Study on the hypoglycemic, hypolipidemic and histological effects of ethylacetate extract of *Combretum platypterum* leaves in alloxan induced diabetic rats.

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ABSTRACT

Background: Diabetes mellitus has become an increase concern in the world. People especially in poor communities have been using medicinal plants to treat diabetes and its complications. Much work has been done to find scientific evidences to support the use of medicinal plants thus ethylacetate extract of *Combretum platypterum* was investigated for its hypoglycemic, hypolipidemic and histological effects in alloxan induced diabetic Wistar albino rats.

Objectives: To investigate the hypoglycemic, hypolipidemic, histological effects and toxicological effects of ethylacetate extract of *Combretum platypterum* leaves in alloxan induced diabetic rats.

Methods: Phytochemical screening was carried out on the crude sample of *Combretum platypterum* leaves. Sequential extraction was carried out on the plant leaves using n-hexane, ethylacetate and methanol. The extracts were concentrated to constant weights using rotary evaporator. Diabetes was induced in the albino rats by administering 150 mg/kg i.p. of alloxan monohydrate. The ethylacetate extract of Combretum platypterum at 1000, 500, 250, 100, 50 mg/kg of body weight were administered orally at a single dose per day to the diabetic induced rats for 14 days. Effect of the extract and Glibenclamide (positive control) on blood glucose, plasma lipid and blood biochemical parameters were determined in the diabetic rats. Histological effect was also carried on the vital organs of the animals.

Results: Phytochemical screening showed the presence of flavonoids, saponnins, cardiac glycosides, cyanogenetic glycosides, tannins and alkaloids while anthraquinones were absent. The hypoglycemic effect showed a significant positive effect when compared with the standard drug ($p<0.05$). Glibenclamide (5 mg/kg) and the extract at 250, 500 and 1000 mg/kg showed significant hypolipidemic effect when compared to reference range (HDL>35 mg/dl) in animals. The extract at all doses caused reduction in total triglyceride and total cholesterol levels when compared with the reference and negative control. The histological result showed that *C. platypterum* has no toxic effect on the vital organs.

Conclusion: The assessment of the ethylacetate extract of *C. platypterum* showed reduction the blood glucose level, hypolipidemic activities and has no toxic effect on the vital organs of the animals.

Key words: *Combretum platypterum* leaves, hypoglycemic, hypolipidemic, histological effect, alloxan, glibenclamide, Wistar rats.

Étude sur les effets hypoglycémiques, hypolipémiques et histologiques de l'extrait d'éthylacétate de feuilles de *Combretum platypterum* chez des rats diabétiques induits par alloxan

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RÉSUMÉ

Contexte: Le diabète sucré est devenu une préoccupation croissante dans le monde. Les habitants des communautés pauvres, notamment, utilisent des plantes médicinales pour traiter le diabète et ses complications. De nombreux travaux ont été réalisés pour trouver des preuves scientifiques à l'appui de l'utilisation de plantes médicinales. Ainsi, l'extrait d'acétate d'éthyle de *Combretum platypterum* a été étudié pour ses effets hypoglycémiques, hypolipidémiques et histologiques sur des rats albinos Wistar diabétiques induits par l'alloxane.

Objectifs: Étudier les effets hypoglycémiques, hypolipidémiques, histologiques et toxicologiques de l'extrait d'acétate d'éthyle de feuilles de *Combretum platypterum* sur des rats diabétiques induits par l'alloxane.

Méthodes: Un criblage phytochimique a été réalisé sur l'échantillon brut de feuilles de *Combretum platypterum.* Une extraction séquentielle a été effectuée sur les feuilles de la plante en utilisant du n-hexane, de l'acétate d'éthyle et du méthanol. Les extraits ont été concentrés à poids constants à l'aide d'un évaporateur rotatif. Le diabète a été induit chez les rats albinos par l'administration ip de 150 mg/kg d'alloxane monohydraté. L'extrait d'acétate d'éthyle de Combretum platypterum à 1 000, 500, 250, 100, 50 mg/kg de poids corporel a été administré par voie orale en une dose unique par jour aux rats induits par le diabète pendant 14 jours. L'effet de l'extrait et du glibenclamide (témoin positif) sur la glycémie, les lipides plasmatiques et les paramètres biochimiques sanguins a été déterminé chez les rats diabétiques. L'effet histologique a également été exercé sur les organes vitaux des animaux.

