EVALUATION OF THE EFFECT OF AQUEOUS LEAF EXTRACT OF JUTE MALLOW Corchorus olitorius ON SOME BIOCHEMICAL PARAMETERS IN ALLOXAN-INDUCED DIABETIC RATS


1Department of Biochemistry, Faculty of Basic Medical Sciences, College of Medical Sciences, Abubakar Tafawa Balewa University (ATBU), Bauchi.
2Department of Biochemistry, Faculty of Medical Sciences, University of Jos.
3Department of Biochemistry, Faculty of Natural and Applied Sciences, Plateau State University, Bokkos. PMB 2012, Plateau, Nigeria.
4Department of Pharmacology, Faculty of Basic Clinical Sciences, College of Medical Sciences, Abubakar Tafawa Balewa University (ATBU), Bauchi.

ABSTRACT
Plants form the main ingredients of medicine in traditional system of healing and have been the source of inspiration for several major pharmaceutical drugs. The study was carried out to investigate the effect of the aqueous leaf extract of Jute Mallow (Corchorus olitorius) on Serum blood glucose, protein, albumin, total bilirubin, direct bilirubin, total cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL) and enzymes activities on normal and alloxan-induced diabetic rats. The aqueous leaf extract was administered orally at a dose of 400mg/kg body weight to both normal and alloxan-induced diabetic rats. Twenty adult male rats were divided into four groups of five rats each, two groups were made diabetic and the other two groups were non-diabetic. One of the diabetic groups was treated with the leaf extract and the second serves as diabetic control. The alloxan was administered intraperitoneal at a dose of 150mg/kg per body weight. The administration of the leaf extract lasted for 28 days. Effect of the extract on blood glucose, protein, albumin, total bilirubin, direct bilirubin total cholesterol; triglyceride, high density lipoprotein and low density lipoprotein concentrations were analyzed. The toxic effect of the leaf extract was determined using biochemical enzyme markers. The photochemical screening of the ethanol extract showed the presences of Alkaloid, Tannins, Resins, Terpenes and steroids, Balsam, Carbohydrate and Phenol, while aqueous extract showed the presences of Alkaloid, Tannins and Resins and chloroform extracts showed the presences of Alkaloid only. Treatment with the extract showed significant (P<0.05) reduction on the serum blood glucose level and other biochemical parameters analyzed. The extract possesses no toxic effect as indicated by the lowering of ALP and ALT levels and may be used for the management of diabetes mellitus.

KEYWORDS: Corchorus olitorius, phytochemical, lipid profile, liver enzymes, hypolipidaemic.

INTRODUCTION
Plants play important role in the cycle of nature. This is because life on earth basically depends on them; plants provide man with all his needs as regards food, shelter, clothing, flavors and fragrance as well as medicine. They are naturally occurring substances that produce almost all the foods that animals as well as humans eat. They have unique potential to make their own food through photosynthesis. All foods that people eat naturally come directly or indirectly from plants.

In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries that has always been associated with cultural behavior, traditional knowledge and considering its bioactive constituent, which have been reported safe, without any adverse side effects especially when compared with synthetic drugs. Thus, a search for new drugs with better and cheaper substitutes from plant origin is a natural choice. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body.[1] Herbal medicine is the major stay of about 75-80% of the world population, mainly in the developing countries, for primary health care due to a better cultural acceptability, better compatibility with human body and few side effects.[2] Plants materials have been known to be used in the management of symptomatic diseases (such as
mental illness, microbial infection, cancers) which is one area in which so many people in developing countries depend on herbal medicine.[5]

General adverse health effects associated with human exposure to carbon tetrachloride (CCl₄) include cardiovascular diseases, developmental abnormalities, neurologic and cardiovascular disorders, diabetes, hearing loss, fibrosis, lung cancer, hematological disorders and black foot disease.[4] The search for new ways to treat them stimulates the investigation of cheap and effective natural compounds as an alternative treatment of the aforementioned diseases.[5] However, strategies have been proposed in an attempt to control the spread of these diseases. The incidence of diabetes mellitus and its complication is due to two major factors. It is due to insulin deficiency for type I or as a result of the body developing resistance against the normal usage of insulin secreted in the body type 2.

