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# Antidiabetic and Safety Properties of Ethanolic Leaf Extract of *Corchorus olitorius* in Alloxan-Induced Diabetic Rats

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## Abstract

Diabetes is a major metabolic disease of global concern. Ethanolic extract of *Corchorus olitorius* leaf was investigated for antidiabetic activity in alloxan-induced diabetic rats. A total of thirty-six albino rats (*Rattus norvegicus*) with body weight  $150.50 \pm 10.50$  g were randomly selected into six groups (A–F). Group A animals were non-diabetic and received 0.5 mL distilled water, groups B, C, D, E and F were made diabetic by administration of alloxan monohydrate (150 mg/kg, body weight i.p). Group B was diabetic untreated, group C was diabetic and treated with glibenclamide, while groups D, E and F received the ethanolic extract of *C. olitorius* leaf at a dose of 200 mg/kg, 400 mg/kg and 800 mg/kg body weight respectively. Phytochemical screening showed the presence of flavonoids, tannins, saponins, phlobatannin anthraquinones, phenol and cardiac glycoside and saponin. The blood glucose of the alloxanized rats after 72 hours which ranged from 17.30–25.33 mmol/L were significantly ( $p < 0.05$ ) and progressively reduced in treated groups which compared favorably with the standard drug group. The significantly ( $p < 0.05$ ) elevated levels of serum and liver bilirubin (direct and total), transaminases (AST and ALT), alkaline phosphatase, urea, creatinine, total cholesterol, triglyceride, LDL-C, as well as reduced levels of total protein, globulin, albumin and HDL-C in the diabetic untreated rats were normalized upon treatment with ethanolic extract of *C. olitorius* leaf. These results suggest that the ethanolic extract of *C. olitorius* leaf possesses antihyperglycemic property with no major side effect hence it could be considered safe for the management of diabetes.

**Keywords:** antioxidants, *Corchorus olitorius*, blood glucose, diabetes

## 1. Introduction

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin (a hormone that regulates blood sugar, or glucose), or when the body cannot effectively use the insulin it produces. Diabetes mellitus (DM) presents enormous and increasingly important public health issues as it is listed among the commonest non-communicable diseases (NCDs) globally, the prevalence of which increased in adults from 4.7% in 1980 to 8.5% in 2014. Diabetes mellitus led to about 1.5 million deaths in 2012. Elevated blood glucose resulted into an additional 2.2 million deaths through complications arising from heart related diseases. Over 43% of these deaths were recorded before the seventh decade of life [1, 2]. Prevalence of DM in Africa is approximately 1% in rural areas and up to 7% in urban sub-Saharan Africa [3]. In Nigeria, DM is estimated to be between 0.9–15% [4].

The percentage of deaths attributable to high blood glucose or diabetes that occurs prior to age 70 is higher in low- and middle-income countries than in high-income countries. The disease is characterized by high blood glucose levels and abnormal metabolism of carbohydrates, proteins, and fat associated with a relative or absolute insufficiency of insulin secretion and with various degrees of insulin resistance. Such alterations result in increased blood glucose causing a chronic state of high blood glucose level (hyperglycemia) that results from an absolute or relative insulin deficiency and is associated with long-term complications affecting the eyes, kidneys, heart and nerves [5].

Cellular stress as a result of reactive oxygen species such as peroxy (ROO), nitrogen dioxide ( $\text{NO}_2^-$ ), superoxide ( $\text{O}_2^-$ ), nitric oxide (NO), hydroxyl ( $\text{OH}^-$ ) and non-free hydrogen peroxide and singlet oxygen radicals play a significant role in the pathogenesis of several disease conditions such as DNA damage, cellular degeneration and oxidation of lipids and proteins. These have been implicated in the development of these diseased conditions associated with diabetes [6–9].

The pathogenesis of diabetes mellitus is managed by insulin and oral administration of hypoglycemic drugs such as sulfonylureas and biguanides which are not without a number of side effects. Moreover, none of the oral synthetic hypoglycemic agents has been successful in diabetes management and controlling long-term microvascular and macrovascular complications [10]. The toxicity of oral antidiabetic agents differs widely in clinical manifestations, severity, and treatment [11].

Optional therapies such as herbal preparations have been used for the management of diabetes. The benefits of these herbal medications are their efficacy, endogenous relativity, cost effectiveness and tolerability [12]. Various parts of medicinal trees have been employed in the third world traditional medicinal system and most have demonstrated pre-clinical or clinical normoglycemic activity [13]. Furthermore, World Health Organization has also recommended the evaluation of traditional plant treatments for diabetes [14].

*Corchorus olitorius* is a plant from the Tiliaceae family from the Mediterranean region, its leaves have been found to be rich in antioxidants, such as vitamin C, vitamin E,  $\beta$ -carotene,  $\alpha$ -tocopherol, glutathione and phenols [15]. The leaves also contain fatty acids, minerals, other vitamins and



**Figure 1.** *Corchorus olitorius*.

mucilaginous polysaccharides, and have been used as traditional folkmedicine. Yokoyama et al [16] reported *C. olitorius* leaves to ameliorate atopic dermatitis in NC/Nga mice [16]. It is called 'ewedu' in Yoruba Language and is a common source of vegetable among Yoruba tribe in Nigeria. This study therefore investigated the anti diabetic and safety potentials of ethanolic leaf extract of *Corchorus olitorius* in alloxan-induced diabetes in rats (**Figure 1**).