Résultats: Le criblage phytochimique a montré la présence de flavonoïdes, de saponines, de glycosides cardiaques, de glycosides cyanogénétiques, de tanins et d'alcaloïdes tandis que les anthraquinones étaient absentes. L'effet hypoglycémique a montré un effet positif significatif par rapport au médicament standard ($p<0,05$). Le glibenclamide (5 mg/kg) et l'extrait à 250, 500 et 1 000 mg/kg ont montré un effet hypolipidémiant significatif par rapport à la gamme de référence (HDL > 35 mg/dl) chez les animaux. L'extrait à toutes les doses a entraîné une réduction des niveaux de triglycérides totaux et de cholestérol total par rapport à la référence et au contrôle négatif. Le résultat histologique a montré que *C. platypterum* n'a pas d'effet toxique sur les organes vitaux.

Conclusion: L'évaluation de l'extrait acétate d'éthyle de *C. platypterum* a montré une réduction de la glycémie et des activités hypolipémiantes.

Mots-clés: Feuilles de *Combretum platypterum*, hypoglycémique, hypolipidémique, effet histologique, alloxane, glibenclamide , rats Wistar.

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by hypoglycemia resulting from defect in the insulin resistance, secretion and/or action. Out of all the several types of diabetes mellitus known to occur, type I and II are predominant. It is characterized by hyperglycemia, lipoprotein abnormalities, raised basal metabolic rates, defects in reactive oxygen species scavenging enzymes and high oxidative stress induced damage to pancreatic β-cells.[1-4] Plants that have medicinal properties are said to be rich in phytochemical compounds such as Saponins, Alkaloids, Tannins, phenolic compounds and antioxidants.[5] Some botanical polysaccharides are considered as important bioactive components responsible for hypoglycemic effect.[6-7] Also, a number of plants are known to have hypolipidemic activity.[8-10] However, there is little information about plants with both hypoglycemic and hypolipidemic activities.

The plant *Combretum platypterum* is a member of the family Combretaceae which occurs mainly in the tropical and subtropical areas.[11] The family has been used extensively in traditional medicine against inflammation and infections.[12] Eloff and McGaw reported and confirmed in 2006 the antimicrobial compounds of *C. platypterum.*[13] From informal discussion with local communities and traditional medical practitioners, the root decoction is drunk to treat lower backache and the leaf decoction is drunk to treat headache while the leaf decoction is taken to treat fever. *Combretum platypterum* possesses a number of local medicinal applications. However, no pharmacological analysis have been done on the species thus, this research is done to evaluate its potential.

MATERIALS AND METHODS

Plant materials

Fresh plants of *C. platypterum* were collected at Arapaja, Odo-Ona Kekere, Ibadan and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. Where voucher specimen with voucher number 110163 was prepared and deposited

Plant extraction

The leaves of *C. platypterum* were carefully separated, air dried for 21 days and powdered. The leaves (500 g) were extracted using soxhlet extraction sequentially with 5000 mls each of n-Hexane, ethylacetate and methanol. The extracts were concentrated under reduced pressure with a rotatory evaporator, dried to constant weights and stored in airtight container prior analysis.

Phytochemical screening

Phytochemical screening was carried out on the powdered sample of *Combretum platypterum* leaves to identify the various secondary metabolites. This was carried out following standard procedures. **(Trease and Evans, 2002).**

Experimental animals

Ethical approval

This was applied for and approved through the chairperson of the Ethical committee, Olabisi Onabanjo University, Sagamu. Ogun State

Thirty-five albino Wistar rats of both sexes weighing between 70 and 100 g obtained from the department of Clinical Pharmacy, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria were used for the study. The animals were fed with standard pellet diet (Sai Durga feeds, Bangalore) and water was provided ad libitum. The animals were maintained under standard laboratory and animal housing conditions at temperature of 25^{o}C ± 2^{o}C, relative humidity 60-70% with 12-hour light/dark cycle for seven days. The baseline weights and blood glucose levels of the animals were carried out before inducing diabetes in the rats. The rats were divided into seven groups of five rats in a group.[11]

Experimental design

The thirty diabetic rats in groups II-VI received 50, 100, 250, 500 and 1000 mg/kg ethylacetate extract of *C. platypterum* respectively. Group VII (positive control) received 5 mg/kg glibenclamide while group I rats were dosed with normal saline only. The plant extract, glibenclamide and normal saline were administered orally using oral canula. Blood was obtained from the tail of the rats by snipping the tips with sterile scissors which had first been sterilized by swabbing with 80% ethanol. Bleeding was then enhanced by gently "milking" the tail from the body towards the tail tip. The blood glucose levels in the rats were then determined using Accucheck Hypo guard glucometer with the appropriate glucometer strips. After the operation, the tips of the tails were

sterilized by swabbing with 80% ethanol.

