Original Research Article

Alleviative role of *Crinum glaucum* bulb against haematological and hepatic enzyme alterations in a rat model

ABSTRACT

Aims: Several parts of medicinal plants have been known to have many health benefits. One of those plants is the *Crinum glaucum* (CG) bulb. Therefore, this research was undertaken to investigate the alleviative role of an aqueous extract of CG bulb (aeCGb) against lipopolysaccharide (LPS)-induced haematological and hepatic enzyme alterations in male and female rat models.

Study design and methodology: Twenty-five male and twenty-five female rats were divided randomly into five groups (n = 5) each. Group 1 is the control group. Group 2 was administered with 1000 mg/kg body weight of aeCGb. Group 3 was exposed to 4 ml/kg body weight of LPS for 4 hours. Group 4 was administered LPS (4 hours) and 1000 mg/kg body weight of aeCGb. Group 5 was administered 1000 mg/kg body weight of aeCGb and LPS for 4 hours. The albumin, total protein, bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and gamma-glutamyl transferase (hepatic enzyme) concentrations and haematological parameters were analysed spectrophotometrically.

Results: The hallmark of LPS is its ability to decrease the concentration of hepatic enzymes and the levels of haematological parameters, as observed. The levels of albumin, total protein, and direct and total bilirubin were significantly (*P*= .05) increased in the aeCGb-treated groups. However, treatment with aeCGb reverses the damaging effect of LPS on hepatic enzyme concentration.

Conclusion: The results suggest that aeCGb has an alleviative role in the LPS-induced alterations by correcting the concentration of hepatic enzyme function as well as regulating the levels of haematological parameters.

Keywords: Alkaline phosphatase, lipopolysaccharide, Crinum glaucum, alleviative, hepatic

1. INTRODUCTION

The liver plays a predominant role in the immune response and ensures the detoxification and elimination of substances found in the blood. Additionally, it plays a crucial role in the metabolism, control of red blood cells, and production and storage of glucose. Blood is a tissue that consists of fluid plasma in which several elements are suspended [1]. When blood levels of toxic substances from the bioactivation of metabolites of drugs, viruses, or alcohol exceed the detoxifying capacity of the liver, the liver cells are attacked, causing hepatitis (inflammation of liver cells) leading to liver damage. Studies have shown lipopolysaccharide (LPS, endotoxin) has been widely used to establish the implications of normal cellular metabolism [2,3]. Exposure to LPS triggers an increase in mediators of inflammation, especially neutrophils, in the liver, which precedes the onset of liver damage [4-6]. Damage to the liver was evaluated as an increase in alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) activities. However, LPS gradually increases endotoxin levels, which can produce haematological and blood biochemical modifications [7]. Long-term liver damage that is not treated leads to cirrhosis and eventually death. Due to the unaffordability of treatment and the various drug side effects, the use of medicinal plants as an alternative therapy for pathologies is being considered now, even though sufficient scientific research is needed to confirm their efficacy [8-10]. The phytochemicals present in plants have a high number of biological properties, which validate their benefits and effects on a lot of metabolic disorders [11,12]. Crinum glaucum (CG) belongs to the Amaryllidaceae family, which possesses

compounds with biological activities that can be developed into drugs [13-16]. Traditional medicine has used CG for several diseases such as cough, asthma, malaria, convulsions, and sexually transmitted diseases, among others [8,17,18]. The previous pharmacological studies have shown that the aqueous extract of CG has analgesic, anti-inflammatory, anti-anaphylactic, and anti-allergic activities, an effect on gastrointestinal smooth muscles, choline esterase inhibitory properties [18,19], and improves the lipid profile [8]. There is, however, insufficient information regarding the mechanism of action of treatment with an aqueous extract of Crinum glaucum bulb in disorders of blood and hepatoxicity. Hence, the alleviative role of an aqueous extract of Crinum glaucum bulb (aeCGb) against haematological and hepatic enzyme alterations in male and female rat models was investigated.

2. METHODOLOGY

2.1 Plant collection and identification, aqueous extraction, and acute toxicity study

The *Crinum glaucum* plant material (bulbs) was purchased, authenticated, an aqueous extract prepared, and refrigerated, as well as the toxicity (LD_{50}), as described in our previous study [8].

2.2 Preparation of lipopolysaccharide (LPS)

The preparation of lipopolysaccharide (LPS) was done according to the preparations of Ogunrinola *et al*. [8], and Rotimi *et al*. [3].