## 2. Materials and methods

Alloxan monohydrate obtained is a product of Sigma Chemical Company, St. Louis, Mo, USA. Kit for the estimation of AST, ALT, urea, creatinine and bilirubin, were produced by Randox Laboratories Ltd., Antrim, UK. All other chemicals were of analytical grades and prepared in all-glass apparatus using distilled water (BDH, UK).

### 2.1. Plant extract preparation

The fresh leaves of *Corchorus olitorius* were obtained from a vegetable farm in Ilorin West Local government, Ilorin, Kwara State, Nigeria. It was taxonomically authenticated at the Department of Plant Biology, University of Ilorin, Ilorin Kwara state, Nigeria where a voucher specimen number 064 was deposited. Fresh leaves of *C. olitorius* was collected and air-dried for 21 days until constant weight was obtained. They were pulverized using an electric blender machine and sieved to obtain a fine powder. Forty grams (400 g) was macerated in 2500 ml of 80% ethanol, shaken at regular intervals to achieve maximum extraction. The solution was filtered using Whatman No.1 filter paper and the filtrate concentrated in water bath at 40°C. The dried extract was later weighed and reconstituted in distilled water to the required dosage for administration.

### 2.2. Experimental animals

A total of thirty-six (36) Albino rats (*Rattus norvegicus*) weighing  $150.50 \pm 10.50$  g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria. Animals were maintained under standard environmental conditions i.e. ambient temperature of  $(27 \pm 2^\circ\text{C})$  and at 45–55% relative humidity for 12 hours,

each of dark and light cycle. The rats were allowed free access to standard laboratory food and water ad libitum throughout the experiment.

### **2.3. Induction of diabetes**

The animals were fasted overnight and diabetes was induced by a single intraperitoneal injection of freshly prepared 150 mg/kg b.w alloxan monohydrate dissolved in (5%) sterile saline. Two days after alloxan injection, rats with blood glucose level of >12 mmol/L were separated and considered diabetic and were used for the study. Blood glucose levels were measured using blood glucose test strips with fine test glucometer (infopia Co. limited Korea). The treatment started 48 hours after alloxan injection and this was considered the first day of treatment. The treatment continued for 14 days.

### **2.4. Animal grouping and extract administration**

Animals were divided into six groups, and for each group, six animals were treated orally once a day for 14 days as follows:

Group A: Control rats received distilled water only.

Group B: Diabetic control.

Group C: Diabetic rats received Glibenclamide at a dose of 5 mg/kg.

Group D: Diabetic rats received 200 mg/kg body weight extract.

Group E: Diabetic rats received 400 mg/kg body weight extract.

Group F: Diabetic rats received 800 mg/kg body weight extract.

### **2.5. Samples preparation**

At the end of the experimental period, food was withdrawn from the rats and they were fasted overnight while the animals had free access to water. They were then euthanized under diethyl ether vapor and sacrificed. Venous blood was collected from the experimental animals and serum was prepared by centrifuging the blood samples at 3000 rpm for 5 minutes and serum collected by pipetting. The animals were quickly dissected and internal organs including liver and kidney were collected, blotted using filter paper to remove traces of blood and then weighed with an analytical balance. The pancreas, liver and kidney were suspended in ice-cold 0.25 M sucrose solution (1:5 m/v) and homogenized as described by Akanji and Yakubu [17].

### **2.6. Statistical analysis**

Comparisons were made using Duncan's multiple range test, and values were considered to be significant at  $p < 0.05$ .

### 3. Results

#### 3.1. Phytochemical constituents of ethanolic extract of *Corchorus olitorius* leaf

**Table 1** shows the results of the preliminary phytochemical analysis of the leaf extract. Analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, phlobatannin anthraquinones, phenol, cardiac glycoside and saponin while Terpenoids, Steroids, Triterpenes were not detected.

#### 3.2. Glycemic effect of ethanolic extract of *Corchorus olitorius* leaf of alloxan-induced diabetic rats

**Table 2** presents the glycemic effects of ethanolic extract of *Corchorus olitorius* leaf in alloxan induced diabetic rats. Single dose of alloxan monohydrate (150 mg/kg) continuously increased the fasting blood glucose from the first day of treatment till the third, while upon oral administration of ethanolic extract of *Corchorus olitorius* and standard drug (Glibenclamide) for 14 days, a significant decrease ( $P < 0.05$ ) in fasting blood glucose was observed particularly at the highest dose of 800 mg/kg of the plant extract.

#### 3.3. Effect of ethanolic leaf extract of *Corchorus olitorius* on body weight of alloxan-induced diabetic rats

In diabetic rats, continuous reduction in body weight was observed as shown in **Table 3**. Glibenclamide (5 mg/kg) as well as the extract treatment groups at the dose of 400 and 800 mg/kg b.w showed improvement ( $P < 0.05$ ) improvement in body weight of diabetic rats.