Induction of diabetes

Diabetes was induced in thirty rats in groups II-VII by a single intraperitoneal administration of freshly prepared 150 mg/kg alloxan monohydrate in normal saline solution.[14] After 72 hours of alloxan administration, the blood glucose level was monitored using glucometer. Rats with blood glucose level of 140 mg/dl and more were used for the study since the level of serum glucose considered to be normal in male Wistar rat ranges from 50-135 mg/dl.[15] The body weights of all the animals were recorded at the start of the experiment and on the 14th day of the experiment.

Biochemical analysis of the blood samples

Collection of bood samples

The diabetic rats were sacrificed by decapitation under light diethyl ether anesthesia for about three minutes on the 14th day of the experiment. The rats were dissected and 3-5 mls of blood samples were collected directly from the heart by cardiac puncture using 5 ml syringe and needle. The blood samples collected into well labeled lithium heparin bottles to prevent clotting and separated by centrifuge for 20 mins at 630 rpm. The obtained serum was kept at -20^{o}C.

(a) Estimation of creatinine and high density lipoprotein concentrations

The creatinine and high density lipoprotein concentrations were analyzed by modified UV spectrophotometric method using wet reagent diagnostic kits according to the Randox and Dialab manufacturers' protocol kits respectively.[16]

(b) Estimation of total cholesterol

Cholesterol determination was carried out as described by CHOD/PAP method. Working reagent (1.0 ml) and 0.01 ml of distilled water were transferred into a test tube, mixed and labeled blank. Another 0.1 ml of the working reagent and 0.01 ml of cholesterol standard were transferred into another test tube, mixed and labeled standard. 0.1 ml of the working group and 0.01 ml of the samples of all the groups were measured into different test tubes, mixed and labeled accordingly. All the mixtures were allowed to stand at 25^{o}C for 15 minutes and the absorbance were determined at 505 nm against distilled water as blank using UV spectrophotometer within 60 minutes. The absorbance readings were then recorded.

(c) Estimation of the total triglyceride

This was carried out as described by DIALAB manual kit. The working reagent (100 µl) and 100 µl of distilled water was measured into a test tube, mixed and labeled blank. Another 100 µl of the working reagent and 100 µl of standard reagent were measured into a test tube, mixed and labeled standard. 100 µl of the working reagent and 100 µl of samples of all the groups were measured into different test tubes, mixed and labeled accordingly. These were allowed to stand for 10 minutes at room temperature. The readings were taken at wavelength of 500 nm. The mixture containing distilled water was used to blank the spectrophotometer. The absorbance was measured against the standard within 30 minutes. The absorbance readings were then recorded. The concentration of the triglycerides was then calculated according to the DIALAB manual test kit.

(d) Estimation of High Density Lipoprotein

This was carried out as described by CHOD/PAP method. 100 µl of the cholesterol working reagent and 100 µl of distilled water were measured into a test tube, mixed and labeled blank. Another 100 µl of the cholesterol working reagent and 100 µl of standard reagent were measured into a test tube, mixed and labeled standard. 100 µl of the cholesterol working reagent and 100 µl of samples of all the groups were measured into different test tubes, mixed and labeled accordingly. These were allowed to stand for 10 mins at room temperature. The readings were taken at wavelength of 550 nm. The mixture containing distilled water was used to blank the spectrophotometer. The absorbance was measured against the standard within 30 mins. The absorbance readings were then recorded. The concentration of the triglycerides was then calculated according to the DIALAB manual test kit. Other biological analysis carried out includes the Total protein, Albumin, Alobulin, Agglutinin Ratio and Total Bilirubin.

Histological analysis

This was performed following a midline laparotomy to remove the organs (kidney, liver, spleen, pancreas and heart). The selected organs were immediately fixed in 10% buffered formalin solution, processed and

embedded in paraffin. The organs were sectioned at 5 microns and stained using haematoxylin and eosin (H and E) technique for the general tissue-organ architecture. Sections were viewed under light microscope. The photomicrographs were obtained using a scope exe camera attached to a computer.

Statistical analysis

Data were expressed as Mean ± Standard deviation. Statistical comparisons were performed by one-way analysis of variance (ANOVA).

RESULTS

The extraction procedure gave the result.

500 g of weighed sample yielded 16.98% of n-Hexane fraction, 17.54% of ethylacetate fraction and 17.78% of methanol fraction.

