al, 2014. Antidiabetic plants improving insulin sensitivity. *J Pharm Pharmacol*. 66(9), Pages 1197-214. Doi: 10.1111/jphp.12243.
34. Restivo I, Attanzio A, Tesoriere L, et al, 2022. Anti-eryptotic activity of food-derived phytochemicals and natural compounds. *Int J Mol Sci*. 23(6), Page 3019. Doi: 10.3390/ijms23063019.
35. Sharifi-Rad J, Quispe C, Zam W, et al, 2021. Phenolic bioactives as antiplatelet aggregation factors. *Oxid Med Cell Longev*. Doi: 10.1155/2021/2195902.