Carbohydrate food sources form the greatest percentage (50-60%) of the daily diet for different segments of the population.[6] In Nigeria, starchy staples (Cereals, roots/tubers) and legumes constitute the major part of the traditional diets, up to 10% and 25% respectively.[7] Plant foods are the most important dietary sources to meet the nutritional needs of majority of the population in sub-Saharan Africa and Asian sub-continent.[8] The roles of plant phytochemicals and antioxidant constituents of plant foods were reported.[9] On this basis, plant foods hold good promise for diabetes management. There are mounting scientific evidence to date on their various health-promoting properties.

The global concern for the diversification of the uses of the plant foods to improve normal and therapeutic nutrition for diabetes control has shifted scientists interest to enhancing the potential sources of beneficial constituents in plant foods have generated increasing research interest because of their anti-diabetic potentials. Currently, documentaries on Nigerian indigenous plant food with anti-diabetic properties are either scattered or lacking. Most reviews on anti-diabetic potentials of plants both locally[10][11][12], and internationally[13][14] were done on medicinal plants.

MATERIALS AND METHODS

Animals Used
Twenty (20) adult male albino rats weighing approximately 80-150g were obtained from Animal House Unit Department of Pharmacology, University of Jos. The rats acclimatized to the laboratory condition for two weeks before any experimental work was undertaken, they were fed with standard feed.

Collection of plant Extracts
The plant Jute mallow (Corchorus olitorius) fresh leaf was obtained from Mazah area in Jos North Local Government, Plateau State. The plant was identified and authenticated at the Federal College of Forestry Jos North, Plateau State. The leaf of Corchorus olitorius was collected and air-dried for 7 weeks, 4 days in the laboratory. The dried leaf were pounded into fine greenish powder and packed into an air tight container, stored until required.

Preparation of plant Extracts
The Corchorus olitorius powder was poured into a beaker mixed properly using a shaker and water as a solvent, it was heated for 15 minutes, then the mixture was filtered and the filtrate was placed in an evaporating dish and evaporated to dryness and the dried extract was stored in a clean, air tight container. Appropriate weights of the extract were prepared to obtain the concentration needed for the study.

Preparation of Alloxan Solution
One gramme of Alloxan monohydrate was dissolved in 10mls of distilled water for standardization and was used at once for inducing the experimental rats.

Induction of Experimental Diabetes
Diabetes was induced in groups A and B rats by intraperitoneal injection (IP) of Alloxan at doses of 150mg/kg body weight. Diabetes was confirmed in the animal after 48 hours by estimation of blood glucose level. Animals with blood glucose level above 120mg/dl were selected.[15]

Administration of the Extract
The Corchorus olitorius aqueous leaf extract solution was administrated through the oral route at a dose of 400mg/kg body weight daily for 28days.

Experimental Design
Twenty male rats were randomly divided into four groups of five rats each and fed with standard feed as follows:

Group A- Diabetic control rats with no administration of extract (negative control).
Group B- Diabetic rats given extract (400mg/kg) body weight daily for 28 days.
Group C- Normal rats with no administration of extract (positive control).
Group D- Normal rats given extract (400mg/kg) body weight daily for 28.

Sample Collection and Preparation
At the end of 28 days of extract administration, blood from the animals (both treated and control groups) was collected from the jugular vein into plan bottles. The blood in the plain bottle was allowed to clot at room temperature. The clotted blood sample was ringed and centrifuged for 10 minutes at 5,000 r.p.m. Pasteur pipette was used to separate the serum (supernatant) into clean bottles. The serum was used for the biochemical assay.

Statistical Analysis
All data are expressed as mean (X) ± Standard deviation (SD). Comparison of the data from test control groups of
animals was analysed by one-way analysis of variance (ANOVA) at the confidence of 95% and where applicable, least significant difference (LSD) was used to determine significant results; differences between groups were considered statistically significant at P<0.05.

**RESULTS**
The table below shows result obtained when *Corchorus olitorius* leaf extract was screened for phytochemicals in which Alkaloids, Flavonoids, Tannins, Saponins, Terpenes, Steroids, Cardiac Glycosides, Balsam, Carbohydrate, Phenol and Resins presence was tested for.