The phytochemical screening of the *Combretum platypterum* leaves is shown in Table 1

Table 1: Phytochemical screening of *Combretum platypterum* leaves

PHYTOCHEMICALS	INFERENCE
Alkaloids	+
Anthraquinones	
Combined	-
Free	-
Cardiac glycosides	+
Cyanogenetic glycosides	+
Saponins	+
Flavonoids	+
Tannins	+

Key: + Positive, - Negative

Figure 1: Body weight changes in the control, glibenclamide and diabetic induced rats treated with ethylacetate extract of *Combretum platypterum* leaves.

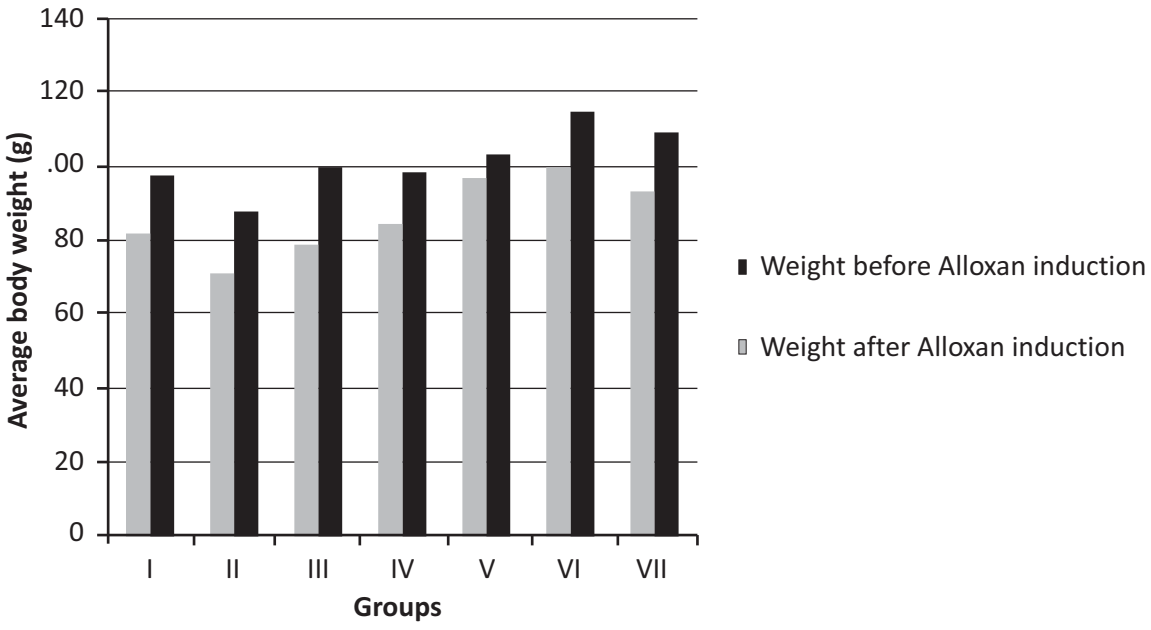


Figure 2: Change in the Blood glucose level in Control, Glibenclamide and diabetic induced rats groups with ethylacetate fraction of *Combretum platypterum* leaves.

