African Journal of Pharmaceutical Research and Development
Available online at https://ajopred.com
Vol. 16 No.2; pp. 125-137 (2024)

Original Research Article

INVESTIGATIONS ON THE TOXICOLOGICAL EFFECTS OF AQUEOUS EXTRACT OF *OCIMUM GRATISSIMUM* LEAVES ON BIOCHEMICAL, HAEMATOLOGICAL, AND HISTOPATHOLOGICAL PARAMETERS IN WISTAR ALBINO RATS

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ABSTRACT

An ethnobotanical survey found over 50,000 flowering plants used to treat ailments. The medicinal properties of these plants are due to the bioactive compounds they contain. However, these bioactive components can potentially be harmful when taken at certain concentrations. *Ocimum gratissimum* is commonly used in Africa to treat various diseases, but its toxicity level is often overlooked. An investigation was conducted into the adverse impacts of the aqueous extract derived from *Ocimum gratissimum* leaves (AEOGL) on rats at varying concentrations. The acute toxicity of AEOGL was evaluated using Lorke's method with minor modifications. The animals were exposed to AEOGL at 100, 200, 300, and 400 mg/kg doses for 4 weeks to evaluate sub-acute toxicity effects on rats. The acute toxicity analysis revealed the death of one animal following a decrease in appetite and activity. The LD_{50} was determined to be 6245 mg/kg of body weight. In the subacute toxicity investigation, there was a significant decrease ($p<0.05$) in the percentage of weight gain with rising AEOGL concentration. Kidney function parameters revealed changes in serum urea (control: 6.23±1.02; 400 mg/kg: 10.40±1.18 mmol/L), BUN (control: 3.40±0.60; 400 mg/kg: 5.86±0.55 mmol/L), and creatinine (control: 41.79±2.68; 400 mg/kg: 61.63±1.00 µmol/L). Liver function parameters also varied: AST (control: 125.94±12.39; 400 mg/kg: 165.65±8.79 U/L), ALT (control: 46.61±2.66; 400 mg/kg: 48.05±4.09 U/L), direct bilirubin (control: 0.37±0.05; 400 mg/kg: 1.15±0.06 mg/dL), and GGT (control: 0.39±0.01; 400 mg/kg: 1.03±0.05 U/L). The cardiac function parameters exhibited changes in LDH (control: 1334.68±87.20; 400 mg/kg: 1684.35±73.13 U/L), CK (control: 20.64±1.95; 400 mg/kg: 31.92±3.73 U/L), and troponin (control: 0.32±0.02; 400 mg/kg: 0.45±0.02 ng/mL). The histopathological results showed heart with myocardial vascular congestion and lungs with tissue inflammation in rats treated with high doses (400 mg/kg). The investigation concludes that high doses and prolonged oral use of AEOGL may pose health risks. Caution is advised, especially in herbal medicine.

ARTICLE INFO

Received 13 June, 2024
Accepted 04 August, 2024
Published 21 August, 2021

KEYWORDS

Acute toxicity,
Sub-acute toxicity,
Haematological indices,
Ocimum gratissimum,
Histopathological index

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https://doi.org/10.59493/ajopred/2024.2.13 ISSN: 0794-800X (print); 1596-2431 (online)

INTRODUCTION

An ethnobotanical survey estimated that the number of flowering plants utilized globally for medicinal purposes is between 50,000 and 80,000 [1]. More than 85% of ailments in humans and animals are managed by plant extracts or compounds obtained from plants, and approximately 70% of modern therapeutic compounds are derived from plant products [2]. The bioactivities of these plants have been attributed to their active principles [3], which, at certain concentrations, may be toxic to biological systems [2]. Therefore, it is imperative to determine the level of toxicity of these plant products before administration. A crucial phase to ensure the safety of medicinal plant extracts is to conduct toxicity tests in suitable animal models to ascertain the levels of extract toxicity.

An acute toxicity study is a commonly used test to assess the adverse effects of a substance upon first exposure to a large single dose [2]. The acute toxicity study is used to investigate animals' mortality and unusual behaviour, as explained in [4] toxicity scale. The assessments of sub-acute toxicity, muscle mass and histopathology are crucial for detecting the long-term effects of medication on vital organs (heart, liver, lungs and kidney) [5].

Ocimum gratissimum is commonly used in Africa to treat various diseases. However, its toxicity level is not well-determined, especially in aqueous form. [6]. In this study, the toxic impacts of the aqueous extract (AEOGL) from the leaves of *Ocimum gratissimum* on albino rats were examined in acute and subacute studies. *Ocimum gratissimum,* belonging to the *Lamiaceae* family, is an herbaceous plant frequently encountered in the savannah and tropical rainforests of West Africa [7]. It is referred to as 'scent leaf' in Nigeria and is commonly employed as a flavouring component in culinary preparations [8]. Oils extracted from *Ocimum gratissimum* are reported to be promising and potent mosquito repellents [9]. *Ocimum gratissimum* is referred to by different local names in Nigeria such as 'Daidoyatagida' in Hausa, 'Efinrin' in Yoruba, and 'Nchanwu' in Igbo. Other names for it among Nigerian tribes include 'Ebe-amwonkho' in Edo, 'Tamwotswagi' in Nupe, 'Anyeba' in Igala, and 'Ntong' in Ibiobio or Efik [10]. Research has shown that extracts of *Ocimum gratissimum* leaves have effectively treated diabetes mellitus by reducing blood glucose levels and alleviating key indicators of diabetes mellitus such as polydipsia, polyphagia, and decreasing weight in streptozotocin-induced diabetic rats. [11]. Moreover, studies have shown that the extract of *Ocimum gratissimum* alters the oxidative harm induced by lead acetate in the lymphoid tissues and haematological parameters of adult Wistar rats. [12]. Important components including flavonoids, alkaloids, tannins, saponins, steroids, terpenoids, cardiac glycosides, phenolic compounds, and phlobatannin have been identified by phytochemical investigation of *Ocimum gratissimum* leaf extracts. [13]. Within our literary exploration, no satisfactory study has been conducted on the concentration-dependent toxicity of AEOGL. Therefore, the purpose of this investigation was to determine a safe concentration for utilization, particularly when using the extract to treat various diseases.

