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Effect of *Ficus glumosa Delile* Leaves on some Biochemical and Haematological Indices of Rats Fed with Kerosene Contaminated Feed

Magdalene Obi-Abang[1], Jonathan O. Enyike[1], Margaret A.Eno[2], Godwin E. Egbung[2]

[1]*Department of Biochemistry, Cross River University of Technology, Calabar, Nigeria*
[2]*Department of Biochemistry, University of Calabar, P.M.B 1115, Calabar, Nigeria*

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ABSTRACT

Ficus glumosa is used extensively in traditional medicine to manage and remedy some health conditions. This study investigated the effect of powdered *Ficus glumosa* leaves on some biochemical and haematological parameters of wistar rats fed with kerosene-contaminated (4 mL/100g) feed. Twenty-five female rats were divided into five groups (n = 5) accordingly. Group A: uncontaminated feed only, B: contaminated feed only, C: contaminated feed + 5g of leaves, D: contaminated feed + 10g of leaves and E: contaminated feed + 30g of leaves. The rats consumed water and feed *ad libitum* for 28 days. They were anesthetized (after treatment period) using ketamine, blood samples and organs were harvested for some haematological and biochemical analyses. Results showed that the leaves facilitated the feed intake and hence the body weights of the rats almost at par with the positive control. Also, the administration of the leaves to rats fed with kerosene-contaminated feed caused a significant increase in RBC, HGB, ALP, Na^+, K^+, LDLc, VLDLc, triacylglycerol and liver weight when compared to the control groups. The levels of serum albumin, urea, total protein and total cholesterol were observed to decrease ($P< 0.05$) compared to the control groups. Also, concomitant administration of the leaves did not significantly alter kidney/heart relative weights, globulin, AST, ALT, HDLc, Cl^-, creatinine, HCO_3^-, WBC and PLT level when compared with the controls. We therefore conclude that *Ficus glumosa* leaves may attenuate kerosene-induced haematotoxicity, hepatotoxicity as well as maintained the body weight in rats.

Keywords: *Ficus glumosa*, Traditional medicine, Kerosene, Toxicity, Contaminated-feed.

Introduction

Nigeria ranks as one of the major petroleum producing countries. Statistics from the government indicates that about 9-11 million litres of kerosene are consumed daily by millions of people in Nigeria for lamp lighting, cooking and other domestic purposes.[1] Kerosene is a pale-yellow oily liquid with a strong odour and a boiling point of 175-325^{0}C.[2] It is one of the fractions obtained from fractional distillation of petroleum.[3] Kerosene contains aliphatic, aromatic and a variety of other branched saturated and unsaturated hydrocarbons.[4] Although petroleum products (notably kerosene, petrol and diesel) are important energy sources they are also hazardous to the health of living things.[5,6] Humans are exposed to kerosene fumes at the filling stations or from spilled oils and chemicals used at home such as pesticides. Moreover, some amount may leak from underground storage tanks and enter the ground waters.[4] These toxic compounds somehow get into the body of human and animal directly or indirectly through inhalation, skin contact or ingestion of contaminated food or water. Besides, in folkloric medicine some people have ignorantly misused petroleum products in the hopes of treating some ailments, thereby exposing themselves to these toxicants[7]. Pollutants such as carbon monoxide, carbon dioxide, sulphur dioxide, nitric oxides, fine particulates and various volatile organic compounds (VOCs) are emitted during the combustion of kerosene.[8] A number of health conditions has been associated with the use of kerosene in particular.[9] Studies have shown that emissions from kerosene combustion increase the risk of asthma, pulmonary tuberculosis, cardiovascular disease, cerebrovascular disease, cancer, stillbirth, etc.[10]

Plant-based remedies have proven to be useful in the treatment and management of diseases and specifically the toxicity caused by hydrocarbons and other constituents of petroleum and petrochemical products.[11] It is estimated that up to 80% of the population in developing countries rely on traditional medicine for their primary healthcare need. This is perhaps due to the affordability and availability of herbal therapies compared to conventional medicine.[12,13] *Ficus glumosa* is a multi-stemmed, evergreen (or sometimes semi-deciduous depending on the amount of rainfall) and hardy tree with a dense, rounded to spreading crown.[14] The common wild fig, *F. glumosa*, is one of the many fruit-bearing trees that have traditionally been used for treating diseases in Africa and beyond.[15] Macerations of fresh *F. glumosa* leaves, taken orally, have been used by traditional healers for treating diarrhoea, gonorrhoea and diabetes mellitus.[16] The leaves can also be used for treating liver disorders and disease conditions associated with jaundice.[17] The expression of toxicity of hydrocarbons and other constituents of petroleum and petrochemical products, just like other xenobiotics are usually determined by assessing certain biochemical and hematological indices.[18] This study investigated the effects of *F. glumosa* leaves-supplemented diet on some biochemical and haematological indices of rats fed with kerosene contaminated feed.

