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Hypoglycemic and hypolipidemic properties of *Casuarina equisetifolia* leaf extracts in alloxan induced diabetic rats



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ABSTRACT

Background: *Casuarina equisetifolia* is a plant used traditionally as a remedy for various disorders such as diabetes, hyperlipidemia, gastric problems and muscle weakness. The aqueous, ethanol and methanol extracts of *C. equisetifolia* leaves were studied for hypoglycemic and hypolipidemic potentials.

Methodology: Phytochemical analysis was carried out using standard procedures. Rats of both sexes, weighing (145–255 g) were randomly allotted to six groups with each group having four rats. Rats in group A were the normoglycemic, while those in groups B, C, D, E, and F were rendered diabetic by the administration of 110 mg/kg bodyweight of alloxan monohydrate. Group C rats were treated with 15 mg/kg bodyweight of standard drug (Glidazide), while groups D, E, and F rats were respectively treated with 150 mg/kg bodyweight of aqueous, ethanol, and methanol extracts for 15 days. The group B rats were untreated (negative control). Blood glucose was checked after every three days using glucometer. At the end of the treatment, the rats were anaesthetized under chloroform vapor and blood samples were collected by jugular puncture and used for analyzing biochemical parameters.

Results: Extracts showed presence of tannins, flavonoids, saponins, phenols, alkaloids and reducing sugars. There was 64%, 59% and 58% reduction in blood glucose concentration of diabetic rats treated with ethanol, aqueous, methanol extracts respectively. Serum cholesterol and triacylglycerol of the three treated groups reduced ($p < 0.05$) with the aqueous and methanol extract treated groups having the most significant reductions in both variables (147.93 ± 8.04 , 152.91 ± 10.85 mg/dL) when compared with the diabetic group (203.21 ± 6.45 , 355.16 ± 11.25 mg/dL) respectively. AST and ALP activities reduced significantly ($p < 0.05$) in the aqueous extract treated group (33.18 ± 4.48 , 52.60 ± 7.23 u/L) with no significant differences when values were compared to that of the normal control group (25.64 ± 4.18 , 44.13 ± 5.80 u/L) respectively. Urea concentration decreased in all extract treatment groups except the group treated with the aqueous extract. Creatinine also decreased in the extract treated groups apart from the group treated with the aqueous and methanol extracts when both compared with the normal control group.

Conclusion: The extracts from the leaves of *C. equisetifolia* have hypoglycemic and hypolipidemic effects, and can be further explored as a natural novel lead for drug development in the management of diabetes and dyslipidemia.

1. Introduction

Diabetes mellitus (DM) is the most prevalent endocrine disorder affecting up to 500 million people worldwide, while the total number of people suffering from diabetes is approaching 700 million by 2040 [1], with larger number in developing countries. The disease which is characterized by high levels of glucose in the blood; accompanied by dys-

lipidemia, free-radical production, atherosclerotic vascular disease and high blood pressure [2]. It is a metabolic disorder linked to abnormal metabolism of carbohydrates, whereby there is insufficiency in either insulin secretion or insulin action, (or both) [3].

Longstanding diabetes is associated with alterations in mitochondrial metabolism that results in both increased formation of reactive oxygen species (ROS) and failure of bioenergetics. Reactive oxygen species gen-

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erated by mitochondria are responsible for the activation of major, independent but interrelated pathogenic mechanisms for diabetic complications as modeled in endothelial cells exposed to hyperglycemia in vitro [4]. In particular, diabetes causes dysfunction of mitochondria in those tissues highly dependent on aerobic metabolism, such as heart, brain and skeletal muscle. The degree of mitochondrial failure has been correlated with the duration of diabetes [5].