MATERIALS AND METHODS

Medicinal Plant

The study utilized *Ocimum gratissimum* leaves, a medicinal plant, which were sourced from the botanical garden of the Department of Biological Sciences at Bayero University Kano, Nigeria. At the herbarium unit of the department, Dr. Yusuf Nuhu identified and verified the plant. After identification, the plants were assigned reference number BUKHAN00306.

Animals

This research involved the procurement of 38 healthy male Wistar albino rats, weighing between 70 and 75g, from the zoological division of the Department of Biological Sciences at Bayero University Kano, Nigeria. Prior to the commencement of the study, the animals were given a one-week acclimatization period to their new environment. They had unrestricted access to food and water and were exposed to a 12-hour light-dark cycle. The cages were cleaned twice daily, in the morning and evening.

Preparation of AEOGL

The method employed by [14] was utilized to prepare AEOGL. The leaves were washed with running water and allowed to dry naturally in the shade for seven days until they reached a constant weight. The plant was then dried and crushed with a mortar and pestle into a rough powder. A 500 millilitre (500 mL) of deionised water was used to soak fifty grams (50 g) of the powder. After 48 hours of periodic shaking, the liquid was filtered through a mesh cloth. The liquid mixture was centrifuged for ten minutes at 8000 rpm. The supernatant was collected as the raw extract, then dried in an oven at 45 °C to produce a solid residue, named "AEOGL," and kept in the refrigerator for further use.

Treatment of Animals

Acute Toxicity

Lorke's method, as outlined in reference [15], was employed with slight adjustments. The method comprises two stages: Phase 1 and Phase 2. Phase 1 entails three sets of three rats each. During this phase, the groups received doses of 10, 100, and 1000 mg/kg body weight of AEOGL, respectively. The rats were then observed for 24 hours to detect any mortality or unusual behaviours. In Phase 2, rats were categorized into five groups and given varying AEOGL doses (1600, 2900, 5000, 6000, and 6500 mg/kg body weight). They were monitored for 24 hours to identify any abnormal behaviour or fatalities. Upon observing no mortality in the fourth group, the fifth group was added to ascertain the LD_{50} for the extract. Equation 1 was applied to calculate the LD_{50}.

$$LD_{5o} = \sqrt{(D_0 \times D_{100})} \quad \ldots\ldots\ldots Equation\ 1$$

D_{100} represents the minimum dose that resulted in death, and D_0 represents the maximum dose that did not result in any deaths.

Sub-Acute Toxicity

Subacute toxicity of AEOGL was evaluated by assessing liver, kidney, cardiac function indices, and haematological parameters.

Ethics Approval

The Nigeria Police Academy Ethics Committee (Research) in Wudil, Kano, has approved and assigned this research work with reference number PEC/HS01/00152. Strict adherence to the institutional guidelines for animal care and use in research was maintained.

Experimental Design

(a) Grouping of Animals

The rats were randomly assigned to five groups, each containing five rats, to assess the impact of AEOGL concentration on the rats. The following procedure was then used for treating the rats.

Group I (control group) (n = 5): This group did not receive any AEOGL treatment. The rats in this group were only provided with food and water.

Group II (n = 5): This group was administered AEOGL orally at a dosage of 100 mg/kg of body weight.

Group III (n = 5): The rats in this group were orally given 200 mg/kg of body weight of AEOGL.

Group IV (n = 5): This group received oral administration of 300 mg/kg of body weight of AEOGL.

Group V (n = 5): The rats in this group were orally administered 400 mg/kg of AEOGL based on their body weight,

where "n" represents the number of rats in each group.

Note: All animal groups received their respective treatments for 28 days, with feed and water provided *ad libitum* throughout the study period.

Determination of Weight Changes in Rats After 28 Days of AEOGL Treatment

The animals were not given food from the evening of day 28 to ensure they fully digested their previous meal. Before their sacrifice on day 29, the rats in the various groups were weighed again in the morning. The difference in weight between day 1 of the AEOGL treatment and day 29 was calculated, and then the percentage weight change was determined using Equation 2.

$$\%\, Weight\ gain = \frac{Final\ Weight - Initial\ Weight}{Initial\ Weight} \times 100 \quad \ldots\ldots equation\ 2$$

(b) Collection of Blood Samples

Animals in each group were euthanized on the 29th day, a day after the final AEOGL administration. They were anaesthetized, and blood samples were collected by puncturing their jugular veins. The blood samples were divided into plain bottles for serum collection for biochemical analysis and ethylenediamine tetraacetate (EDTA) bottles for haematological examination.

(c) Harvest of Organs

The sacrificed rats' liver, kidney, lung, and heart were instantly removed, and the blood was quickly cleaned with ice-cold physiological saline. The organs were then weighed, transferred to individual bottles, and stored in 10% formalin for histological analysis.

Biochemical and Haematological Analysis

Jen-drassik's colorimetric (Diazo) method [16] was used to estimate serum bilirubin (total and direct). ALP (serum alkaline phosphatase) was measured using Roy's colorimetric end-point technique [17]. Gamma-glutamyltransferase (GGT) estimation was performed by [18] colorimetric approach. As directed by [19], the Randox Kit from Randox Laboratories Limited in the UK was used to measure the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Serum Albumin (ALB) was estimated using Keyster's technique [20]. Berthelot's [21] urea-colorimetric method was used to estimate serum urea/BUN. The concentration of serum creatinine was measured by the use of the Bartels and Bohmer [22] technique. Serum sodium was estimated using the Maruna [23] technique. Terri and Sesin's turbidimetric (tetraphenylborate, TPB) method [24] was used to estimate the potassium ion in serum. The colorimetric method of Tietz was used to estimate serum chloride [25]. The Tietz [26] method was used to estimate the total protein in serum. Using the Etievent *et al.,* [27] approach, the serum troponin (Immunoenzymometric Assay) was estimated. The method of Decker and Lohmann [28] was utilised to estimate the level of lactate dehydrogenase (LDH) in the serum. Utilising the immuno-inhibition approach, the serum creatine kinase (CK-MB) was estimated using the Bayer *et al*. method [29]. The Jain principle [30] was used to do the haematological analysis.