*Corresponding author. E mail: drmegobiabang@gmail.com
Tel: +2348055629545

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Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Materials

The kerosene used was purchased from Total fuel station, Yellow Duke street, Calabar, Cross River State. Other chemical reagents used

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in the course of the study were of high-quality analytical grade and procured from a registered laboratory.

Plant collection, identification and preparation

Ficus glumosa leaves were collected from Cross River University of Technology (CRUTECH) Calabar, farm and was re-confirmed by Prof. Abraham Joesphant of the Department of Plant and Biotechnology, Faculty of Biological Sciences, CRUTECH. The plant with an assigned voucher number of BOT/FG/2015/1 deposited in the herbarium was previously identified by Mr. Frank Apojeye (Botanist} of the Department of Botany, University of Calabar. Thereafter, the leaves were thoroughly washed, dried at 25ºC for two weeks, ground into powder form using a manual grinder and stored in an air-tight clean container for subsequent use.

Experimental animals

A total of twenty-five female rats (weighing 60-80g) were obtained from the animal house of the Department of Biochemistry, CRUTECH. These animals were maintained in standard conditions according to the procedure of the Animal Ethics Committee with approval number/code: CRUTECH/FBMS/IREC/2020-A1102, housed in well-ventilated standard cages and allowed to acclimatize to the environment and on grower's mash (rat chow) and clean water for 14 days.

Experimental design

Twenty-five rats were divided randomly into five groups (n = 5). All the experimental groups except the positive control were fed with feed (rat chow) contaminated with 4ml of kerosene per 100g of feed (methods of Achuba *et al*., 2016 as modified). Groups; A (positive control): uncontaminated feed only, B (negative control): contaminated feed only, C: contaminated feed + 5g of *F. glumosa* powdered leaves, D: contaminated feed + 10g of the leaves and E: contaminated feed + 30g of the leaves. The rats in all the groups were given drinking water and feed *ad libitum* for 28 days. The feed for all the groups were freshly prepared daily and stale remnants were discarded regularly. Measurements of their feed intake were taken daily while that of body weights was done in alternate days.

Collection of blood samples

At the end of the treatment period, the rats were anesthetized using ketamine afterwards blood samples were collected via cardiac puncture. The required organs were harvested using sterilized surgical blades. Serum was obtained from the clotted blood and used for the determination of certain biochemical indices while the whole blood samples in the heparinized tubes were used for haematological indices.

Biochemical and haematological analyses

Activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in serum were determined using Randox kits from Randox laboratories (55 Diamond road, Crumlin, County Antrim BT29 4QY, United Kingdom). Total proteins (by Biuret method) and albumin concentrations were evaluated using Randox kit. However, the concentration of globulin was estimated from the difference between total protein and albumin concentrations. Randox kits were also used to assessed serum triacylglycerol (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDLc) concentrations. Furthermore, concentrations of low-density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLc) were calculated using Friedewald (1972) formulae.[19] Concentrations of urea and creatinine were also evaluated using Randox kits. However, TECO kits were utilized to determine the concentrations of sodium (Na^+), potassium (K^+), chloride (Cl^-) and bicarbonate (HCO_3^-) ions in serum. Haematological parameters were analysed based on the methods adapted from Elarabany (2018).[20]

Statistical analysis

The data are presented as mean ± SEM (n = 5). Data obtained were analysed using one-way ANOVA followed by least square difference (LSD) post-hoc comparison test to evaluate significant difference between the mean values of the experimental and control groups. Differences at $P< 0.05$ were regarded as significant. Graphpad prism version 7 and SPSS software package version 23.0 were used for the statistical analyses.

Results and Discussion

Effect of Ficus glumosa on the feed intake, body and relative organ weights of rats fed kerosene contaminated feed

Feed consumption pattern showed that group B consumed the least ($P< 0.05$) amount of feed. The feed intake in groups A, C, D and E did not significantly differ from one another during the course of the treatment (Figure 1a). However, at the onset of the research their body weights were quite similar. During the course of the study, the weight of the positive control spiked ($P< 0.05$) and this trend was maintained till the end of the study. On the other hand, group E had the lowest body weight almost throughout the treatment period (Figure 1b).