2.3 Experimental animals and design

The experiment was carried out at the Department of Biochemistry, Drug Discovery Lab, Faculty of Science, Lagos State University, Ojo, in April 2021. Twenty-five male and twenty-five female Wistar rats, weighing between 100 and 200 g, were kept and acclimatize (for fourteen days) in the animal house for the experiment. They were fed a standard diet (Livestock Feeds, Plc, Lagos, Nigeria) and water ad libitum. The rats were distributed randomly into five groups (n = 5) for both males and females, respectively, as described below. This is to understand the mechanism of action of aqueous extract of *C. glaucum* bulb (aeCGb) to alleviate the effects of LPS-induced toxicity on male and female haematological parameters and hepatic enzymes.

Group 1: Control, fed with water and an animal standard diet only.
Group 2: administered with aeCGb (1000 mg/kg body weight) for 7 days.
Group 3: Injected with lipopolysaccharide (LPS) (4 ml/kg body weight) for 4 hours before they were sacrificed.
Group 4: Injected with LPS (4 ml/kg body weight) for 4 hours and aeCGb (1000 mg/kg body weight) for 7 days.
Group 5: aeCGb (1000 mg/kg body weight) for 7 days and 4 hours of LPS (4 ml/kg body weight).
The animals were sacrificed under light anaesthesia, after the experimental period. Blood was drawn from the animals' hearts into plane, heparinized and ethylene diamine tetraacetic acid (EDTA) anticoagulated tubes and processed as previously described by Ogunrinola *et al*. [8].
All procedures involving the animals were conducted with adherence to the guidelines of the ethical guide for laboratory animal care [20] and approved by the Ad Hoc Animal Ethical Committee of the Department of Biochemistry, Ojo, Lagos, Nigeria.

2.4 Biochemical Analysis

2.4.1 Assay of hepatic enzyme concentration

The concentrations of hepatocellular (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) and cholestatic (alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT)) enzymes, albumin, total protein, and direct and total bilirubin were evaluated in the plasma according to the procedure of the diagnostic agape reagent kit (Agape Diagnostics, Switzerland GmbH).

2.4.2 Haematological Assay

The haematological assay was carried out by the modified method of Wararut, [21]. Briefly, the total red blood cell counts (RBC) were determined by diluting blood samples with Grower's solution, and then red blood cells were counted at 5 red blood cells per square hemocytometer. Total white blood cell counts (WBC) were counted in Neubauer's hemocytometer after the blood samples were diluted (1:20) in Turk's solution. The hematocrit (HCT) was measured by filling blood into

heparinized capillary tubes and centrifuged at 11,000 rpm for 5 minutes, then measured with a hematocrit reader. The haemoglobin (HGB) concentration was determined by adding 20 µl of the blood sample to 5 ml of Drabkin's solution. The optical density was measured in a spectrophotometer at 540 nm and calculated from the HGB standard curve. Some haematological parameters, such as mean cell volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), were also calculated. For the determination of the percentage of leukocytes, the blood smear was made and stained with Wright's dye, and the percentage of lymphocytes (Lymph) and platelets (PLT) were counted under a light microscope.

2.5 Statistical Analysis

The statistical evaluation of the data was performed using one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS 21). The data were expressed as mean ± S.E.M (standard error of mean). The significance was considered at *P*= .05.

3. RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Acute toxicity (LD_{50}) test

As reported by our previous research [8], the acute toxicity (LD_{50}) of aeCGb is not toxic, for there has been no death recorded.

3.1.2 The effect of aeCGb on the hepatic enzyme concentration of male and female rat with LPS-induced hepatotoxicity

The effect of aeCGb on the albumin, total protein, and direct and total bilirubin concentration of male and female rat with LPS-induced hepatotoxicity: Figure 1 shows the effect of aeCGb on albumin, total protein, and direct and total bilirubin concentrations in LPS-induced male and female rats. The concentrations of albumin, total protein, and direct and total bilirubin in male and female rats increased significantly (*P*= .05) in the aeCGb group compared to the control, except for the male total protein and female direct bilirubin, respectively. The hallmark of LPS-induced toxicity is a significant (*P*= .05) reduction in the concentrations of albumin, total protein, and direct and total bilirubin in both male and female rats compared to the control group. The administration of the aeCGb to the male and female rats before and after LPS-induction significantly (*P*= .05) increased the albumin, total protein, and direct and total bilirubin concentrations. And there is an up and down in the increase of these concentrations in the animal model.