Histopathological analysis

The Auwioro [31] protocol was employed. This involved using 10% formal saline to fix the biopsies of rats' liver, kidney, and heart. Melted paraffin wax (melting point: 56°C) was used to penetrate the tissues after they had been dried using progressively higher alcohol concentrations (70%, 90%, and 95% v/v). Toluene was then used to clean the tissues. A rotating microtome was used to cut thin slices with a thickness of 5 µm. Using a Leica ICC50HD microscope, these sections were stained using the haematoxylin and eosin staining method. A Leica ICC50HD camera was used to take the pictures.

Statistical Analysis of Data

The data collected from each group of five animals was analysed and presented as mean ± standard deviation ($x \pm SD$). To confirm differences in the collected data within and between the groups, a One-way analysis of variance (ANOVA) followed by the Tukey-Kramer Multiple Comparisons Test was used. Statistical analysis was performed using GraphPad Instat software (version 3.05), and a *P-value* of less than 0.05 ($p<0.05$) was considered significant.

RESULTS

Result of Acute Toxicity

The findings from the acute toxicity (LD50) testing can be found in Table 1. During Phase I, there were no instances of mortality or unusual behaviour observed in the animals. However, when given higher doses in Phase II, various strange behaviours such as sluggishness, fragility, hypoactivity, and anorexia were observed among the rats. In addition, one animal was found dead at the end of 24-hour observation in group V of Phase II (i.e., the animal that was given 6500 mg/Kg body weight).

Sub-Acute Toxicity

Percentage Weight Gain by Rats during 28 Days of AEOGL Treatments

Figure 1 presents the changes in weight of animals treated with AEOGL for twenty-eight (28) days. The results show that the animals given AEOGL experienced a significantly ($p<0.05$) reduced percentage of weight gain compared to the control group following the four-week treatment.

Results of Effect of AEOGL on Haematological Parameters

The results of haematological analysis for animals that received varying concentrations of AEOGL for 28 days are displayed in Table 2. There were no significant variations ($p>0.05$) observed in any of the parameters tested when compared to their respective control groups.

Results of the Impact of AEOGL on Kidney Function Parameters

Figure 2 illustrates the outcomes of the kidney function examination for rats administered with AEOGL over a period of 28 days. Rats treated with a high dose of 400 mg/kg body weight exhibited significantly elevated levels ($p<0.05$) in all measured parameters, except albumin, potassium, and chloride.

The Impact of AEOGL on Liver Function Indicators

The liver function test results for rats given AEOGL are depicted in Figure 3. Animals treated with AEOGL exhibited significantly elevated levels of AST, bilirubin (both direct and total), and GGT compared to their corresponding control groups ($p < 0.05$).

Results of Effect of AEOGL on Cardiac Function Parameters

The results from the cardiac function test for rats administered with AEOGL are depicted in Figure 4. Serum levels of LDH, CK-MB, and troponin significantly increased ($p<0.05$) at high doses compared to their respective control groups.

Results of Effect of AEOGL on Histopathological Parameters

The histopathology results for the liver, kidney, heart, and lungs of rats treated with AEOGL are shown in Figures 5, 6, 7, and 8, respectively. Each figure shows the control group in section A, and rats treated with 100, 300, and 400 mg/kg body weight of AEOGL are represented in sections B, C, and D, respectively.

The results of the histopathological examination of rat livers treated with different AEOGL concentrations are illustrated in Figure 5. The results show no significant liver lesions in any examined liver sections.

Histopathological results of the rat kidneys treated with various concentrations of AEOGL are shown in Figure 6. The findings indicate no significant renal lesions in any of the sections examined.

Histopathological findings of heart tissue samples from rats exposed to various doses of AEOGL are illustrated in Figure 7. The findings reveal regions of congested myocardial blood vessels, as pointed out by the arrow, in section D, which received a dosage of 400 mg/kg body weight of AEOGL. Sections A, B, and C exhibit no abnormalities.

The histopathological findings of lung tissues from rats exposed to different concentrations of AEOGL are depicted in Figure 8. The results reveal an area of inflammation in lung tissue in Section D, which was treated with 400 mg/kg body weight AEOGL (as indicated by the arrow). No visible abnormalities were found in sections A, B, and C of the lungs.

DISCUSSION

Oral acute toxicity refers to the harmful effects that occur after a substance is taken by mouth in a single dose or multiple doses within 24 hours [32]. AEOGL is classified as practically non-toxic because the LD_{50} of AEOGL exceeds 5000 mg/kg of body weight in rats. [5]. However, animals treated with higher doses of AEOGL (6000 and 6500 mg/kg) showed major signs of toxicity, such as loss of appetite (anorexia) and general weakness (hypoactivity). This suggests that AEOGL may be hazardous at higher doses, which is further supported by one recorded death at the 6500 mg/kg dose.