The relative liver weight of the negative control and treated groups (i.e. groups B, C, D and E) were significantly higher ($P< 0.05$) compared to group A. However, there were no significant differences among the groups with respect to the relative weight of the kidneys and heart. The relative weights of the liver, kidneys and heart in group D were the lowest among the groups fed with kerosene-contaminated feed (Figure 1c).

The effect of *F. glumosa* powdered leaves on selected biochemical and haematological indices of female Wistar rats fed with kerosene-contaminated feed for a period of 28 days was investigated. Generally, the feed intake of the positive control was more than those fed with kerosene-contaminated feed (especially the negative control). It has been severally proven that organoleptic properties of food (such as taste, aroma, colour, texture, appearance, etc) can impart on the acceptability, appetite and hence the amount of food an organism consumed.[21,22] However, *F. glumosa*-treated groups consumed significantly higher amounts of feed compared to the negative control. Perhaps *F. glumosa* enhanced the sensory and nutritional characteristics of the formulated feed thereby enabling the rats to eat more of the feed. The enhancement of the feed by *F. glumosa* maybe attributed to the high content of phenolic compounds in the leaves.[23] Recent studies have indicated that phenolics are important metabolites for the sensory and nutritional qualities of vegetables and fruits.[24,25] Consequently, rats fed with kerosene-contaminated feed showed significant decrease in body weight compared to the positive control. This reduction in body weight may have been due to loss of appetite that the animals may have experienced. However, the body weights of the *F. glumosa*-treated groups were stabilized. Scientific evidence affirmed that *F. glumosa* compared favourably with some useful medicinal plants in the maintenance of body weights of rats.[26] Furthermore, the relative weight of liver of the rats fed with kerosene-contaminated feed increased significantly relative to the positive control. Our findings are quite similar to the work of Patrick-Iwuanyanwu *et al*.[27] that reported a significant decrease in body weight compared to the control of animals fed kerosene and petroleum contaminated diets. The increase in the hepatic weight of the experimental groups observed in the present study was most likely triggered by high rate of metabolism and detoxification of the kerosene-laden compounds. Metabolism and detoxification of exogenous substances occur predominantly in the liver.[28]

Effect of F. glumosa on liver function indices of rats

There were insignificant increases in activities of aspartate aminotransferase (AST) in the sera of groups (B, C and E) fed with kerosene-contaminated feed than in group A. The trend in the activities of serum alanine aminotransferase (ALT) among the groups was similar to that observed in serum AST. The activities of alkaline phosphatase (ALP) in the sera of the various groups indicated a pattern akin to their AST and ALT activities. These observations were however significantly different ($P< 0.05$). Notably, the ALP activity in group C was the highest among the groups (Figure 2a). Group D had the lowest concentrations of serum total protein, albumin and globulin.

The other groups tend to compare favourably with the positive control (P> 0.05) as indicated in Figure 2b.

In this study, ALP serum activities in all the groups administered with kerosene-contaminated feed were higher than the positive control suggesting that the liver tissues of the experimental groups were damaged to some extent by the kerosene. Most radicals attack microsomal lipids thereby causing lipid peroxidation. Products of lipid peroxidation often change the fluidity of membranes, increase membrane permeability and cause damages to the biological membranes leading to serious cellular injury and leakage of liver marker enzymes[29] (like ALT, AST and ALP). These liver marker enzymes are localized in the cytosol of hepatic cells and thus are extruded into the serum when cells are damaged or necrotic.[30] However, activities of AST and ALT in the sera of the *F. glumosa*-treated groups were maintained close to the level of the positive control (albeit insignificantly- probable due to the short duration of the treatment period). This is an indication that *F. glumosa* can potentially ameliorate liver damage. This result was similar to the work reported by Raheim *et al*.[31] that *Ficus ingens* decrease the level of these enzymes indicating the stabilization of plasma membrane as well as repair of liver damage caused by hydrocarbons.[31]

Studies opined that *F. glumosa* is not cytotoxic rather it exhibits cellular protective effect (owing to its numerous antioxidative phytochemical composition) against oxidative damage by free radicals.[32] Owing to the observation that the total protein, albumin and globulin concentrations of experimental groups did not significantly vary from those of the positive control, it can therefore, be inferred that the synthetic function of the liver was not severely impaired in the course of this work. Concentrations of serum total protein, albumin and globulin are commonly used to assess the functionality of liver.[33]

Effect of F. glumosa on serum lipid profile

The concentration of serum triacylglycerol (TG), very low-density lipoprotein cholesterol (VLDLc) and low density lipoprotein cholesterol (LDLc) in the groups fed with kerosene-contaminated feed were markedly (P< 0.05) higher than in the positive control. Furthermore, group C showed the highest concentration of TG. Moreover, serum total cholesterol (TC) concentration in group B was the least (P< 0.05) among the groups. Also, TC in group E was observably higher (P< 0.05) than in group C. However, amongst groups B, C, D and E, group D had the lowest VLDLc concentration. Remarkably, *F. glumosa* may have probably influenced the simultaneous reduction of LDLc and increase in HDLc concentrations (although non-significantly) relative to the negative control (Figure 3).