Phytochemicals	Crude extracts
Anthraquinones	+
Tannins	+
Phenolics	+
Saponins	+
Terpenoids	-
Alkaloids	+
Steroids	-
Cardiac glycoside	+
Flavonoids	+
Triterpenes	-

Where: (+) indicates present; (-) indicates not present

**Table 1.** Phytochemical composition of the crude extract of *Corchorus olitorius*.



Treatment groups	Fasting blood glucose level after diabetes induction			
	Day 0	Day 5	Day 10	Day 14
Control	5.13 ± 0.60 <sup>a</sup>	4.00 ± 0.70 <sup>a</sup>	4.38 ± 0.20 <sup>a</sup>	4.13 ± 0.29 <sup>a</sup>
Diabetic rats + distilled water	17.03 ± 1.70 <sup>b</sup>	19.18 ± 1.11 <sup>b</sup>	18.83 ± 1.25 <sup>b</sup>	20.41 ± 1.07 <sup>b</sup>
Diabetic rats + Glibenclamide	21.68 ± 1.93 <sup>b</sup>	16.05 ± 0.72 <sup>b</sup>	12.10 ± 0.29 <sup>ab</sup>	5.80 ± 0.35 <sup>a</sup>
Diabetic rats +200 mg/kg body weight of the extract	18.43 ± 1.04 <sup>b</sup>	14.58 ± 0.55 <sup>b</sup>	13.08 ± 0.44 <sup>ab</sup>	9.43 ± 0.26 <sup>ab</sup>
Diabetic rats +400 mg/kg body weight of the extract	20.80 ± 2.46 <sup>b</sup>	13.63 ± 0.21 <sup>b</sup>	12.30 ± 0.81 <sup>ab</sup>	7.88 ± 0.63 <sup>ab</sup>
Diabetic rats +800 mg/kg body weight of the extract	25.33 ± 1.91 <sup>b</sup>	22.95 ± 1.41 <sup>b</sup>	13.43 ± 1.10 <sup>ab</sup>	6.05 ± 0.66 <sup>a</sup>

Values are expressed as mean of six replicates ±SD and those with different superscripts down the column are statistically different ( $p < 0.05$ )

**Table 2.** Effect of ethanolic extract of *Corchorus olitorus* leaf on fasting blood glucose level (mmol/L) of alloxan-induced diabetic rats.

Treatment groups	Initial body weight (g)	Final body weight (g)
Control	136.25 ± 10.33 <sup>a</sup>	180.07 ± 13.07 <sup>b</sup>
Diabetic rats + distilled water	172.67 ± 5.10 <sup>b</sup>	134.01 ± 13.17 <sup>a</sup>
Diabetic rats + Glibenclamide	153.33 ± 1.55 <sup>ab</sup>	184.22 ± 8.46 <sup>b</sup>
Diabetic rats +200 mg/kg body weight of the extract	157.25 ± 3.07 <sup>ab</sup>	164.08 ± 10.56 <sup>ab</sup>
Diabetic rats +400 mg/kg body weight of the extract	175.67 ± 14.06 <sup>b</sup>	183.19 ± 14.79 <sup>b</sup>
Diabetic rats +800 mg/kg body weight of the extract	141.42 ± 4.47 <sup>ab</sup>	172.69 ± 10.70 <sup>b</sup>

Values are expressed as mean of six replicates ± SD and those with different superscripts down the column are statistically different ( $p < 0.05$ )

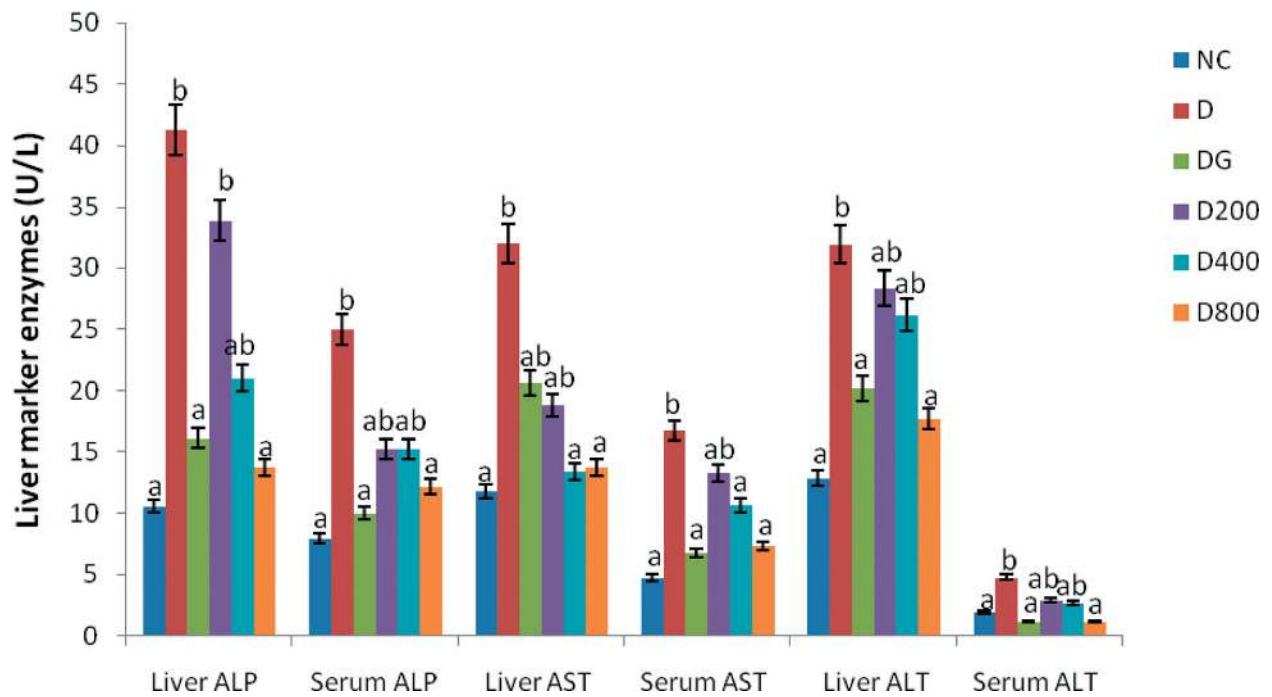
**Table 3.** Effect of *Corchorus olitorus* leaf extract on total body weight of alloxan-induced diabetic rats.