The adipose tissue's increased lipid oxidation might be the cause of the significant decrease in weight gain percentage in subacute tests for the groups treated with AEOGL. This effect could be advantageous for individuals struggling with obesity [33]. Haematology, which involves the study of blood morphology and cellular components, is valuable for identifying blood damage and diagnosing various diseases

[33]. The haematological parameters of the groups of rats treated with AEOGL did not exhibit significant differences ($p>0.05$) compared to each other or the control group. This indicates that AEOGL did not have an adverse impact on the blood components of the treated rats [34]. The kidney function test assesses the normal functioning of the kidneys, including their tubular activities, concentration and dilution capacity, and glomerular filtration rate. Any deviation in the values of the kidney function test parameters may indicate kidney dysfunction or injury [35]. The blood urea test measures the amount of urea nitrogen in the blood, which is produced in the liver during protein breakdown, to assess kidney function. Under normal circumstances, the kidneys filter this waste and

Table 1: Result of acute toxicity test (LD_{50}) of AEOGL on the Wistar Albino Rats

Phase	Group	Oral Dose (mg/Kg)	Number of rats/group	Strange behaviour	Mortality	LD_{50} mg/Kg
Phase I	I	10	3	None	0	
	II	100	3	None	0	
	III	1000	3	None	0	
Phase II	I	1600	1	None	0	6245
	II	2900	1	Feeble	0	
	III	5000	1	weak	0	
	IV	6000	1	Hypoactive	0	
	V	6500	1	Anorexia	1	

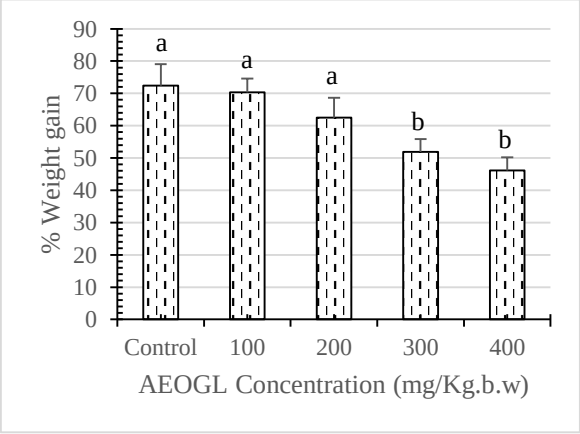
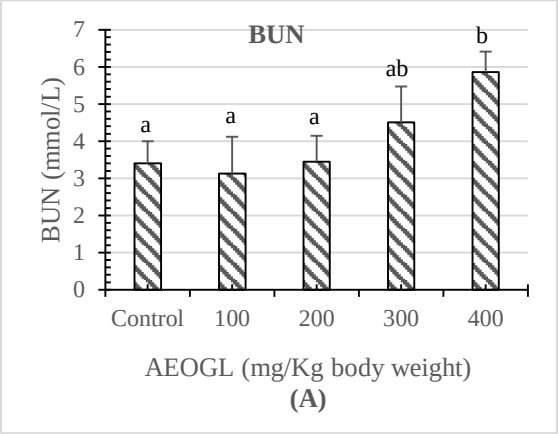


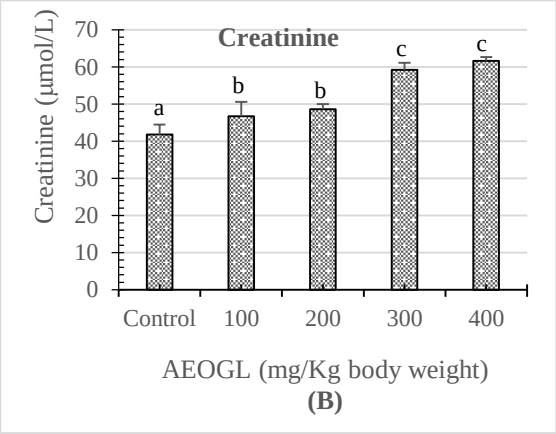
Figure 1: Percentage weight gain in rats exposed to various concentrations of AEOGL over a 28-day period
Note: *Bars with identically labelled alphabets are not significantly different, whereas bars with distinctly labelled alphabets are significantly different at a confidence level of $p<0.05$ for each chart.*

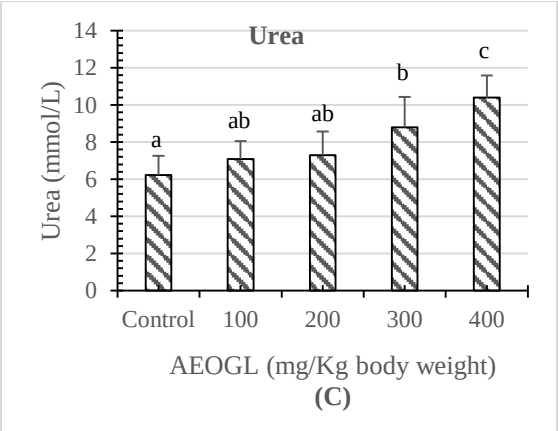
Table 2: Haematological parameters of rats subjected to various concentrations of AEOGL for 28 days

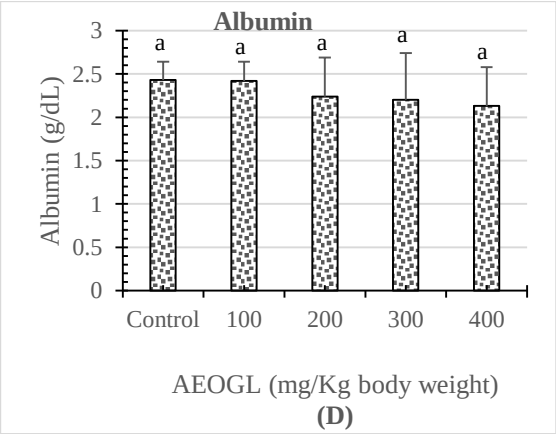
Blood Parameters	Control	AEOGL (100 mg/Kg)	AEOGL (200 mg/Kg)	AEOGL (300 mg/Kg)	AEOGL (400 mg/Kg)
PCV (%)	44.66±3.11	45.18±1.13	49.00±3.12	48.00±2.13	45.70±2.31
WBC (10^9/L)	10.44±3.86	8.80±1.87	9.70±1.50	7.70±1.20	7.16±1.71
PLT (10^9/L)	577.20±83.71	570.44±77.32	540.98±68.20	557.87±81.02	563.67±87.00
RBC (10^{12}/L)	7.38±0.30	7.71±0.86	7.27±0.17	7.12±0.71	7.16±0.84
HGB (g/dL)	14.94±0.78	15.02±0.64	14.50±0.44	14.40±0.54	15.10±0.56
MCH (pg)	20.24±1.21	21.42±1.21	20.47±1.32	20.42±2.21	20.70±1.04
MCHC (g/dL)	33.50±1.64	34.45±1.46	33.63±1.22	34.36±2.20	33.03±1.44
MCV (fL)	60.66±5.31	62.67±6.67	60.87±6.98	61.78±5.96	62.90±4.85
Lym (%)	66.32±2.38	70.37±3.45	67.93±5.40	70.39±4.50	69.53±6.51
MID (%)	9.98±1.58	8.17±1.82	7.60±1.08	7.66±1.80	7.73±1.56
GRA (%)	28.28±3.47	30.93±5.00	29.82±2.21	29.28±2.24	32.50±1.50