There has been increasing demand for the use of plant products with antidiabetic potentials. The high cost, low availability, uncertainty of use during pregnancy and undesirable side effects of synthetic drugs have been some of the factors leading to a strong preference for hypoglycemic drugs of plant origin, which are believed to be suitable for chronic treatments [6]. Plants which have been shown to have hypoglycemic properties act through different mechanisms. Some of them may inhibit endogenous glucose production or interfere with gastrointestinal glucose absorption (dietary fiber from plants). Some may have insulin mimicking substances, may inhibit insulinase activity and may increase secretion of insulin from the β cells of the pancreas i.e., pancreatotropic action examples include, the spinach leaves, *lemna* plants, *Coriandrum sativum*, while others may increase β cells in pancreas by activating regeneration of these cells; *Gymnema sylvestre*, *Nigella sativa* [7]. Very few traditional treatments for diabetes mellitus have been scientifically scrutinized. The herbal extracts which are effective in lowering blood glucose level with minimal or no side effects are known to be used as antidiabetic remedies. Hundreds of plants are known to be useful in treating diabetes in different corners of the world.

Casuarina equisetifolia contains many active secondary metabolites including carbohydrates, alkaloids, proteins, glycosides, saponins, phenolics, flavonoids, tannins, steroids, gum, reducing sugars and triterpenoids [8]. It is used for the folkloric treatment of constipation, cough, impaired glucose, diarrhea, gonorrhoea, nervous disorders, rash, throat infections and stomach ulcers. In addition, the bark is used as an astringent and for abdominal pain [9]. The leaf is used as an antispasmodic for colic, while the aerial components are used as hypoglycemic agents and the seeds are used as anthelmintic, antispasmodic [10]. Previous studies have reported that extracts (methanol, ethanol, aqueous, ethyl-acetate, and hexane) from various parts of the plant (leaf, root, stem bark, seed) showed biological activities viz; antioxidant, antimicrobial, hypoglycemic, hypolipidemic, antihistamine, anti-inflammatory and hepatoprotective [11,12,13,14], and [15]. However, the hypoglycemic and hypolipidemic effects studied were on the seed extracts (hexane, ethyl-acetate and ethanol) and the bark extract (ethanol) of the plant.

The research is aimed at investigating the hypoglycemic and hypolipidemic properties of *Casuarina equisetifolia* leaf extracts and its effects on activity of liver biomarker enzymes and some kidney function parameters of diabetic rats.

2. Materials and methods

2.1. Plant material

Fresh *Casuarina equisetifolia* leaves were collected in July 2020 from Farindoki hotel along GRA Minna, Nigeria. The plant was identified and authenticated at the herbarium section of Plant Biology Department, Federal University of Technology Minna, where voucher specimen was deposited with number FUT/PLB/CAS/001.

2.1.1. Preparation of plant material

The fresh leaves were washed under running tap water, air dried and homogenized to fine powder using clean sterile mortar and pestle and stored in polythene bags till ready for use.

2.1.2. Extraction of plant material

Fifty grams (50 g) each of the powdered plant sample were weighed and added to 400 ml of distilled water, ethanol and methanol then refluxed using Medline extractor Mantle at 50 °C for 3 hrs. The extracts ob-

tained were filtered hot with muslin cloth and then solvents were evaporated using water bath (Galleukamp, Britain, CAT. NO BJE 80,001,013, APP NO.954005E) at 60 °C. Extracts were stored in sterile plastic tubes in the refrigerator until required for use.

2.1.3. Phytochemical screening

Qualitative phytochemical screening of the aqueous, ethanol and methanol extracts to determine their chemical constituents was carried out using standard methods according to Trease and Evans, [16] and Sofowora, [17].