### 3.4. Effect of ethanolic leaf extract of *Corchorus olitorus* on liver function enzymes of alloxan-induced diabetic rats

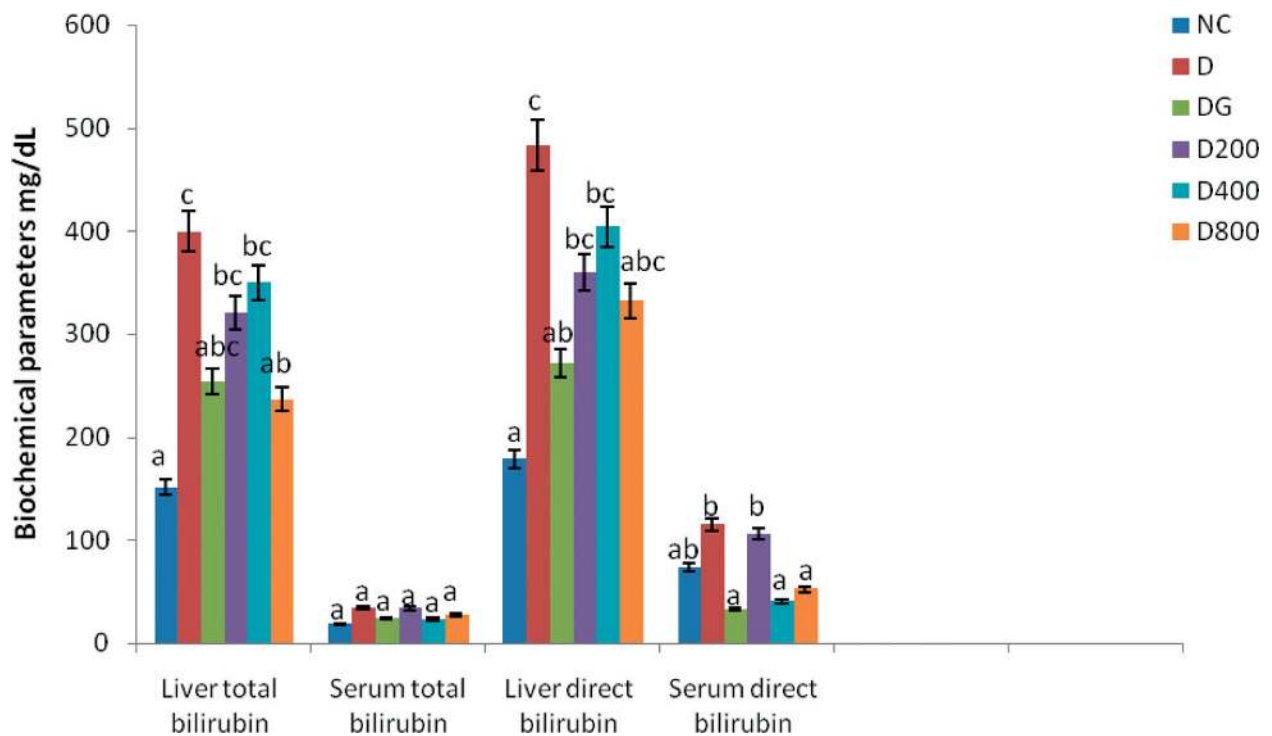
The effect of ethanolic leaf extract of *Corchorus olitorus* on liver function enzymes is represented in **Figure 2**. ALT, AST and ALP levels were significantly elevated in alloxan induced diabetes. The rats treated with ethanolic leaf extract of *Corchorus olitorus* showed significant ( $P < 0.05$ ) reduction in the activity of liver and serum ALT, AST and ALP in the groups administered 800 mg/kg b.w and standard drug (Glibenclamide) when compared with the control while there was no significant difference ( $P > 0.05$ ) in other treatment groups.