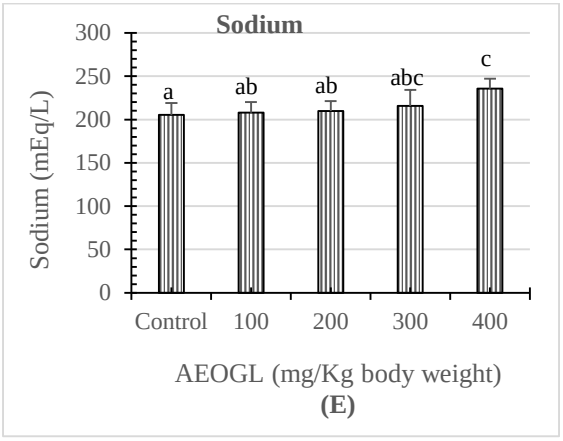
The data presented are $(\bar{x} \pm SD)$ *and* $n = 5$

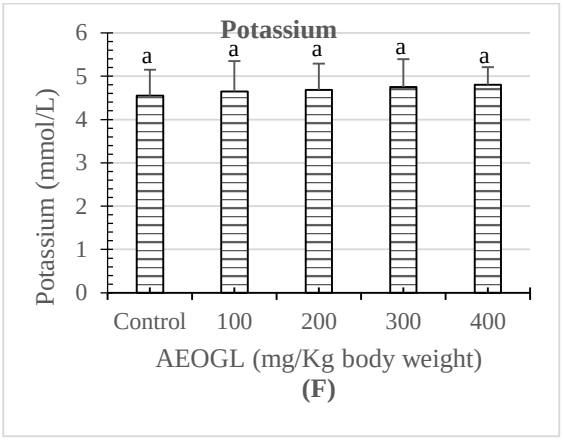


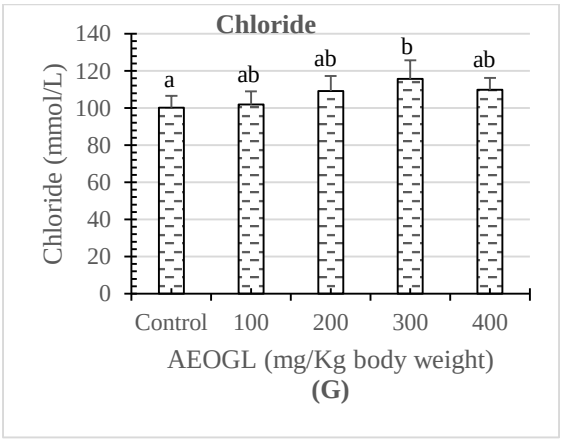












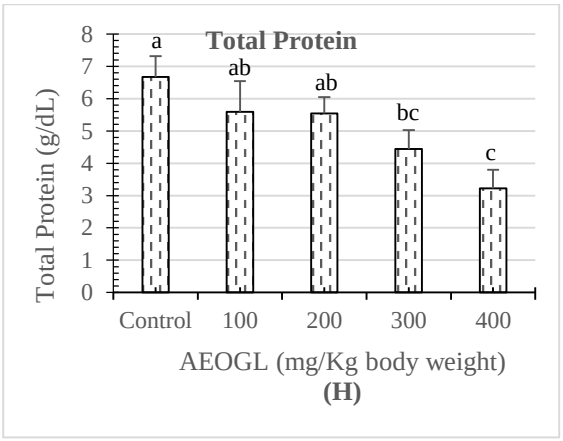
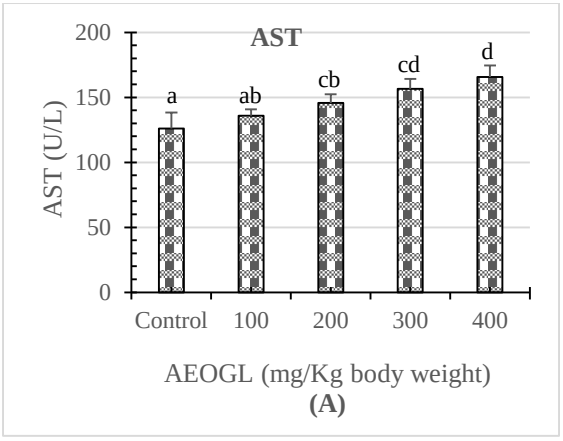
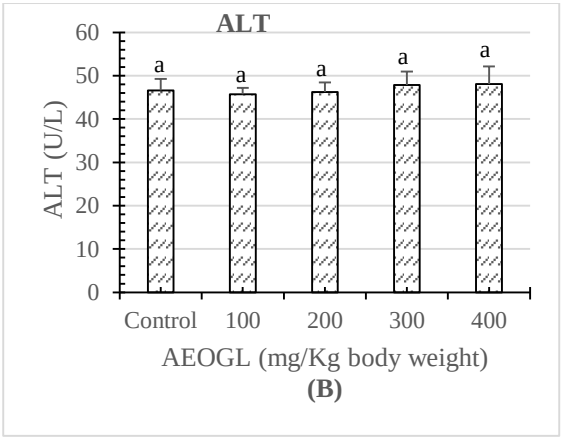


Figure 2. Results of the Impact of AEOGL on renal function markers in the bloodstream of rats following 28 days of AEOGL treatment

A, B, C, D, E, F, G, and H represent the findings from AEOGL treatment on blood urea nitrogen (BUN), creatinine, serum urea, albumin, sodium, potassium, chloride, and total protein, respectively.