**Table 1: Phytochemical Screening of *Corchorus olitorius* leaf Extract.**

<table>
<thead>
<tr>
<th>Photochemical</th>
<th>Ethanol</th>
<th>Chloroform</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenes and Steroids</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Balsam</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Key:** + = Present - = Absent

Figure 1 showed the effect of *Corchorus olitorius* aqueous leaf extract on weight changes in diabetic and normal groups of rats on day 1, 4 and 7.

Table 2 showed the serum blood glucose levels in (mmol/L) in diabetic and normal groups. The diabetic administered with aqueous leaf extract showed a significant decrease (P<0.05) in the Serum glucose, total protein and albumin levels when compared to the diabetic control.

**Table 2: Effect of aqueous leaf extract of *Corchorus olitorius* on serum glucose, protein and albumin on alloxan-induced diabetic rats.**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Glucose (mmol/L)</th>
<th>Protein (g/L)</th>
<th>Albumin (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic control</td>
<td>10.30±0.14a</td>
<td>71.00±0.82a</td>
<td>42.00±0.82a</td>
</tr>
<tr>
<td>Diabetic treated</td>
<td>5.20±0.14ab</td>
<td>65.75±3.52ab</td>
<td>30.00±0.82ab</td>
</tr>
<tr>
<td>Normal control</td>
<td>4.60±0.08b</td>
<td>59.00±0.82b</td>
<td>31.00±0.82ab</td>
</tr>
<tr>
<td>Normal treated</td>
<td>4.50±0.08</td>
<td>55.00±0.82</td>
<td>36.00±0.82</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD, n= 5 for each group
* values are significantly different from normal control (p<0.05)
ab values are significantly different from the diabetic control group (p<0.05)

Table 3 showed the results of total and direct bilirubin levels. The diabetic administered with aqueous leaf extract showed statistically significant differences (P<0.05) when compared to the diabetic control.
Table 3: Effect of aqueous leaf extract of Corchorus olitorius on bilirubin concentrations on alloxan-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Bilirubin (g/l)</th>
<th>Direct Bilirubin (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic control</td>
<td>16.00±0.82\textsuperscript{a}</td>
<td>4.08±0.46</td>
</tr>
<tr>
<td>Diabetic treated</td>
<td>9.00±0.82\textsuperscript{ab}</td>
<td>3.40±0.08\textsuperscript{ab}</td>
</tr>
<tr>
<td>Normal control</td>
<td>13.00±0.82</td>
<td>4.10±0.08</td>
</tr>
<tr>
<td>Normal treated</td>
<td>8.00±0.82\textsuperscript{ab}</td>
<td>3.20 ±0.08\textsuperscript{ab}</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD, n= 5 for each group
\textsuperscript{a}values are significantly different from the normal control (p<0.05)
\textsuperscript{ab}values are significantly different from the diabetic control group (p<0.05)

Table 4 showed the result of enzyme activity. The diabetic administered with aqueous leaf extract shows statistically significant differences (P<0.05) when compared to the diabetic control.

Table 4: Effect of aqueous leaf extract of Corchorus olitorius on selected enzyme markers on alloxan-induced diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic control</td>
<td>36.00±0.82\textsuperscript{a}</td>
<td>80.00±0.82\textsuperscript{a}</td>
<td>753.0±300.00\textsuperscript{a}</td>
</tr>
<tr>
<td>Diabetic treated</td>
<td>28.00±0.82\textsuperscript{ab}</td>
<td>69.00±0.82\textsuperscript{ab}</td>
<td>875.0±0.82\textsuperscript{ab}</td>
</tr>
<tr>
<td>Normal control</td>
<td>31.00±0.82</td>
<td>68.00±0.82</td>
<td>749.0±0.82</td>
</tr>
<tr>
<td>Normal treated</td>
<td>20.00±0.82\textsuperscript{ab}</td>
<td>27.00±0.82\textsuperscript{ab}</td>
<td>711.0±0.82\textsuperscript{ab}</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD, n= 4 for each group
\textsuperscript{a}values are significantly different from the normal control (p<0.05)
\textsuperscript{ab}values are significantly different from the diabetic control group (p<0.05)

Table 5 showed the analysis of total cholesterol, triglyceride, high density, and low density lipoprotein levels respectively. The groups administrated with the plant aqueous leaf extract shows statistically significant differences when compared to the control groups (P<0.05).