It was observed that groups fed with kerosene-contaminated feed had higher concentrations of triacylglycerol (TG), low density lipoprotein cholesterol (LDL-c) and very low lipoprotein cholesterol (VLDL-c) relative to the normal control. It is widely believed that hyperlipidemia and altered lipoprotein metabolism have been identified as risk factors for the development of several disorders[34,35] thus maintaining the levels of lipid fractions within normal range will improve the health status of the individual. On the other hand, *F. glumosa*-treated groups showed marked reduction in the concentrations of serum TG, LDL-c and VLDL-c compared to the negative control. This outcome agrees with a report by Ntchapda *et al*.[36], who reported that the levels of LDL-c and VLDL-c in male rats with hypercholesterolemia were said to be significantly decreased upon treatment (four weeks) with aqueous extract of *F. glumosa* leaves. However, the *F. glumosa*-treated groups showed high levels of total cholesterol (TC) than the negative control, this may have been a transient rather than a sustained occurrence. Remarkably, the HDL-c concentration in the *F. glumosa*-treated groups were increased (although non-significantly) than both the positive and negative controls. Numerous studies have proven that the higher the levels of HDL-c, the lower the risk of some diseases.[37] Hence it can be assumed that *F. glumosa* has anti-hyperlipidaemic potentials as alluded to by Umar *et al*.[38]