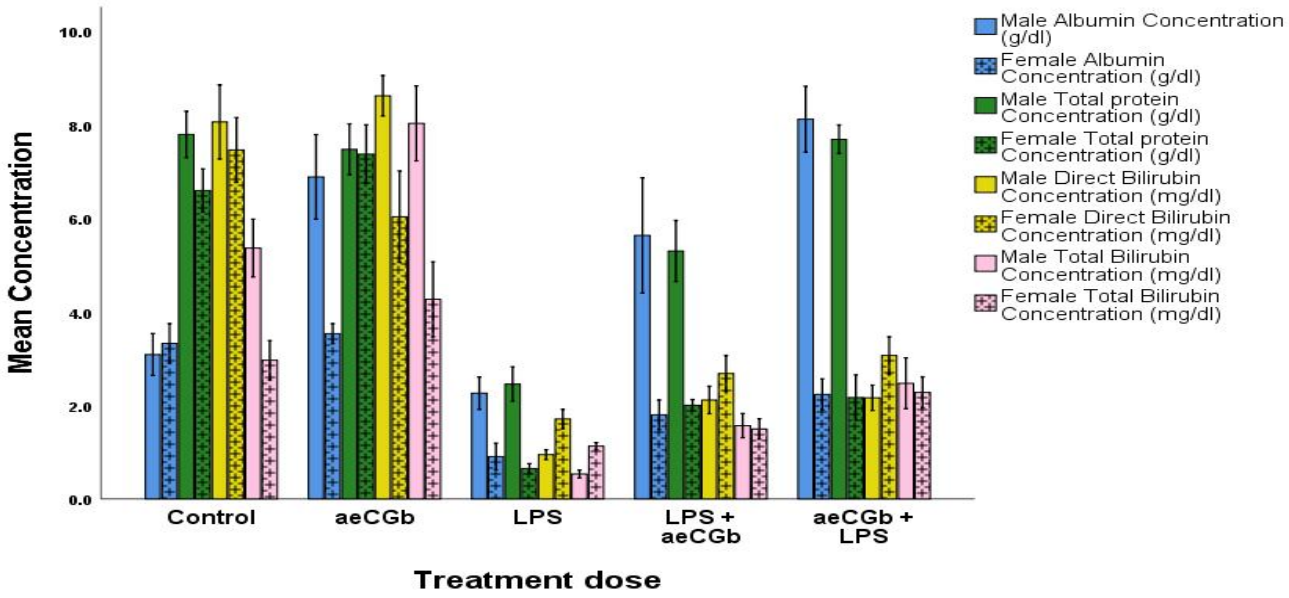

Fig. 1. Effect of 1000 mg/kg body weight of aqueous extract of *Crinum glaucum* bulb (aeCGb) on the albumin, total protein, and direct and total bilirubin concentration of male and female rat with LPS-induced hepatotoxicity

Significant from normal control, P= .05; LPS = Lipopolysaccharide
Mean ± S.E.M = Mean values ± Standard error of means of five animals

The effect of aeCGb on the hepatic enzyme concentration of male and female rat with LPS-induced hepatotoxicity: As depicted in figure 2, the effect of aeCGb on the concentrations of hepatocellular (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)), and cholestatic (alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT)) enzymes in the LPS-induced male and female rat models. The result shows that the concentrations of ALT, AST, ALP, and GGT in male and female rats slightly decreased with the administration of aeCGb compared to the control group. Induction of the male and female rats with LPS resulted in a significant (*P*= .05) increase in the concentrations of ALT, AST, ALP, and GGT compared to the control and aeCGb groups, respectively. The administration of aeCGb to the male and female rats before and after LPS-induction decreased the concentrations of ALT, AST, ALP, and GGT.