### 3.5. Effect of ethanolic leaf extract of *Corchorus olitorus* on some biochemical parameters of alloxan-induced diabetic rats

**Figures 3 and 4** show the effect of administration of ethanolic leaf extract of *Corchorus olitorus* on total bilirubin, conjugated bilirubin, total protein, albumin and globulin in alloxan induced diabetic rats. The concentration of both total bilirubin and conjugated bilirubin level in serum and liver was

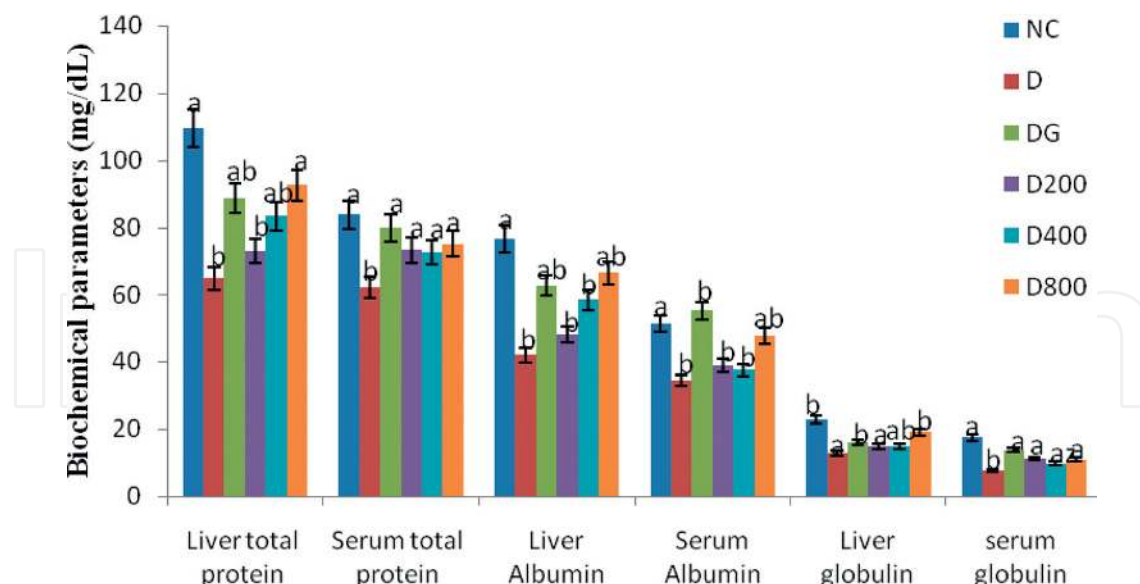


**Figure 2.** Effect of ethanolic leaf extract of *Corchorus olitorius* on liver function marker enzymes of alloxan-induced diabetic rats. Values are given as mean  $\pm$  SD from six rats in each group. Bars not sharing a common superscript differ significantly at  $p < 0.05$ .



**Figure 3.** Effect of ethanolic leaf extract of *Corchorus olitorius* on some biochemical parameters of alloxan-induced diabetic rats. Values are given as mean  $\pm$  SD from six rats in each group. Bars not sharing a common superscript differ significantly at  $p < 0.05$ .





**Figure 4.** Effect of ethanolic leaf extract of *Corchorus olitorius* on serum and liver protein of alloxan-induced diabetic rats. Values are given as mean  $\pm$  SD from six rats in each group. Bars not sharing a common superscript differ significantly at  $p < 0.05$ .

increased significantly ( $P < 0.05$ ) in diabetic untreated group compared to the control but was reduced upon administration of ethanolic leaf extract of *Corchorus olitorius* for 14 days.

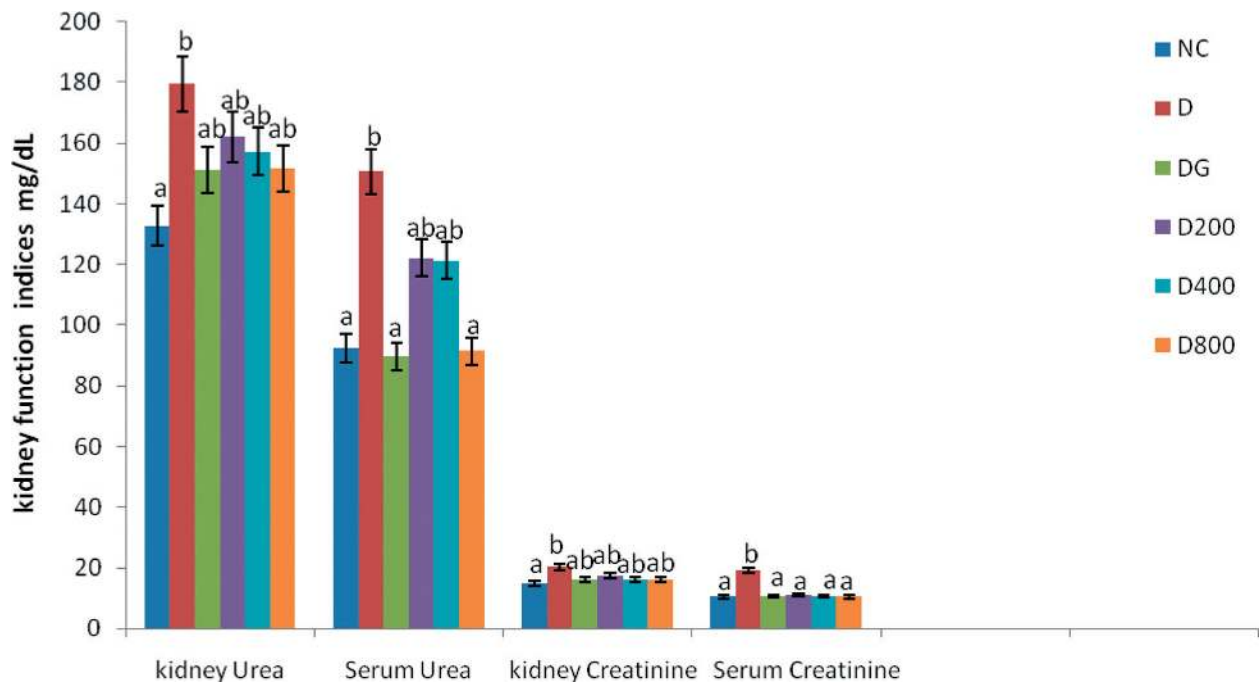
The diabetic untreated rats group had decreased levels of serum and liver total protein, albumin and globulin when compared with normal control rats. After treatment for 14 days, liver and serum total protein, albumin and globulin levels were restored to normalcy especially in the groups treated with 800 mg/kg body weight of the extract and reference drug (glibenclamide).