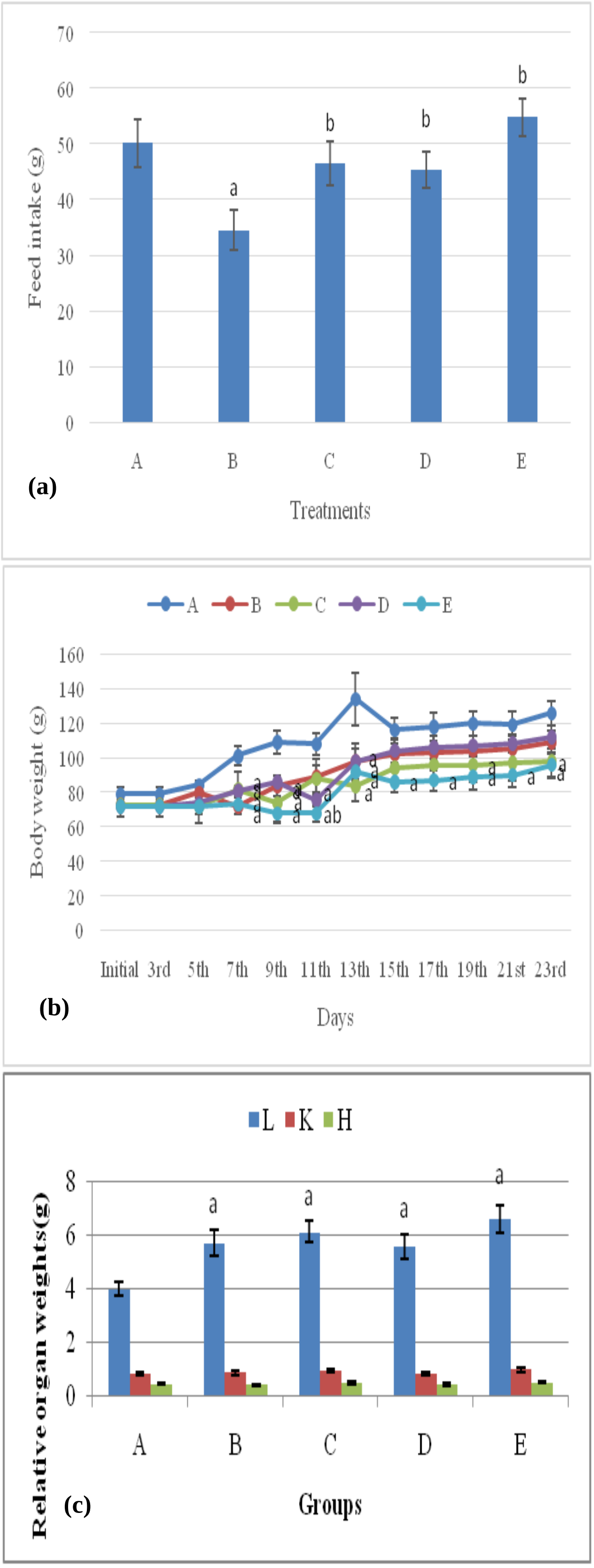

Figure 1: Feed intake/body weight/organ weights (g). Groups A: administered with uncontaminated feed only, B: kerosene-contaminated feed only, C: kerosene-contaminated feed + 5g of *Ficus glumosa* powdered leaves, D: kerosene-contaminated feed + 10g of *Ficus glumosa* powdered leaves and E: kerosene-contaminated feed + 30g of *Ficusglumosa* powdered leaves. a = P < 0.05 vs group A; b = P < 0.05 vs group B; c = P < 0.05 vs group C. Values are expressed as mean ± SEM, n = 5.

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Effect of F. glumosa on kidney function indices

Concentration of serum sodium in the groups fed with kerosene-contaminated feed was significantly higher ($P< 0.05$) than in the positive control. The opposite trend was observed with respect to their serum potassium concentration. However, comparisons of the serum chloride and bicarbonate concentrations among the groups indicated no significant difference as depicted in Figure 4a. Data in Figure 4b suggest that the higher the dose of *F. glumosa* administered, the lower the concentration of serum creatinine (group E had the least concentration of creatinine). Furthermore, concentrations of serum urea in *F. glumosa*-treated groups were considerably lower than in the negative control (Figure 4c).

However, considering the electrolytes, rats fed with the kerosene-contaminated feed showed increased concentration of sodium compared to the normal control. The opposite scenario was obtained in the case of potassium concentration. Sodium is the most abundant cation in the extracellular fluid whereas potassium is the predominant cation in the intracellular fluid.[39] However, the anions (such as chloride and bicarbonate) of all the groups were within the same range. Awad *et al.*[18] attested that nine weeks treatment of hypercholesterolaemic rats with leaf extracts of *Ficus spp.* can improve kidney function indices.

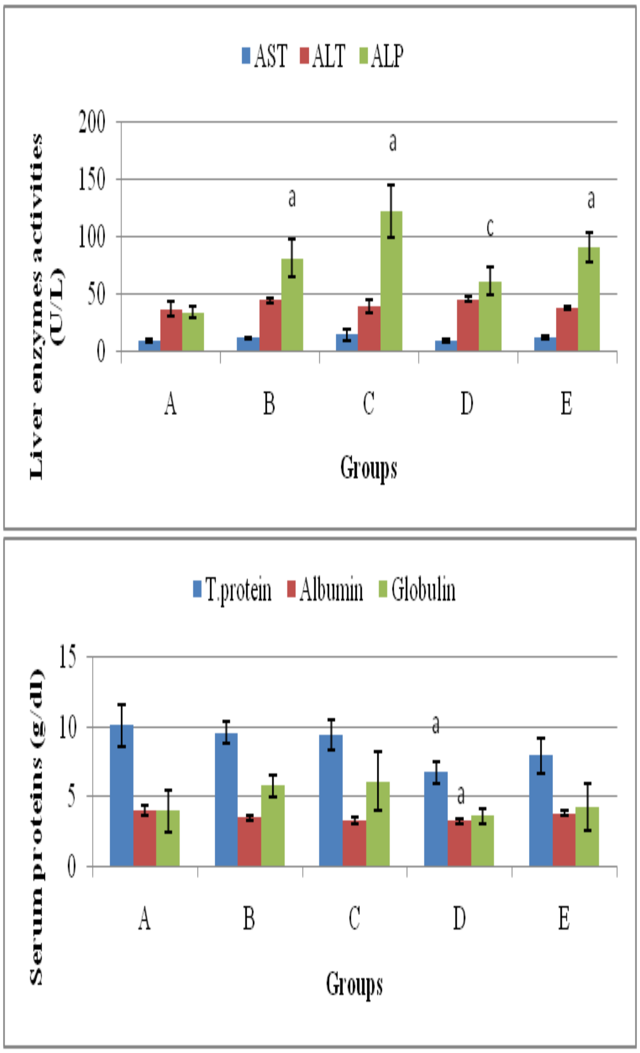

Figure 2: Liver function indices. Groups A: administered with uncontaminated feed only, B: kerosene-contaminated feed only, C: kerosene-contaminated feed + 5g of *Ficus glumosa* powdered leaves, D: kerosene-contaminated feed + 10g of *Ficus glumosa* powdered leaves and E: kerosene-contaminated feed + 30g of *Ficus glumosa* powdered leaves. a = $P < 0.05$ vs group A; c = $P < 0.05$ vs group C. Values are expressed as mean ± SEM, n = 5.