Note: Bars with the same letter are not significantly different, while bars with different letters are significantly different at a confidence level of p<0.05 for each chart.





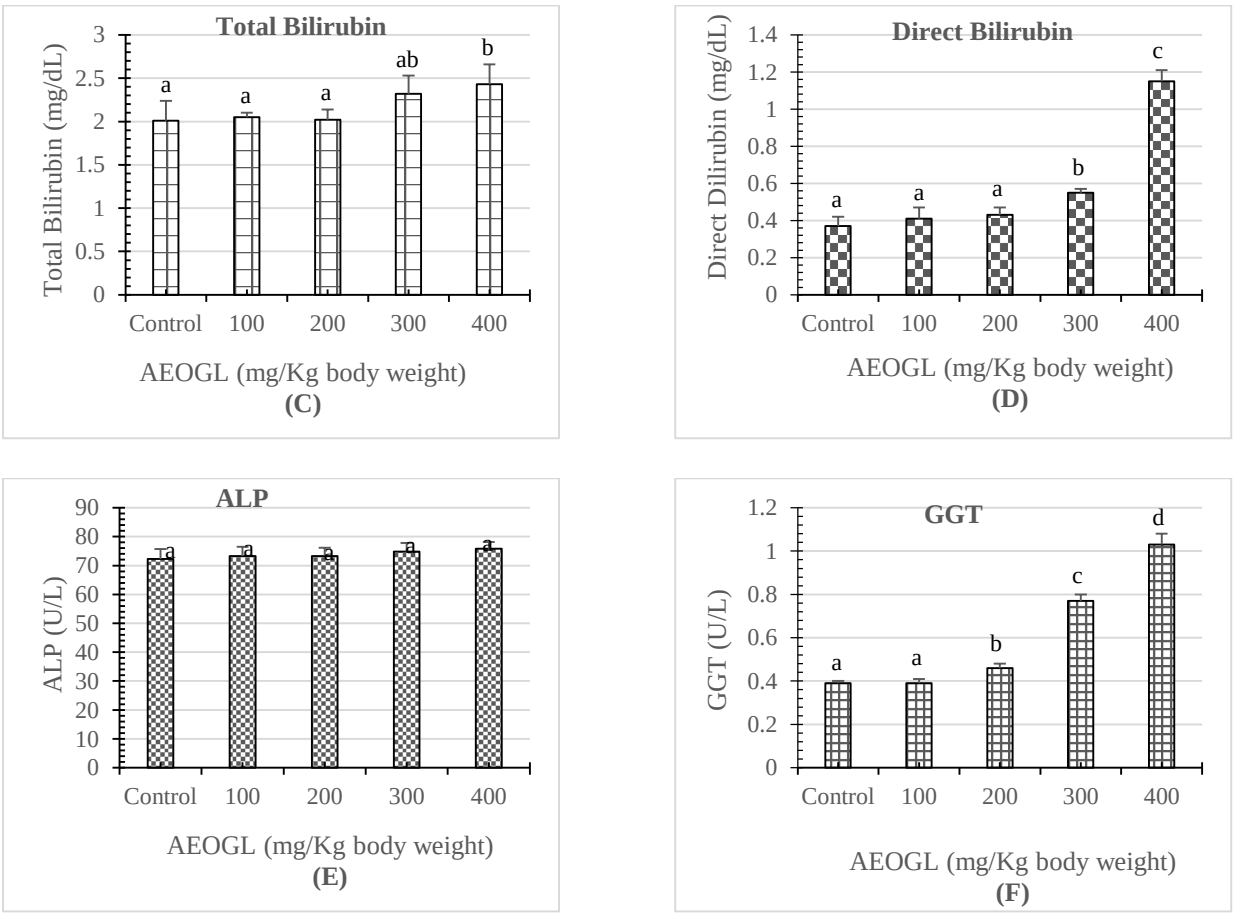
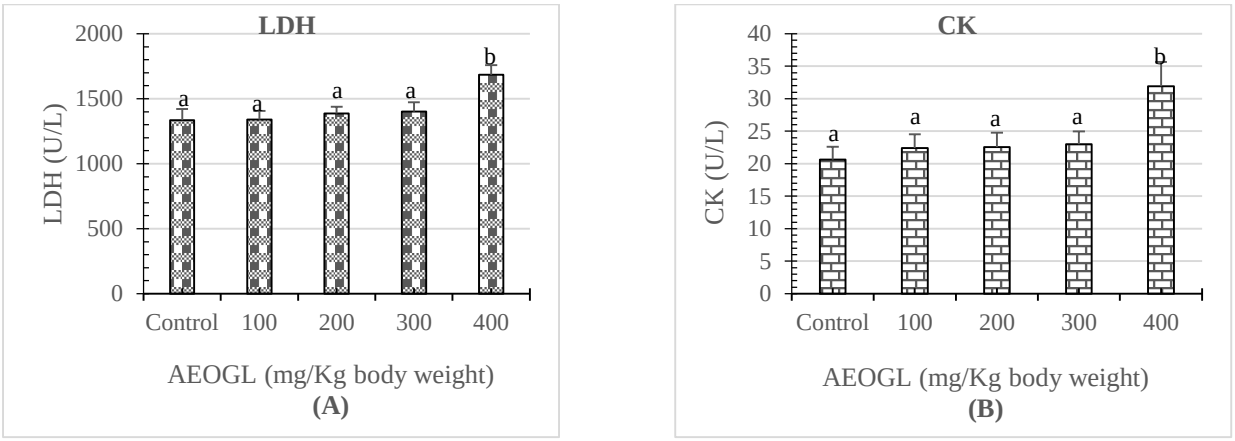


Figure 3: Results of the Effect of AEOGL treatment on liver function indices of rats for 28 days.

The results, shown in charts A, B, C, D, E, and F, indicate significant differences in AST, total bilirubin, direct bilirubin, and GGT levels compared to their respective control groups. In contrast, ALT and ALP levels did not show significant differences.