### 3.6. Effect ethanolic leaf extract of *Corchorus olitorius* on kidney function indices of alloxan-induced diabetic rats

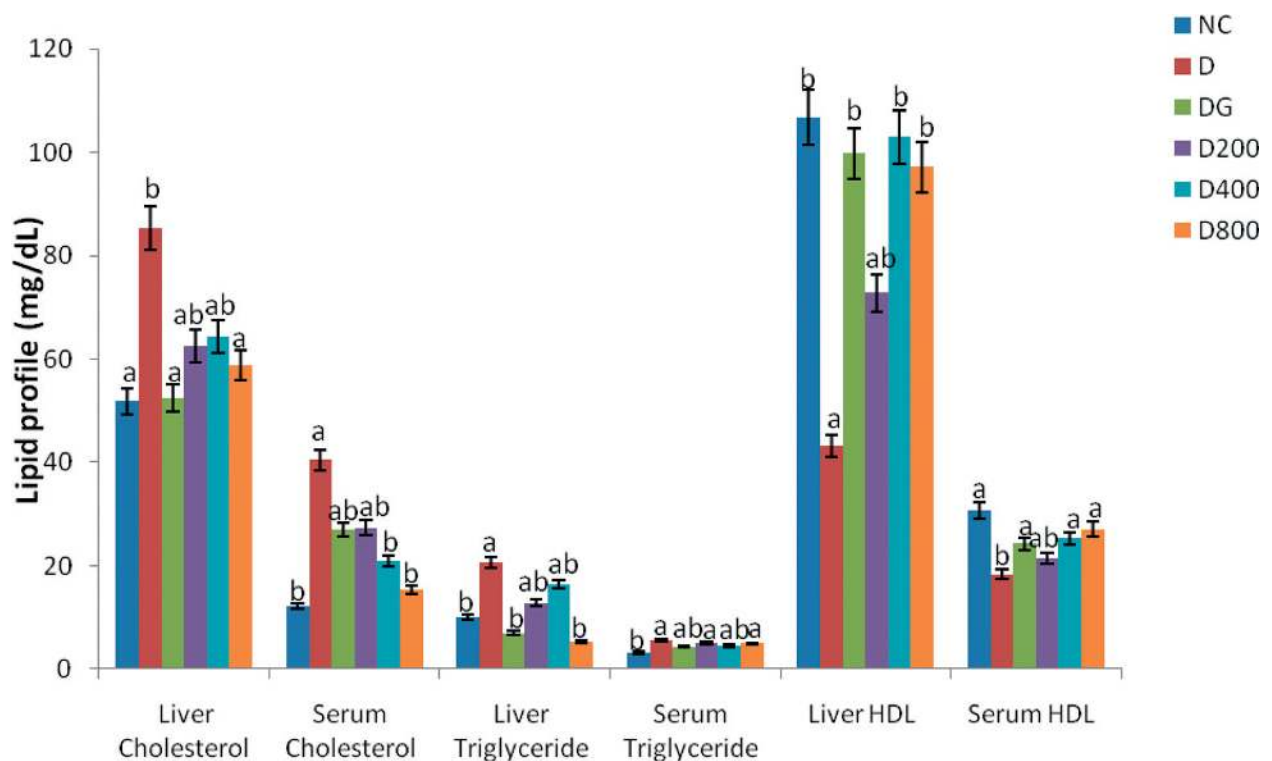
The influence of administration of ethanolic leaf extract of *Corchorus olitorius* on kidney function indices is shown in **Figure 5**. In this study, urea and creatinine levels showed significant ( $p < 0.05$ ) increase in diabetic rats group when compared with the control but showed no significant ( $p > 0.05$ ) difference at all doses of treatment when compared with the control.