2.2. Determination of safe dose (Acute toxicity study)

Safe dose determination of the aqueous, ethanol and methanol extracts of *Casuarina equisetifolia* was carried out using Wistar albino rats of a single sex adopting the guideline of the organization for economic corporation and development standards [18]. Lorke's method [19] was used to study the toxicity effect of the extracts in Wistar albino rats. The study involves the oral administration of different doses of the extracts to 54 rats of 18 groups consisting of 3 animals each of a single sex. Labeled rats in the first nine groups were respectively administered the extracts orally at graded doses of 10, 100, 1000 mg/kg bodyweight (phase I). Similarly, the last labeled nine groups were administered respective extracts orally at graded doses of 1600, 2900, and 5000 mg/kg bodyweight in the second stage (phase II). Signs accompanying poisoning; indicating the state of adverse effect by the interaction of the toxicants and the cell (such as drowsiness, respiratory distress, diarrhea, convulsion, lethargy, coma and possible death of animals) were monitored for 24 h in an interval of 2 h and the safe dose was established to be up to 2000 mg/kg bodyweight.

2.3. Experimental animal model

A total of twenty-four (24) Wistar albino rats of both sexes weighing between (145–255 g) were obtained from Ahmadu Bello University Zaria, Kaduna State, Nigeria. They were housed in the Animal House at the Department of Biochemistry, Federal University of Technology Minna, Nigeria. The rats were maintained in experimental conditions (temperature of 25±2 °C, 12 h light/dark cycles) and allowed access to food and water *ad libitum* for two weeks to acclimatize. This was in compliance with internationally accepted principles for use and handling of laboratory animals as stated in the Canadian Council of Animal Care guidelines and Protocol Review [20], adopted by the ethical committee of Federal University of Technology Minna.

2.4. Preparation of alloxan monohydrate

Fresh alloxan solution was prepared by dissolving an appropriate amount of alloxan monohydrate in an appropriate volume of cold citrate buffer solution at pH of 4.5.

2.5. Animal induction of diabetes with alloxan monohydrate

Diabetes was induced in rats by intraperitoneal administration of 110 mg/kg bodyweight of alloxan monohydrate to 40 rats of both sexes, after an overnight fast but with access to water. The rats were allowed access to food, the blood glucose was checked 96 h after induction and rats with blood glucose levels \geq 200 mg/dl were considered diabetic and used for the experiment.

2.6. Grouping of animals

A total of 24 rats (20 with blood glucose levels \geq 200 mg/dl) were divided into six groups of 4 rats each. Group A rats were normoglycemic, labeled: (NGLC), Group B rats: diabetic untreated, labeled (DUNT), Group C rats: diabetic rats treated with 15 mg/kg bodyweight standard

drug (Glidazide), labeled (STND), Group D, E and F rats: diabetic rats treated with 150 mg/kg bodyweight aqueous, ethanol and methanol extracts labeled (AQCE), (ETCE), and (MTCE) respectively.

2.7. Monitoring of blood glucose

Blood glucose levels of rats were monitored using rapid Accucheck (Roche Diagnostics, Germany) glucometer. Blood samples were withdrawn from the tail vein of the animals, placed on the strip and the glucose levels were read few seconds after the application of the blood sample. Blood glucose levels of all animals were checked every three days in the morning at 8:00 am before treatment. The treatment lasted for 15 days.

2.8. Collection of serum

At the end of the treatment, rats were anesthetized under mild chloroform vapor, euthanized and the blood samples were collected by jugular puncture. The blood samples were allowed to stand for 1 hour, then centrifuged at 3000 rpm for 5 min and the sera obtained were used for the determination of biochemical parameters.

2.9. Estimation of biochemical parameters

The serum total cholesterol (TC), triacylglycerol (TG), total protein (TP), urea, and creatinine were determined using Spectrum diagnostic kits while aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) were estimated using Agape diagnostic kits. The absorbance was determined and concentration calculated using Rayto semi- auto chemistry analyzer. All analyses were carried out in triplicates of four analyses ($n = 4$), and according to manufacturer's instruction.

2.10. Statistical analysis

Results are expressed as mean \pm standard error of mean ($n = 4$). One way analysis of variance (ANOVA), followed by Dunnett Duncan's test was used to analyze the data obtained from the study using SPSS software version 20. The level of significance was set at $p < 0.05$.