Note that within each chart, bars marked with distinct letters exhibit significant differences at a confidence level of $p<0.05$. Bars indicated with identical letters do not display significant differences.



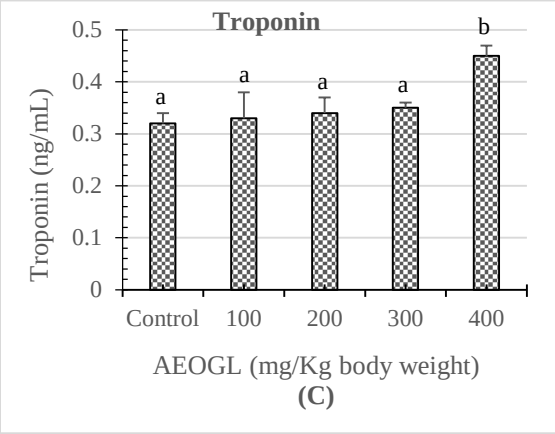


Figure 4: Results of cardiac function indices of rats treated with AEOGL for 28 days.
Charts (A), (B), and (C) represent the results of the effect of AEOGL treatments on the LDH, CK, and troponin status of the treated rats, respectively. *Note: Bars with different letters in each chart are considered to be significantly different ($p<0.05$), while bars with the same letters are not significantly different ($p>0.05$).*

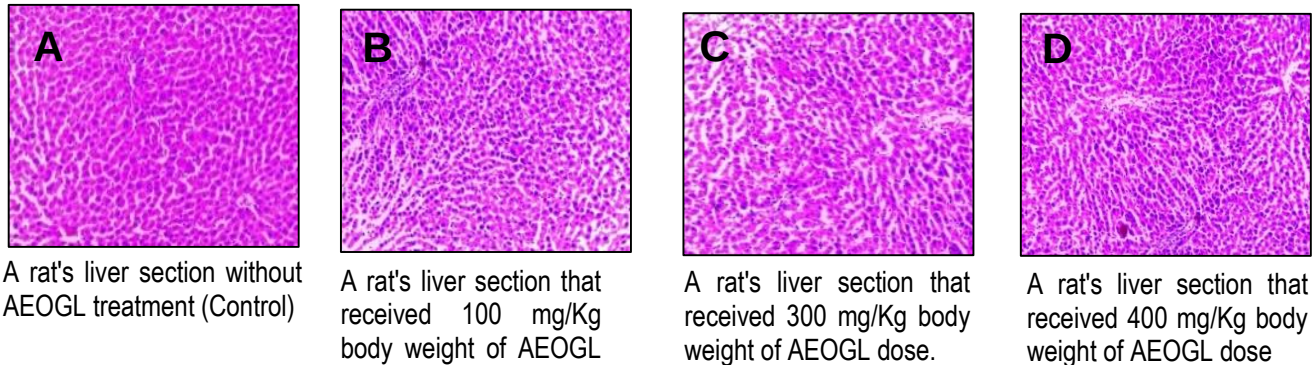


Figure 5: Sections of rats' liver tissue examined for histopathology
Group A was the control group, whereas Groups B, C, and D received treatments of 100 mg/kg, 300 mg/kg, and 400 mg/kg of AEOGL, respectively (H&E, Magnification: ×100).

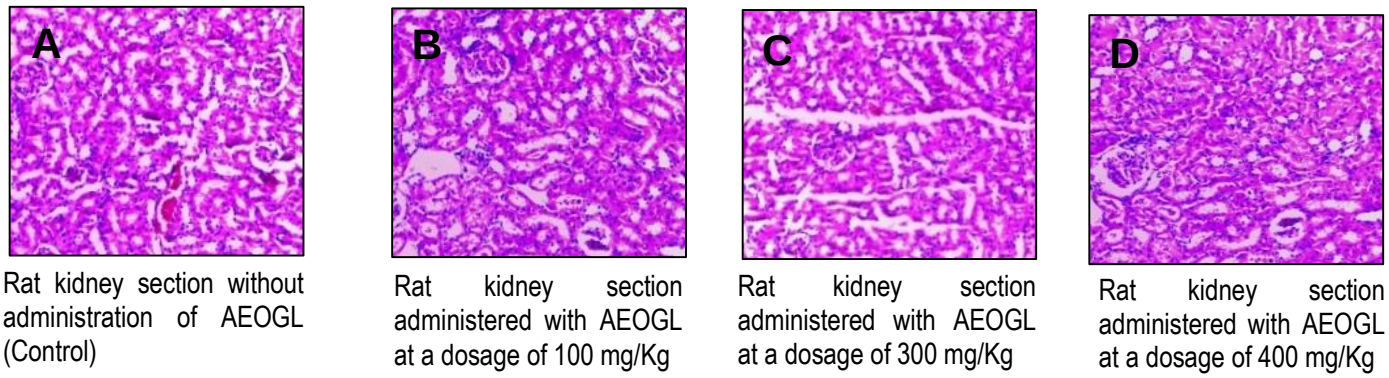
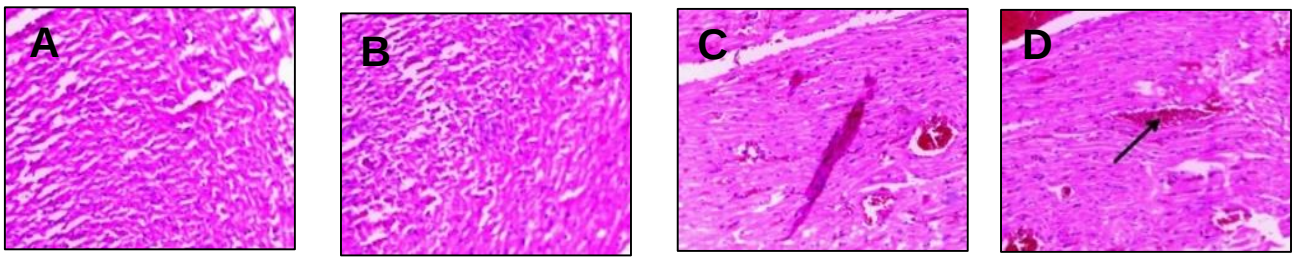