### 3.7. Effect of administration of ethanolic leaf extract of *Corchorus olitorius* on liver lipid profile of alloxan-induced diabetic rats

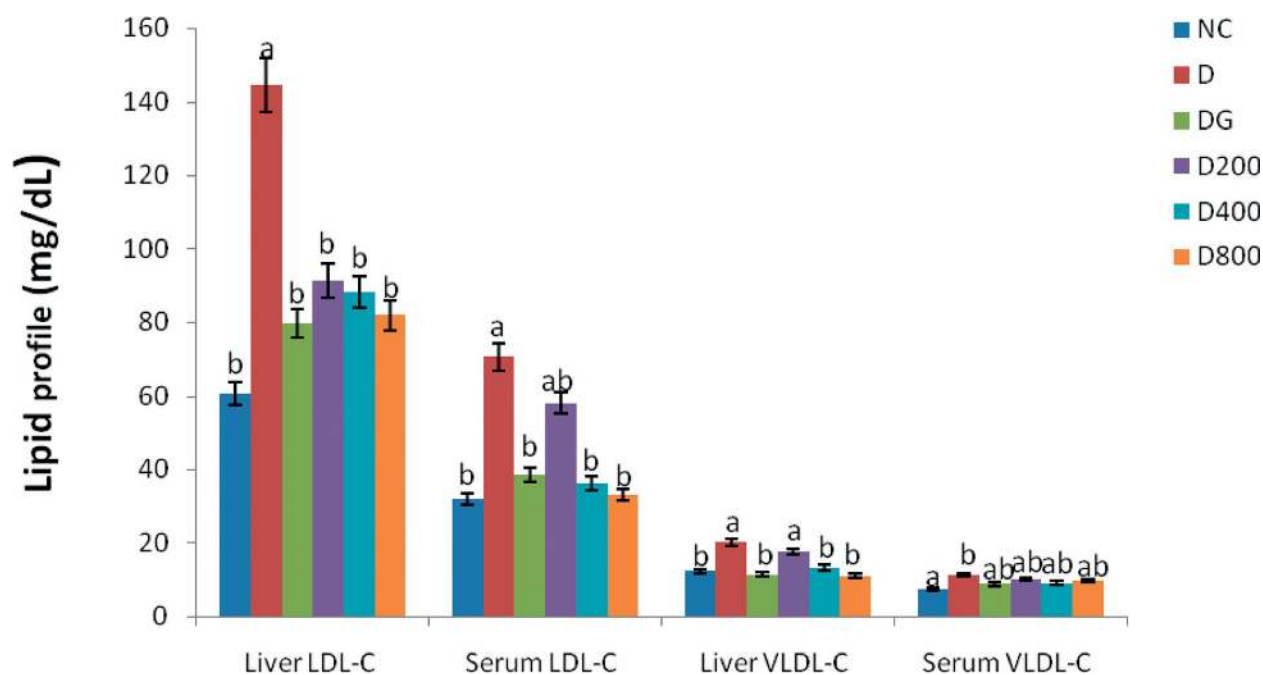
The effect of oral administration of ethanolic leaf extract of *Corchorus olitorius* on the levels of total TC, TG, HDL, LDL-C, and VLDL-C in the serum and liver of diabetic rats are shown in **Figures 6** and **7**. In alloxan-induced diabetic rats, TC, TG, LDL, and VLDL levels were increased and HDL level was decreased significantly ( $p < 0.05$ ) when compared with normal control rats. In diabetic rats group, administration of ethanolic leaf extract of *Corchorus olitorius* at 800 mg/kg body weight dose particularly, showed significant ( $p < 0.05$ ) reduction in elevated TC, TG, LDL and VLDL levels while at doses 200 and 400 mg/kg body weight of the extract no significant ( $p > 0.05$ ) difference was observed when compared to diabetic rats group. Also, a



**Figure 5.** Effect of ethanolic leaf extract of *Corchorus olitorius* on kidney function indices of alloxan-induced diabetic rats. Values are given as mean  $\pm$  SD from six rats in each group. Bars not sharing a common superscript differ significantly at  $p < 0.05$ .



**Figure 6.** Effect of ethanolic leaf extract of *Corchorus olitorius* on lipid profile of alloxan-induced diabetic rats. Values are given as mean  $\pm$  SD from six rats in each group. Bars not sharing a common superscript differ significantly at  $p < 0.05$  (Duncan's multiple range test).



**Figure 7.** Effect of ethanolic leaf extract of *Corchorus olitorius* on liver and serum LDL-C and VLDL-C. Values are given as mean  $\pm$  SD from six rats in each group. Values not sharing a common superscript differ significantly at  $p < 0.05$  (Duncan's Multiple Range Test).

significantly ( $p < 0.05$ ) increased level of HDL was observed in diabetic rats treated with the plant extract at doses 400 mg/kg body weight and 800 mg/kg body weight and glibenclamide compared to diabetic control rats.

#### 4. Discussion

The therapeutic cure for diabetes mellitus has remained elusive despite the discovery of an array of medications that can ameliorate the symptoms of the disease [18]. Phytotherapies have remained a veritable source for drug discovery the world over [19], and for some decades have played an important role in the management of diabetes especially in resource poor countries.

Alloxan acts as diabetogenic by the destruction of  $\beta$ -cells of the islets of langerhans and causes massive reduction in insulin release, thereby inducing hyperglycaemia [20]. Insulin deficiency leads to various metabolic alterations in the animals viz. increased blood glucose, increased levels of alkaline phosphate and transaminases etc. [21].

Phytochemical investigation of ethanolic leaf extract of *Corchorus olitorius* as shown in **Table 1** reveals the presences of alkaloids, flavonoids, tannins, saponins, phlobatannin anthraquinones, phenol and cardiac glycoside and saponin. These secondary principles are known to be bioactive for the management of diabetes. It is well known that certain flavonoids exhibit hypoglycemic activity and pancreas beta cell regeneration ability. Thus, the significant antidiabetic effect of ethanolic leaf extract of *Corchorus olitorius* may be due to the presence of more than one antihyperglycemic principle and their synergistic properties [22].