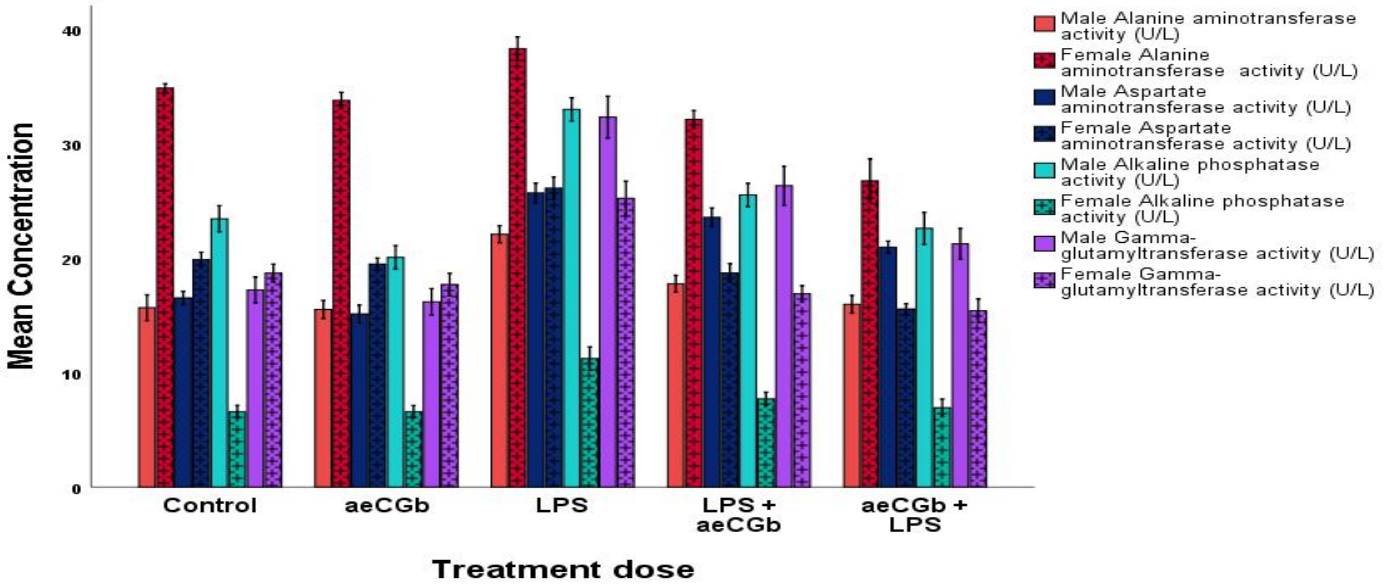

Fig. 2. Effect of 1000 mg/kg body weight of an aqueous extract of *Crinum glaucum* bulb (aeCGb) on the hepatic enzyme concentration of male and female rat with LPS-induced hepatotoxicity

Significant from normal control, P= .05; LPS = Lipopolysaccharide
Mean ± S.E.M = Mean values ± Standard error of means of five animals

3.1.3 The effect of aeCGb on the haematological parameters of male and female rat with LPS-induced hepatotoxicity

The effect of aeCGb on the haematological parameters of male and female rat with LPS-induced hepatotoxicity is shown in Table 1. The rats given aeCGb show a significant (*P*= .05) increase in concentrations of white blood cells (WBC), haemoglobin (HGB), red blood cells (RBC), mean corpuscular volume (MCV), lymphocytes (LYMPH) (female), hematocrit (HCT) (female), and platelets (PLT) and a decrease in concentrations of lymphocytes (LYMPH) (male), hematocrit (HCT) (male), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), respectively compared to the control group. The LPS induction of male and female rats significantly (*P*= .05) reduced the concentrations of WBC, HGB, RBC, HCT, MCH, and MCHC and decreased the concentrations of LYMPH, MCV, and PLT compared to the control group, respectively. Treatment with aeCGb after LPS induction caused a significant (*P*= .05) activation of the WBC, HGB, RBC, HCT, MCH, MCV (female), and PLT, and significantly (*P*= .05) deactivated the concentrations of LYMPH, MCV (male), and MCHC compared to the LPS-induced group. In aeCGb treatment before LPS induction, the concentrations of WBC, HGB, RBC, HCT, MCH, MCHC (female), and PLT (male) showed a significant (*P*= .05) increase and a reduction in LYMPH and MCV (male), MCHC (male), LYMPH, MCV, and PLT (female), respectively, compared to the LPS-induced group.