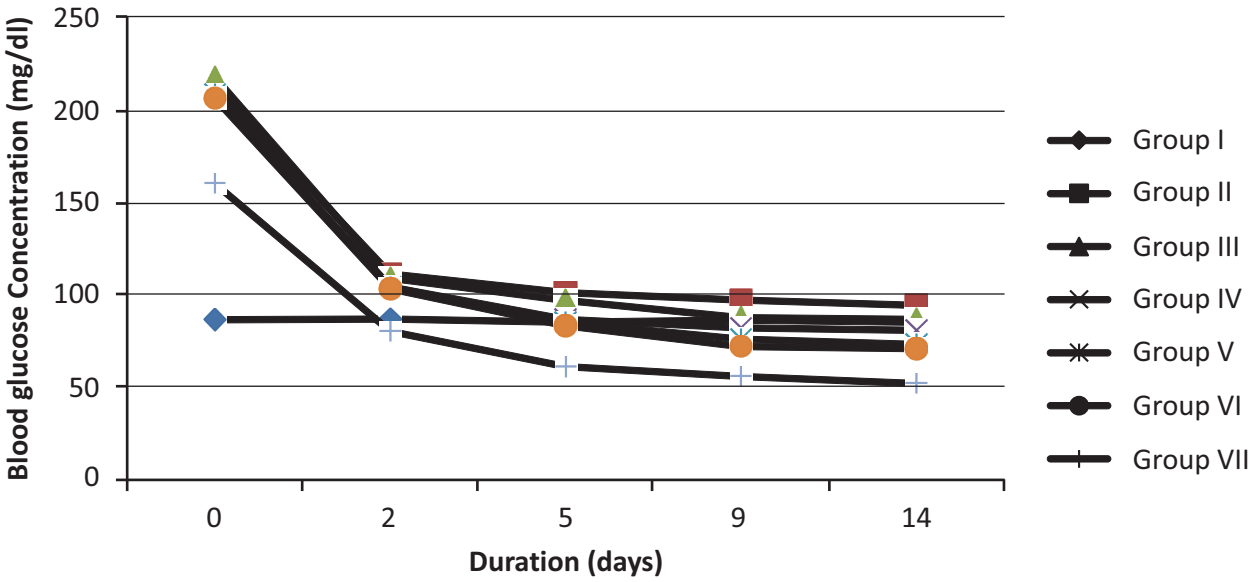


Figure 3: Effect of ethylacetate extract of *Combretum platypterum* leaves and Glibenclamide on lipid concentration of diabetic induced rats.

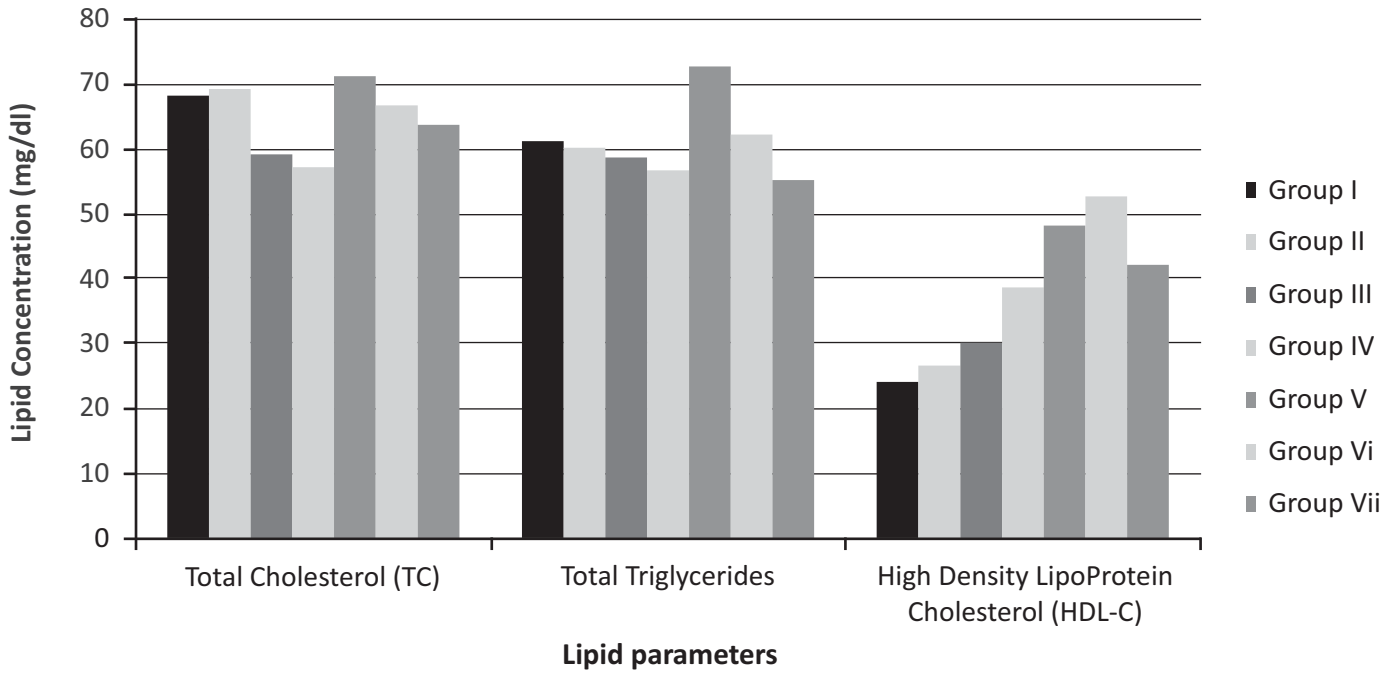


Figure 4: Effect of ethylacetate extract of *Combretum platypterum* leaves and Glibenclamide on Biochemical parameters concentration of alloxan induced rats.