Figure 6: Sections of rats' kidney tissues that were examined for histopathology
Group A was the control group, and Groups B, C, and D received treatments of 100 mg/kg, 300 mg/kg, and 400 mg/kg of AEOGL per kilogram of body weight, respectively. (H&E, Magnification: ×100)



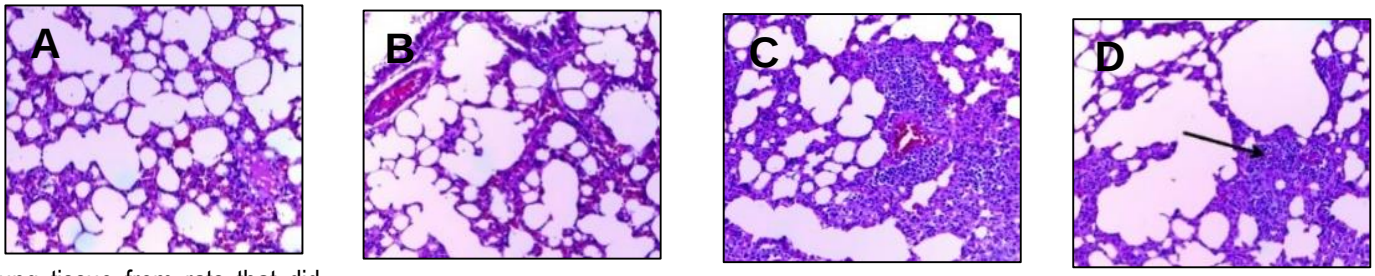
Heart section of an untreated rat (Control)

Heart section of a rat given 100 mg/Kg body weight of AEOGL dose

Heart section of a rat given 100 mg/Kg body weight of AEOGL dose

Heart section of a rat given 100 mg/Kg body weight of AEOGL dose

Figure 7: Sections of rats' heart tissues that were examined for histopathology
The control group was Group A, and Groups B, C, and D received treatment with AEOGL at doses of 100, 300, and 400 mg/kg respectively. (H&E staining, Magnification: ×100)



Lung tissue from rats that did not receive AEOGL treatment (Control)

Lung tissue from rats that received AEOGL at a dosage of 100 mg/Kg

Lung tissue from rats that received AEOGL at a dosage of 300 mg/Kg

Lung tissue from rats that received AEOGL at a dosage of 400 mg/Kg

Figure 8: Sections of rat lung tissues that were examined for histopathology
The control group was represented by Group A, whereas Groups B, C, and D received AEOGL at doses of 100, 300, and 400 mg/kg body weight, respectively. (H&E staining, Magnification: ×100).

excrete it from the body through urine [35]. The substantial increase in blood urea nitrogen (BUN) and serum creatinine observed in the rats receiving AEOGL treatment could indicate uraemia, possibly resulting from renal impairment and damage to nephron function [36]. Liver function tests are a useful screening method for identifying hepatic dysfunctions. The rats treated with AEOGL displayed evidence of liver injury or dysfunction, as evidenced by significant increases in blood GGT, ALT, ALP, direct, and total bilirubin levels, which could be attributed to hepatocellular damage [37].

The heart's ability to supply oxygen-rich blood and remove waste products from cells, which are essential for life, is known as cardiac function [38]. Significant increases in blood LDH, CK, and troponin levels in rats treated with AEOGL indicate heart damage and myocardial infarction ($p<0.05$) [38]. The histopathological findings in the treated rats support the study's results, showing areas of myocardial vascular congestion, which indicate heart tissue damage. Histopathology is a branch of pathology that focuses on studying diseases in tissue sections [39]. Examination of lung and heart tissues from rats treated with higher doses of AEOGL revealed tissue inflammation in the lungs and myocardial vascular congestion in the heart. This is consistent with the findings of [40], who reported that high doses of AEOGL caused functional damage to vital organs such as the liver, heart, kidney, and lungs.

CONCLUSION

After analysing the available data, it can be inferred that exposure to AEOGL led to harm in the kidneys, liver, heart, and lungs of rats, particularly at high doses. There was a notable rise in serum LDH, CK, and troponin levels, suggesting myocardial infarction. This was further evidenced by histopathological findings indicating myocardial vascular congestion. Furthermore, there was a significant increase in serum GGT, AST, and bilirubin levels, implying damage to the liver cells. The observed elevation in serum urea, BUN, and creatinine levels may indicate renal dysfunction, which was also supported by histopathological findings of lung tissue inflammation. These findings emphasize the importance of

being cautious when using high doses of AEOGL for medication, particularly in traditional medicine.

LIST OF ABBREVIATIONS

AEOGL: Aqueous extract of *Ocimum gratissimum* leaves
ALB: Albumin
ALP: Alkaline phosphatase
ALT: Alanine transferase
AST: Aspartate transferase
BUN: Blood urea nitrogen
CK: Creatine kinase
CK-MB: Creatine kinase of heart origin
D_0: Highest dose that gave no mortality
D_{100}: Lowest dose that produced mortality
EDTA: Ethylenediaminetetraacetic acid
GGT: Gamma-glutamyl transferase
LD_{50}: Lethal dose for 50% of the population
LDH: Lactate dehydrogenase
TPB: Tetraphenyl borate

CONFLICT OF INTEREST

All the authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

TAL designed the investigation methodology, supervised the research project, and prepared the published work. He specifically wrote the initial draft. QOS provided the resources for the study and conducted a statistical analysis of the data obtained from the investigation. KS participated in the investigation process and assisted in collecting data for statistical analysis. MF was involved in typesetting, editing, formatting, investigation, and statistical analysis of the data. He greatly contributed to the revision of this work. TJH coordinated the work and was responsible for the research activities, planning, and execution. He is the research administrator.

FUNDING

None declared.

ACKNOWLEDGMENT

The authors of this article appreciate the efforts of all technicians and technologists of the Department of Biochemistry, Bayero Kano University (BUK) for their roles in this investigation.

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