Table 5: Effect of aqueous leaf extract of Corchorus olitorius on serum lipid profile on alloxan-induced diabetic rats.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>TG (mmol/L)</th>
<th>TC (mmol/L)</th>
<th>HDL (mmol/L)</th>
<th>LDL (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic control</td>
<td>2.00±0.04\textsuperscript{a}</td>
<td>2.30±0.33</td>
<td>0.60±0.08</td>
<td>1.00±0.01</td>
</tr>
<tr>
<td>Diabetic treated</td>
<td>1.70±0.08\textsuperscript{a}</td>
<td>1.90±0.01\textsuperscript{a}</td>
<td>1.00±0.06</td>
<td>0.60±0.08</td>
</tr>
<tr>
<td>Normal control</td>
<td>1.60±0.08</td>
<td>2.00±0.08</td>
<td>0.50±0.08</td>
<td>0.90±0.08</td>
</tr>
<tr>
<td>Normal treated</td>
<td>1.70±0.08\textsuperscript{a}</td>
<td>1.80±0.08\textsuperscript{a}</td>
<td>1.15±0.50\textsuperscript{a}</td>
<td>0.50±0.08</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD, n= 4 for each group
\textsuperscript{a}values are significantly different from normal control (p<0.05)
\textsuperscript{ab}values are significantly different from the diabetic control group (p<0.05)

DISCUSSION

Plant produced different chemical compounds or phytochemical which have been used in a wide range of commercial, Medicinal and industrial applications. The result obtained from the preliminary qualitative phytochemical screening of aqueous, ethanol and n-hexane extracts of Corchorus olitorius showed similar results in a studies conducted on methanol leaf extract found cardiac glycosides, steroids, cholesterol, phenols, and alkaloids from plants. The studies also described the phytochemicals in medicinal plants as the active principles responsible for the pharmacological potentials of medicinal plants and reduction in the effect of diabetic complication. Studies have also shown that tannins and alkaloids also have some pharmacological effects and are used as hypoglycemic, anti-diuretic and anti-diarrhea drugs. They are also used as local anesthetics, analgesics and antimarial drugs.

Alloxan brings about massive reduction in insulin release by destruction of \(\beta\)- cells of the islet of langerhains, hence inducing diabetes. Alloxan and the production of its reduction, dialuric acid establishes a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide and thereafter cause a massive release of cytosolic calcium concentration, which causes rapid necrotic deconstruction of pancreatic \(\beta\)-cells, hence diabetes and its attendant haematotoxicity to the vascular system.

Diabetes tends to increase the oxidative stress in tissue of both humans and animals. The possible sources of oxidative stress in diabetes include high generation of reactive oxygen species by auto-oxidation of glucose and decreased tissue concentration of low molecular weight antioxidant defense enzymes. A great number of traditional medicinal plants are still being used to treat...
diabetes and cardiovascular diseases. Several beneficial roles including correcting altered carbohydrate metabolism, maintaining integrity and function of β-cells, insulin secreting activity and enhancing glucose uptake and utilization. Antioxidant properties present in the traditional medicinal plants and their constituents offer exciting opportunity to develop them into novel therapeutics.[21] In this research study, the results indicated that Corchorus olitorius aqueous leaf extract was able to reduce the blood glucose levels in alloxan-induced diabetic male rats weighting 80kg and above. Similar study by[22] on Dihydrophenantherene Isolated from Khaya senegalensis Stem Bark showed significant anti-hyperglycemic activity in alloxan-induced diabetic group, treated with 50mg/kg body weight.

The study also revealed significant loss of weight of untreated diabetic rats compared to normal control rats and the diabetic treated rats whose weights increased significantly throughout the period of study. Also, Similar study on effect of aqueous extract of Dioscorea bulbifera on some biochemical parameters in alloxan-induced diabetic rats have equally reported significant weight reduction in untreated diabetic rats.[23] The loss in weight observed in the diabetic control may be due to the loss in muscle and adipose tissue resulting from excessive breakdown of tissue protein and fatty acids, weight loss is one of the symptoms of diabetic mellitus occurring especially when glycemic control is poor. However, the rats treated with the plant extract showed appreciable increase in weight compared to the diabetic control group. This appreciable increase in weight indicates that the treatment allowed the tissues to access the glucose both to supply energy and spared some to build tissue materials required for growth. It also showed significant increase in the elevation of the serum albumin in the diabetic control group compared to diabetic treated group. When the diabetic rats were placed on the treatment with Corchorus olitorius aqueous extract the high levels of albumin and total protein reduced significantly. This is also similar to findings of[23] on effect of aqueous extract of Dioscorea bulbifera on some biochemical parameters in alloxan-induced diabetic rats. Also, result for the bilirubin analysis showed no significant difference in total and conjugated bilirubin levels.