Table 1. Effect of an aqueous extract of Crinum glaucum bulb (aeCGb) on the haematological parameters of male and female rat with LPS-induced hepatotoxicity

GROUPS		GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
PARAMETERS		Control	aeCGb	LPS	LPS + aeCGb	aeCGb + LPS
WBC (103/mm3)	M	6.24±0.30	9.2±0.38	5.97±0.02	8.8±0.70	7.9±0.90
	F	5.48±0.29	8.63±0.24	3.90±0.10	6.4±0.25	7.38±0.30
Lymph (%)	M	65.9±2.20	55.50±2.10	85.50±2.10	76.6±2.90	63.8±3.10
	F	74.1±5.25	74.45±2.00	79.00±3.00	70.80±2.80	69.15±1.89
HGB (g/dl)	M	12.68±1.80	13.2±0.80	9.5±2.00	12.50±0.90	14.18±1.00
	F	10.88±0.98	12.13±2.04	8.30±1.05	9.30±2.40	8.63±0.99
RBC (10^6/mm^3)	M	6.75±0.33	7.56±0.25	6.03±0.20	6.54±0.29	7.06±0.30
	F	6.06±0.47	6.98±0.22	4.91±0.20	5.35±0.33	6.53±0.26
HCT (%)	M	41.12±1.00	40.23±0.90	33.70±2.00	49.80±2.20	50.87±0.89
	F	35.68±0.99	42.73±0.55	20.42±1.02	43.9±2.11	32.15±0.96
MCV (fl)	M	61.00±0.50	70.90±0.32	65.00±0.45	60.10±0.70	63.25± 0.85
	F	56.93±1.26	71.03±3.09	61.00±2.78	69.20±2.99	58.30±1.89
MCH (pg)	M	19.52±0.32	18.00±0.40	15.70±0.45	17.20±0.40	17.18±0.33
	F	17.98±0.34	17.33±0.45	15.80±0.27	17.70±0.99	18.07±1.58
MCHC (g/dl)	M	32.08±0.10	29.40±0.30	28.1±0.20	27.10±0.19	29.58±0.54
	F	31.55±0.99	29.60±2.67	27.20±1.00	26.70±1.67	31.25±2.01
PLT (10^3/mm^3)	M	558.80±30.00	672.00±55.00	664.00±60.00	1095.00±37.00	707.33±80.00
	F	575.00±24.00	663.67±35.00	790.00±40.01	835.00±23.56	627.17±20.57

Significant from normal control, P= .05; LPS = Lipopolysaccharide
Mean ± S.E.M = Mean values ± Standard error of means of five animals

3.2 DISCUSSION

The liver has a central role in carbohydrate, protein, and fat metabolism, and it is the site where waste products of metabolism are detoxified. And involved in the destruction of spent red blood cells along with the spleen and the reclamation of their constituents [22,23]. When the membranes of liver cells become permeable, it results in an increase in blood concentrations of intracellular enzymes (aminotransferase (ALT) and aspartate aminotransferase (AST)) in the bloodstream, causing hepatocellular injury (e.g., hepatitis). And when there are obstructed or damaged intra- or extra-hepatic bile ducts, it causes the induction of the synthesis of alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT), known as cholestasis (e.g., biliary obstruction or hepatic infiltration) [22,23,24-27].

Current research supports several earlier reports that have shown that LPS induces hepatic damage, and therefore increases the concentrations of the aminotransferases (ALT, AST, ALP, and GGT) [11,28-33]. The hepatic enzymes are cytoplasmic, and alteration in the membrane permeability will cause dysfunction and damaged structural integrity of hepatocytes and the aminotransferases to leak to the bloodstream, thereby resulting in an increase of the aminotransferases in the plasma of the male and female rat models, characterized by viral hepatitis, diabetes, heart failure, bile duct problems, haemorrhage, inflammation, cardiac, muscular, biliary, and injury, and microabscess formation, activation of the transcription factor nuclear factor-kappa B, and many inflammatory genes [11,27,28,30,33,34-37].

The quantities of plasma proteins (albumin and globulin) present in the fluid portion of the blood are measured by total protein and albumin [38]. Albumin is the plasma protein produced by liver cells. Thus, levels of total protein and albumin display the functional status of hepatocytes [39]. The reports of Berkoz et al. [34], and Wali et al. [40] corroborated the observation of this study that LPS induction in male and female rats inhibits albumin, total protein, and direct and total bilirubin concentrations. The reduction may be due to their increased catabolism or decreased synthesis, which indicate liver injury that indicates several health issues such as liver disease, inflammation, shock, celiac disease, and nephrotic syndrome [34].

Bilirubin (BR) is the final product of heme catabolism [41]. Reports from Ahn et al. [42] also agree with the reduced concentration of total and direct bilirubin by the induction of LPS, as revealed in this present study, which can predict liver damage.