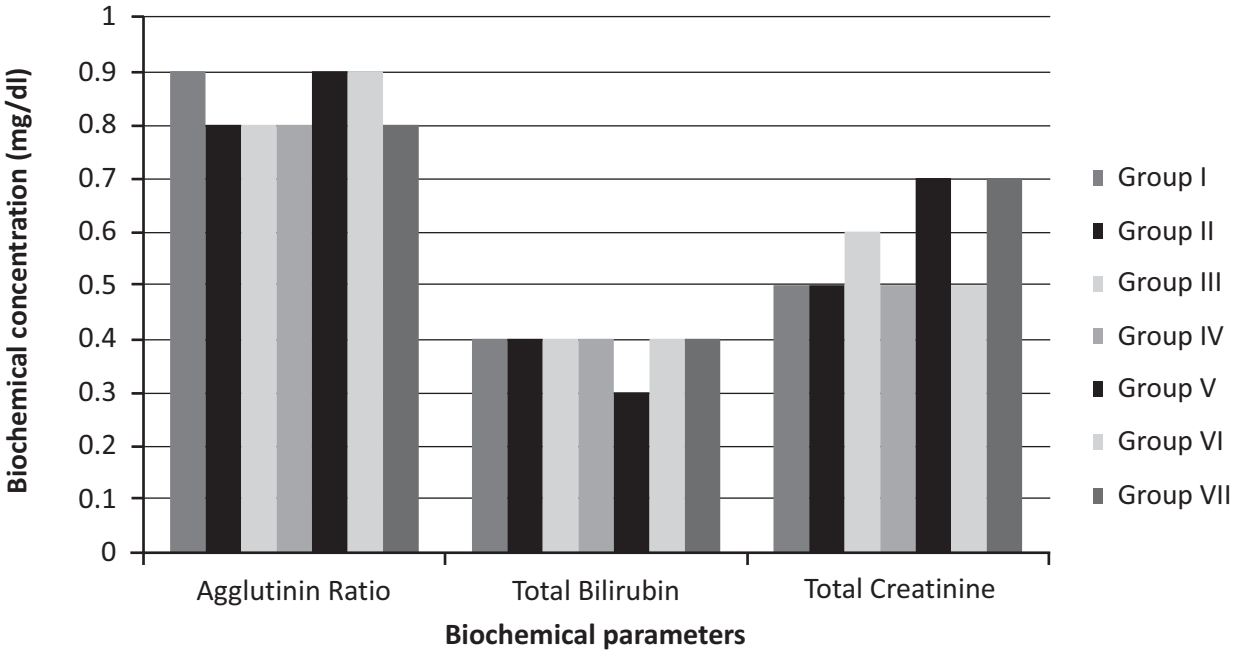
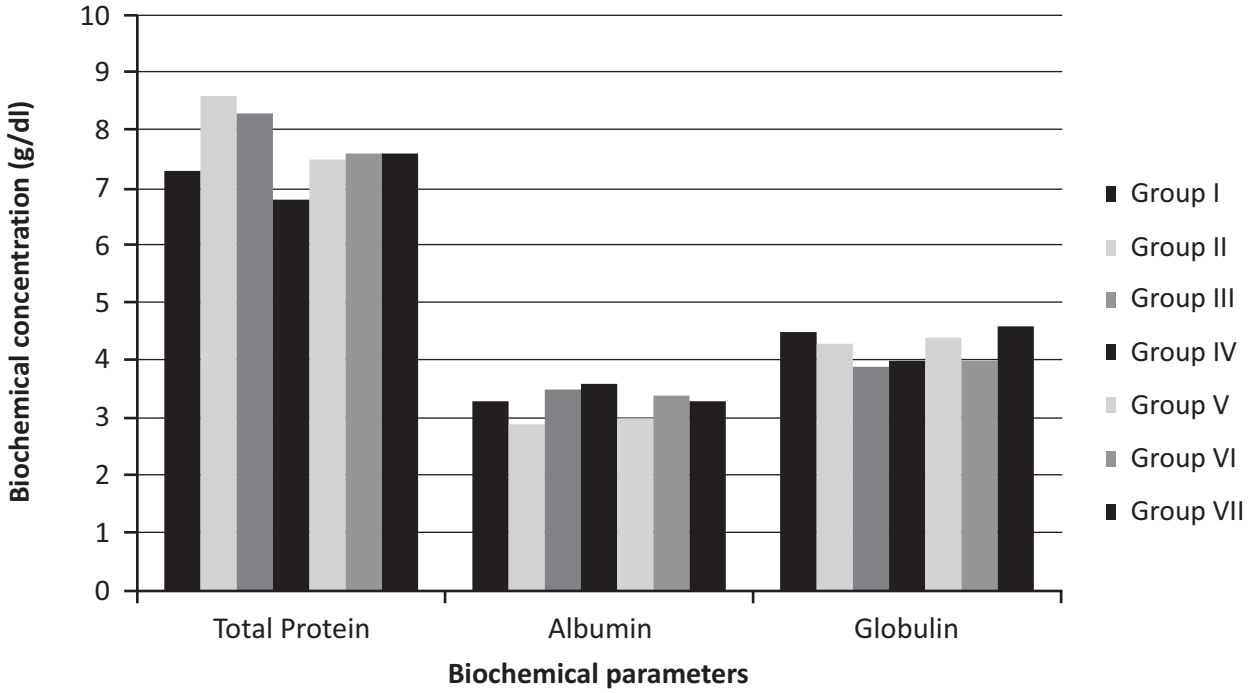
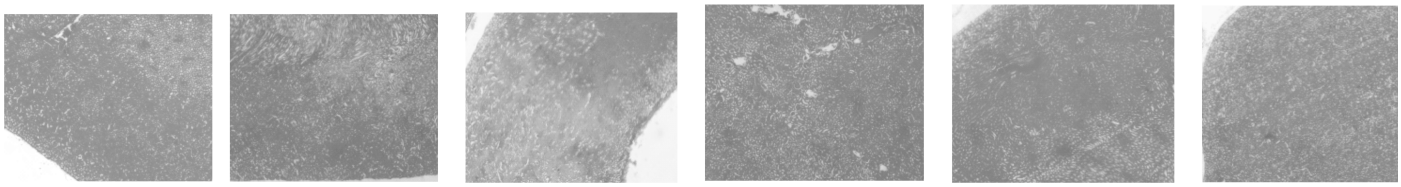