The difference in hypoglycemic effect shown could be attributed to the high concentration of hypoglycemic principles at higher doses of the extract. Generally, alloxan diabetogenesis was shown to suppress liver enzyme and protein expression in the liver. The result reveals that, the activity of serum alanine aminotransferase (ALT), Aspartate amino transferase (AST) and Alkaline phosphatase (ALP) was significantly reduced compared to both diabetic and normal control groups. Similar findings was reported by[24] on Evaluation of the effect of coconut oil (cocos nucifera) on some biochemical parameters in alloxan-induced diabetic rats. This observation may indicate inducing by the leaf extract at that dose level employed. Alkaline phosphatase (ALP) had also been found to be increased by enzyme-inducing drugs. More importantly, at the dose level of 400mg/kg body weight, an increase in ALP activity could be drug induced as observed with overdose of paracetamol, methyl dopa, isoniazid and certain steroids. It shows that at a dose level of 400mg/kg body weight, the leaf extract still maintained an increased Alkaline phosphate (ALP) activity considerably, inducing that at that dose the extract may be acting as an enzyme inducing drug, similar to some anticoagulants. Alkaline phosphate (ALP) has been reported to be involved in protein synthesis, glycogen metabolism and synthesis of certain enzymes and the transport of metabolites across the cell membranes.[25] Triacylglycerols are partly taken up in diets and partly synthesized in the liver. As a major component of various very low density lipoprotein (VLDL) and chylomycrons. It plays a significant role in Metabolism as energy sources and transport of dietary fats. High blood triacylglycerols have been linked to atherosclerosis and by extension, the risk of heart disease and stroke.[26] Result from this research shows that the high level of total cholesterol, triglycerides and low density lipoprotein was obtained in diabetic control rats but was significantly lowered in the diabetic treated rats as compared to the diabetic control. The result is in accordance to findings of[26][27] which shows similar results even though different plants were used. The hyper-cholesterolemaia observed in the diabetic rats generally might by due to increased activity of β-hydroxy—β-methyl glutaryl CoA reductase and cholesterol acyltransferase (ACAT), the major enzymes involved in cholesterol biosynthesis which have been reported to have increased activity in diabetes mellitus.[28] It may also be partly due to increased availability of acetyl CoA which is a key substrate in cholesterol biosynthesis resulting from increased oxidation of fatty acids in diabetes mellitus. The mechanism of hypolipidaemic action of the Corchorus olitorius extract may be due to decreased oxidative load by directly scavenging the reactive oxygen metabolites, due to high concentration of antioxidant molecules. The results may also suggest that the extract could at that dose, reduce hepatic triacylglycerols biosynthesis and favour the redistribution of cholesterol among the lipoprotein molecules,[29], reported that flavonoids have antioxidant properties with effects on endothelial function namely, reducing the oxidation of low density Lipo protein (LDL). A type of flavonoid anthyocyanin helps in reducing the incidence of cardiovascular diseases, cancer, hyperlipidaemia and other chronic diseases.[30] The presence of alkaloid and saponins in plant extract may function in lowering plasma cholesterol level and therefore, serve as a potential remedy for the management of atherosclerosis and other related disorders like diabetes and obesity.[31]
CONCLUSION
In conclusion, the oral administration of the Corchorus olitorius aqueous leaf extract to both diabetic and normal groups of rats have shown a significant decrease in the concentration of the Serum blood glucose, total cholesterol, total protein, enzyme activity, triglycerol and bilirubin levels respectively, this proves its anti-diabetic properties. Thus, the Corchorus olitorius leaf extract may be beneficial in the management of diabetes mellitus at the dose employed.

REFERENCES