The kidney helps to maintain homeostasis in the body as well as work synergistically with the liver to complete the detoxification process of xenobiotics.[32] Additionally, *Ficus glumosa*-treated groups (especially the high doses) showed marked reduction in their levels of creatinine and urea. This corroborates the claim by Ntchapda*et al.*[40] that *Ficus glumosa* aids in the lowering of urea and creatinine concentration in serum. Besides, another study suggested that a possible adaptation over time maybe the reason why the kidneys in the treated groups relative to the control group appeared to be eliminating creatinine from the blood more efficiently as shown by their lower serum creatinine levels.[41]

Serum concentration of both urea and creatinine is reflective of the glomerular filtration rate (GFR) of the kidney. Increased plasma creatinine is almost invariably a consequence of GFR.[42] Phytochemical screening showed that *Ficusglumosa* is laden with many bioactive components such as flavonoids, tannins, saponins, triterpenes, etc,[32] and these secondary metabolites amongst other substances may be responsible for the potency of *Ficusglumosa* to help reduce toxicity in biological systems.

Effect of F. glumosa on haematological indices

Data analysis showed that by comparison, there was no significant difference among the groups ($P> 0.05$) with respect to the white blood cell (WBC) counts. The red blood cell (RBC) counts and haemoglobin (HGB) content of the *F. glumosa*-treated groups and the controls were more or less within the same range although group C had the least values among the groups (Figure 5a). Furthermore, haematocrit or packed cell volume (PCV – Figure 5b) and platelet counts of the various experimental groups were not significantly different from one another neither with the controls ($P> 0.05$). However, the negative control had the least count of platelets (Figure 5c).

Moreover, in the present study, the WBC counts were found to be higher (although non-significantly) than the positive control. Results of the platelet count showed a similar pattern like the WBC. Increase in the WBC counts in the test groups may have been triggered by the immune system as a defensive mechanism in response to the presence of kerosene fumes (which the body considers as xenobiotics). Inhalation of petroleum products have been reported to change blood chemistry.[41] These exogenous compounds are usually transported via the bloodstream to the respective organs or tissues of the body for metabolism.

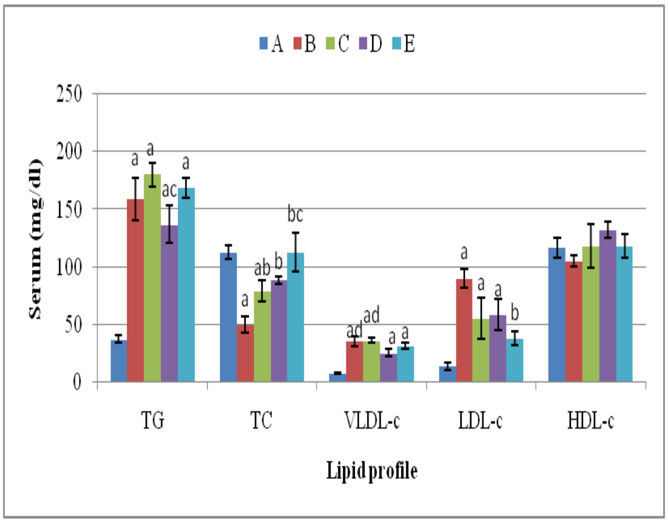

Figure 3: Serum lipid profile. Groups A: administered with uncontaminated feed only, B: kerosene-contaminated feed only, C: kerosene-contaminated feed + 5g of *Ficus glumosa* powdered leaves, D: kerosene-contaminated feed + 10g of *Ficus glumosa* powdered leaves and E: kerosene-contaminated feed + 30g of *Ficus glumosa* powdered leaves. a = $P < 0.05$ vs group A; b = $P< 0.05$ vs group B; c = $P < 0.05$ vs group C, d = $P < 0.05$ vs group D. Values are expressed as mean ± SEM, n = 5.