Figure 5: Effect of ethylacetate extract of *Combretum platypterum* leaves and Glibenclamide on Biochemical parameters concentration of alloxan induced rats.

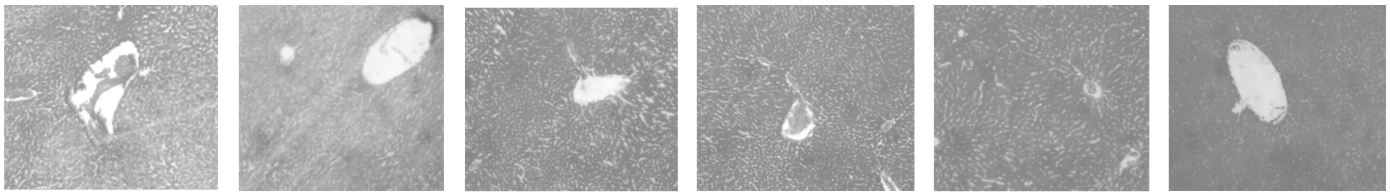




(ak) (bk) (ck) (dk) (ek) (fl)

Figure 6: Photomicrographs (x40) of the Kidney of control and groups II-VI diabetic rats respectively.

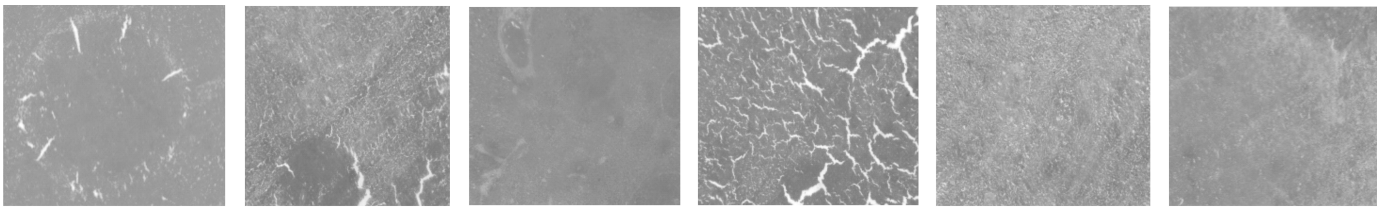
Key: ak - Group II, bk -Group III, ck - Group IV, dk- GroupV, ek - GroupVI, fk -GroupVII



(al) (bl) (cl) (dl) (el) (fl)

Figure 7: Photomicrographs (x100) of the Liver of control and groups II-VI diabetic rats respectively.