Single dose intra-peritoneal (i.p) treatment of rats with alloxan monohydrate (150 mg/kg) caused an increase in the blood glucose. Ethanolic leaf extract of *Corchorus olitorius* and glibenclamide were found to reduce the elevated glucose level significantly in alloxan induced diabetes animals during the 14 days treatment. This suggests the hypoglycaemic effect of the plant. As suggested by Ekpenyong et al [23] that normal protein level reflects normal synthesis while high level is common in high protein diet.

The concentration of total protein globulin, albumin and bilirubin may indicate the state of the liver and type of damage. Protein molecules that are regularly employed to assess the state of health of the liver are albumins and globulins (Total Proteins). The blood circulated albumin is the main carrier protein produced in the liver. The larger globulins are responsible for immunogenic activities [24]. Decreased serum albumin and globulin concentrations in the untreated diabetic rats suggests reduced synthetic function of the hepatic cells. Oral administration of ethanolic leaf extract of *Corchorus olitorius*, however, normalized the serum albumin and globulin concentration. This is a further proof of the protective potential of ethanolic leaf extract of *Corchorus olitorius* on the liver of diabetic rats.

Bilirubin is a useful index of the excretory function of the liver. It is an important breakdown product of blood with biological and diagnostic values [25] Elevated bilirubin is an indication of liver cell impairment. The gradual increase in the serum levels of unconjugated (total and conjugated) bilirubin in diabetic rats when compared with the normal control may be an indication that the rats had liver function impairment, resulting in diminished ability of hepatocytes to conjugate bilirubin. The insignificant decrease in total and conjugated bilirubin of both the serum and liver in all the treated animals suggest the ability of the plant extract to ameliorate liver impairment caused by diabetes induction.

Liver enzymes e.g. alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alanine phosphatase level (ALP) were increased in diabetic rats which is responsible for the liver damage. The elevated serum level of these enzymes was significantly reduced by ethanolic leaf extract of *Corchorus olitorius* treatment particularly at the dose of 800 mg/kg bw, suggesting the protective effect of the plant extract against diabetes- induced hepatocellular damage especially at high dose. The diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated enzymes [26]. The restoration of transaminases to their normal levels also treatment also indicates revival of insulin secretion.

The kidney removes metabolic wastes such as urea and creatinine, the concentration of which are usually required to assess the normal functioning of different parts of the nephrons [27]. The serum creatinine and urea concentrations are widely interpreted as measures of the glomerular filtration rate (GFR) and are used as indices of renal function in clinical practice. The concentration of these metabolites increase in blood during renal damage associated with uncontrollable diabetes mellitus. On the contrary those treated with ethanolic leaf extract of *Corchorus olitorius* effected decrease in creatinine and urea levels, indicating ameliorative effect of the plant extract on kidney functions in diabetic rats. This may suggest that the damage caused on renal function indices by the disease had been restored by the plant extract, thus the proper function of the nephrons at the tubular and glomerular level.

Inbalances in serum lipid levels are usual occurrences in a diabetic state [28]. Since changes in lipoproteins concentrations is an inherent property of diabetes mellitus, such changes are usually triggered by diabetes induced obesity and renal complications [29]. As observed in this study, administration of ethanolic leaf extract of *Corchorus olitorius* led to a reduction in cholesterol, triglycerides and low density lipoprotein (LDL) concentrations while it led to the normalization of high density lipoprotein (HDL) concentration in diabetic rats when compared to the untreated diabetic group. The serum concentration of cholesterol is usually elevated in diabetes, and such an increase is a risk factor for cardiovascular diseases. The observed high concentration of serum cholesterol during diabetes is mainly attributable to pronounced mobilization of free fatty acids from the peripheral depots, because the hormone-sensitive lipase is usually inhibited by insulin [30]. Administration of ethanolic leaf extract of *Corchorus olitorius* to diabetic rats significantly decreased the plasma cholesterol level to near normalcy and therefore reduces the risk of cardiovascular disease [31]. An increase in the concentrations of LDL-cholesterol and reduced HDL-cholesterol as observed during diabetes are associated with raised risk of myocardial infarction [32]. Administration of ethanolic leaf extract of *Corchorus olitorius* led to an increased concentration of HDL-cholesterol and depleted VLD-cholesterol levels which are characteristic of reduced risk of myocardial infarction. Convincing evidence from laboratory, clinical and epidemiologic data have confirmed that increased serum concentration of triglyceride is a standalone risk factor for cardiovascular complications. Hyper triglyceridemia is a characteristic condition observed in diabetics, in this study, treatment with ethanolic leaf extract of *Corchorus olitorius* has prevented the elevation of triglycerides, signifying that myocardial membrane is intact and not damaged.

## 5. Conclusion

The present study showed that the ethanolic extract of *Corchorus olitorius* leaf exhibited antihyperglycemic and anti-dyslipidemic effects and there was no significant changes in the toxicological parameters and marker enzymes evaluated hence it could be considered safe for use as an antidiabetic recipe.

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