Blood is composed of plasma and several kinds of cells, such as red blood cells (RBC), white blood cells (WBC), hemoglobin (HGB), mean corpuscular volume (MCV), lymphocytes (LYMPH), hematocrit (HCT), platelets (PLT), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), that exist at constant levels,

suggesting the existence of feedback regulatory mechanisms in the body system. They perform many important roles, such as supplying oxygen and nutrients to cells and tissues, transporting hormones and signals, removing wastes, regulating body pH and temperature, and helping in immunological functions and coagulation [1,43]. Therefore, these parameters are crucial factors in the determination of hemodynamics in the body, and changes in their concentrations are associated with abnormal clinical conditions.

In this study, there was a significant (P= .05) reduction in the concentrations of WBC, HGB, RBC, HCT, MCH, and MCHC and a decrease in the concentrations of LYMPH, MCV, and PLT with the induction of LPS. These alterations lead to hemolysis anemia (due to low levels of RBC and HGB), the formation of lumps that cause blockage in blood vessels (due to high levels of platelets), and lymphocytosis (high levels of lymph), as demonstrated by Brauckmann et al. [44]. Also, LPS-induced blood alterations cause intercalation with the lipid membrane, leading to abnormalities in the membrane. Our recent research shows that LPS-induction increases membrane fluctuation [8]. And LPS affects leukocyte membranes external monolayer by triggering the formation of lipid microdomains and diffusing to their receptors to activate the Toll-like receptor 4 (TLR-4) signal cascade, evoking red cell membrane impairment of cell integrity and hemolysis [44]. Furthermore, the principal determinants of blood viscosity (a function of lumen diameter and flow viscosity) are hematocrit, plasma protein concentrations, erythrocyte aggregation and deformability, and plasma viscosity [45]. An increase in the concentration of these, especially hematocrit, increases high-shear blood viscosity, as demonstrated by this study with LPS-induction. Studies have also demonstrated that induction of LPS causes adverse effects such as decreased osmotic resistance, diminished membrane stiffness, schistocyte formation in the red blood cells, an increase in MCV levels, and a reduction of hemoglobin in the blood [44].

With all these disorders due to LPS (endotoxin) induction, the pre- and post-administration of aeCGb reverses the damage to the hepatic tissues by stimulating hepatocyte regeneration to stabilize and maintain the integrity of the hepatocyte membrane, by preventing leakage of intracellular enzymes, creating membrane stability, as well as regeneration of hepatocellular protein synthesis. This is confirmed by the reversal of the transaminases and total and direct bilirubin concentrations. Likewise, it plays a protective role against sepsis-induced disseminated intravascular coagulation and is responsible for its anti-anemic and immunologic properties. Our results agree with the report of Kassim et al. [46] in the reductions and corrections of the haematological parameters. The presence of different bioactive constituents, such as flavonoids and tannins, that prevent the formation of lipid peroxide, thereby reducing the effect of co-existing substances or averting their oxidation, might be responsible for its mechanism of action. This is in accordance with some previous reports of some polyphenol-rich plant extracts such as *Aspalathus linearis* [11], *Nauclea latifolia* and *Alchornea cordifolia* [27], *Salvia plebeia* [47], and *Hibiscus sabdariffa* [48], among others, respectively.

4. CONCLUSION

From the above observations, it can be concluded that injection of a low dose of LPS at 4 hours resulted in a significant induction of physiological and biochemical changes that are characterised by an increase in transaminases (ALT, AST, ALT, and GGT), reduced albumin, total protein, direct and total bilirubin, and up- and down-regulation of haematological parameter concentrations. This leads to alterations in the function of liver integrity, membrane integrity in hepatocytes, and haematological parameters, suggesting a significant risk of liver damage and haematological disorder. However, the haematological and biochemical dysfunctions were reversed with the pre- and post- treatment with aeCGb due to the presence of phytochemical constituents. Upcoming research will focus on the evaluation of aeCGb on oxidative stress biomarkers. The data gathered in this study shows that aeCGb has ameliorative and protective effects and could be recommended as safe for therapeutic purposes.

ETHICAL APPROVAL

The research was given the approval by the Ad Hoc Animal Ethical Committee of the Department of Biochemistry at Lagos State University, Ojo, Lagos, Nigeria, and all methods adhered to the ethical guidelines for the care of laboratory animals.

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