Key: al- Group II, bl -Group III, cl - Group IV, dl- GroupV, el - GroupVI, fl -GroupVII



(aS) (bS) (cS) (dS) (eS) (fS)

Figure 8: Photomicrographs (x100) of the Spleen of control and groups II-VI diabetic rats respectively.

Key: as - Group II, bs -Group III, cs - Group IV, ds- GroupV, es - GroupVI, fs -GroupVII

DISCUSSION

The steady increase in the mean body weight in Figure 1 showed that the ethylacetate extract of *C. platypterum* was able to protect the rats from tissue wasting associated with diabetes mellitus.[17] The alloxan induced diabetic rats elicited significant rise in blood glucose level but the diabetic rats treated with ethylacetate extract of *C. platypterum* leaves exhibited a decrease in the blood glucose level (Figure 2). This possible mechanism may be by stimulating insulin released from the remnant pancreatic β-cells or its release from the bound form.[18] This might also involve an extra pancreatic action in the alloxan induced hyperglycemic rats which might include the stimulation of peripheral glucose utilization of enhancing glycolytic and glycogenic processes with concominant decrease in glycogenolysis and glyconeogenesis.[19] Phytochemical analysis has shown that the presence of potent phytochemicals like steroids, saponins, flavonoids, tannins, terpenoids possess antihyperglycemic activity as enunciated by reports of Oliver, 1980| which showed that flavonoids, steroids, terpenoids, phenolic acids are known to be bioactive antidiabetic principles.[20] Moreover, flavonoids are known to regenerate the damage β-cells in the alloxan induced diabetic rats and acts as insulin secretagogues.[21] Also saponin has been reported to have hypocholesterotemic effect and thus, may aid lessening metabolic burden that would have been placed in the liver.[22] The hypoglycemic activity of the *Combretum platypterum* may also be due to the presence of the active chemical compounds like the alkaloids, saponins, glycosides, steroids etc which have demonstrated

activity with use in the treatment of diabetes.[23-24] Since the fall in blood glucose levels was different in the models with different response to hyperglycemia, it showed that the hypoglycemic effect of the plant is dosage related to the diabetogenic agent which in turn leads to β-cells destruction.[25] Similar work by Fagbohun *et al* (2017) showed that the Fleurya aestuans was more effective at lower concentration in the treatment of the diabetic induced rats.[9] Studies have shown that hypolipidemia is one of the clinical features of alloxan induced type 1 diabetes mellitus.[26-30] In this study, ethylacetate extract of *C. platypterum* reduced the levels of Total Triglyceride, TG, and Total Cholesterol, TC but the High Density Lipoprotein-Cholesterol, HDL-C, was elevated. Diabetic dyslipidemia which is as a result of abnormalities in glucose and fatty acid metabolism due to altered insulin action in fatty acids may be possible factor causing increased in Total Cholesterol and Total Triglycerides levels on exposure to alloxan as found in the study carried out by Mgbeje *et al*, (2016).[17] Thus the hypolipidemic effect may be attributed to the action of different phytols present in the plant. Creatinine, synthesized in the liver, passes into the blood circulation and taken up almost entirely by the skeletal muscles. The treatment with ethylacetate extract of *C. platypterum* was effective in preventing increase in Total creatinine level in alloxan induced diabetic rats when compared with the control (Figure 4). This was also observed in the study carried out by Alagammal *et al* (2012)[31] and Mohammed *et al* (2019).[32] The levels of Total protein, Albumin, and Globulin of the control and the alloxan induced rat with different concentrations of ethylacetate and the standard drug were presented in Figure 5. Reduction in the level of serum protein, albumin and globulin indicates the occurrence of hypoalbuminemia which is observed in diabetes.[32] At lower concentrations (50 and 100 mg/kg) of the ethylacetate extract, the total protein increased but at higher concentrations, the total protein as well as albumin and globulin did not deviate from the normal range. This showed that insulin deficiency could be corrected at higher doses of the ethylacetate extract as shown by Alagammel *et al*., (2012).[31] The histological photomicrographs obtained using the exe camera attached to a computer showed that there was no damage in the kidney, liver, spleen, pancreas and heart (Figure 6-8) indicating that the extract leads to relieving the injuries with a promising effect towards hyperglycemia and other related disorders.

CONCLUSION

It is concluded that the ethylacetate of *Combretum platypterum* extract exhibit the hypoglycemic and hypolipidemic activities which may be due to the presence of the active phytochemicals contained in the plant. Also, the histological studies showed that the ethylacetate extract of the plant has no toxic effect on the vital organs of the experimental animals.

The authors declare no conflict of interest.

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