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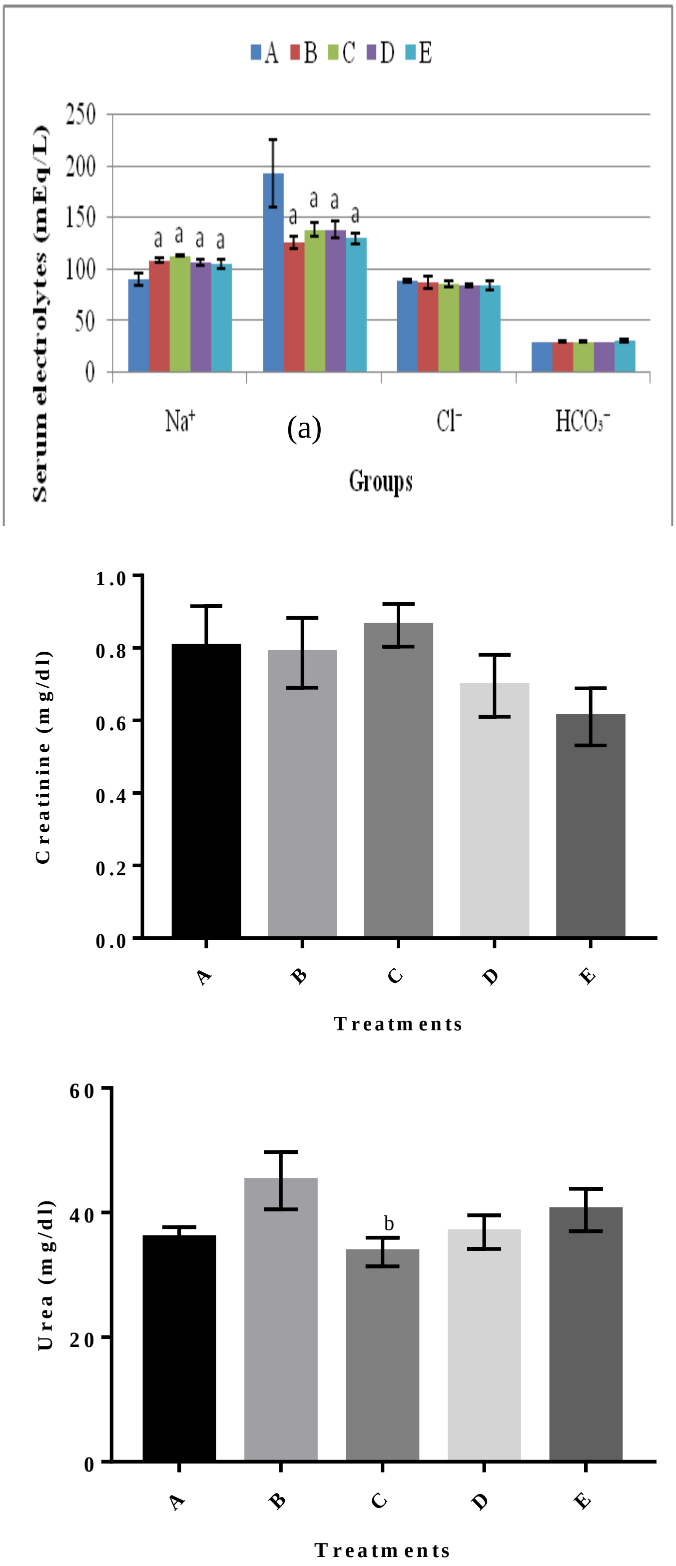

Figure 4: Selected kidney function parameters. Groups A: administered with uncontaminated feed only, B: kerosene-contaminated feed only, C: kerosene-contaminated feed + 5g of *Ficus glumosa* powdered leaves, D: kerosene-contaminated feed + 10g of *Ficus glumosa* powdered leaves and E: kerosene-contaminated feed + 30g of *Ficus glumosa* powdered leaves. a = $P < 0.05$ vs group A; b = $P < 0.05$ vs group B. Values are expressed as mean ± SEM, n = 5.

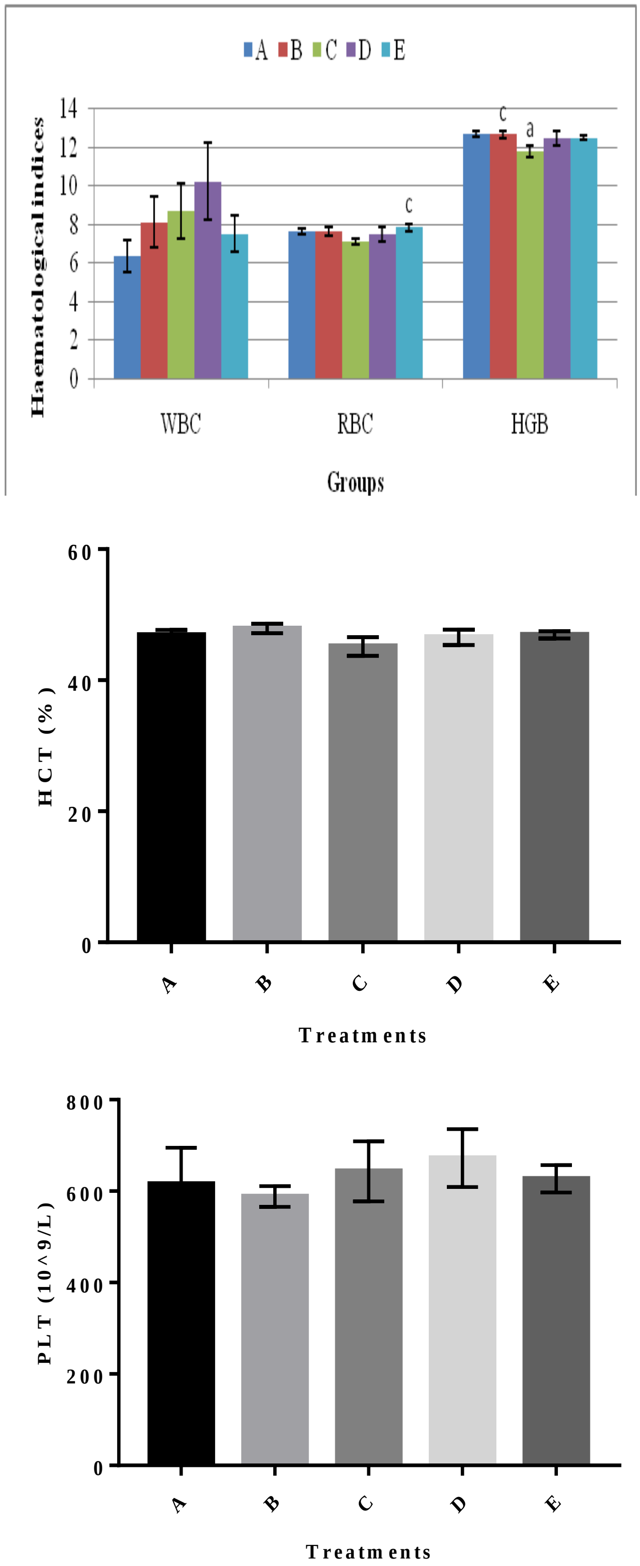

Figure 5: Selected haematological indices. WBC – White blood cell (10^9/L), RBC – Red blood cell (10^{12}/L), HGB – Haemoglobin (g/dl), HCT – Haematocrit (%) and PLT – Platelet (10^9/L). Groups A: administered with uncontaminated feed only, B: kerosene-contaminated feed only, C: kerosene-contaminated feed + 5g of *Ficus glumosa* powdered leaves, D: kerosene-contaminated feed + 10 g of *Ficus glumosa* powdered leaves and E: kerosene-contaminated feed + 30g of *Ficus glumosa* powdered leaves. a = $P < 0.05$ vs group A; b = $P < 0.05$ vs group B. Values are expressed as mean ± SEM, n = 5.

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Interestingly, the group that received the highest dose of the *F. glumosa* showed the lowest WBC count compared to the negative control and other treated groups. It is plausible that certain bioactive substances (in higher concentration) in the leaves may have enhanced the body's capacity to metabolize the xenobiotics at a faster rate hence decreasing the WBC count. However, the group administered with the lowest dose of the leaves (group C) showed a significantly decreased RBC count compared to the group that received the highest dose of the extract (group E). This perhaps was because of a probable high rate of haemolysis experienced by the rats in group C and more quantity of the leaves possibly helped the system to stop possible haemolysis in group E. Ita and Udofia[43] adduced that the reduction in RBC counts could be due to cytotoxic effects of the various constituents in petroleum fractions. These may cause oxidative stress thereby damaging the cell membrane of the erythrocytes. Furthermore, in comparison, the haemoglobin content and haematocrit among the groups were not significantly different. This may have been due to the short duration of the study. Onyeka*et al.*[44] stated that a dysfunction in the secretion of erythropoietin (occasioned by the exposure to heavy metals in crude oil fractions) accounts for the increase in RBC differentials i.e. Hb and PCV. In a diabetic study, extract of *F. glumosa* stem bark was said to have caused a significant increase in RBC counts, Hb and PCV in the treated rats when compared to the negative control group.[45]

Conclusion

Data obtained from this research has further affirmed the fact that exposure to kerosene negatively affects body tissues as well as their functions. However, *Ficus glumosa*leaf has the potential to reduce toxicity induced by kerosene especially if the duration of the administration of the plant is carried out on long-term basis.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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