3. Results

3.1. Qualitative phytochemical composition of aqueous, ethanol and methanol extracts of *Casuarina equisetifolia*

Table 1 shows the phytochemical constituents of *C. equisetifolia*, with abundant tannins and phenols in aqueous extract, tannins, flavonoids and saponins in ethanol extract, while flavonoids and phenols were abundant in methanol extract.

Table 1

Phytochemical constituents of aqueous, ethanol, and methanol extracts of *C. equisetifolia*.

Phytochemicals	Aqueous Extract	Ethanol Extract	Methanol Extract
Tannins	+++	+++	++
Flavonoids	++	+++	+++
Steroids	-	-	-
Terpenes	-	-	+
Anthraquinones	-	-	-
Cardiac glycosides	-	-	-
Alkaloids	++	-	+
Saponins	+	+++	++
Phenols	+++	++	+++
Anthranoids	-	-	-
Reducing sugar	+	+	++

Key: + = Slightly present, ++ = Moderately present, +++ = Significantly present, - = Absent.

3.2. Acute toxicity oral study

No toxicity signs (such as drowsiness, respiratory distress, diarrhea, convulsion, lethargy, coma) or death were recorded following administration of the extracts of *C. equisetifolia*, indicating that the safe dose of extracts was up to 2000 mg/kg bodyweight. The extract of *Casuarina equisetifolia* may be assigned to be class 5 ($LD_{50} > 2000$ mg/kg bodyweight), which was designated to be the lowest class. The animals showed no expression of poisoning, indicating there was no adverse effects as a result of interaction between the extracts and the cells.

3.3. The effect of *C. equisetifolia* extracts on blood glucose level of experimental rats

Fig. 1 shows the blood glucose level of rats across the treatment groups. The fasting blood glucose levels of diabetic untreated group significantly increased progressively in the days of treatment as compared to the normoglycemic, standard drug and extract treated groups. Blood glucose levels in all the treated groups reduced within the treatment days, with the ethanol extract exhibiting the highest hypoglycemic activity (64%). The percentage reduction in blood glucose was 64%, 59% and 58% for the ethanol, methanol and aqueous extracts respectively.

3.4. The effect of *C. equisetifolia* extracts on serum liver marker enzymes of experimental rats

Fig. 2 shows the effect of the extracts on the liver biomarker enzymes. Significant ($p < 0.05$) increase in AST and ALP was observed in both the diabetic untreated group and some of the extract treated group when compared with the normoglycemic group (NGLC).

3.5. The effect of *C. equisetifolia* extracts on total protein, urea and creatinine of experimental rats

Fig. 3 shows the effect of extracts on total protein, urea and creatinine. The concentration of urea and creatinine were significantly ($p < 0.05$) increased in the diabetic untreated group when compared with normoglycemic and the extract treated groups.

3.6. The effect of *C. equisetifolia* extracts on serum total cholesterol and triglyceride level of experimental rats

Fig. 4 shows the levels of total cholesterol and triacylglycerol of diabetic rats in various treatment groups. There was a significant reduction in the concentrations of both parameters in the diabetic treated groups when compared to normoglycemic group.

4. Discussion

Plants produce a large number of natural products which have great contributions in human health that make them important sources for the discovery of new lead molecules [21]. Analysis of extracts for secondary metabolites revealed that steroids, anthraquinones, and cardiac glycosides were absent in the aqueous, ethanol and methanol leaf extracts of *Casuarina equisetifolia* (Table 1), however, alkaloids are common to the ethanol and methanol extracts. Muthuraj *et al.*, [10] and Pawar *et al.*, [22] reported a similar result in addition to glycoside and steroid which were absent in the present study, the difference observed could be attributed to the variation in extraction procedure and plant part used. The secondary metabolites present in the extracts are known to be responsible for the beneficial health effects elicited by many plants, including *C. equisetifolia* [23, 24].

According to the American Diabetes Association, [25], hyperglycemia is a major cause of organ damage in type 2 diabetes thus, it is essential to control hyperglycemia effectively to prevent its serious consequences. More so, for a plant to be a good antidiabetic agent, it

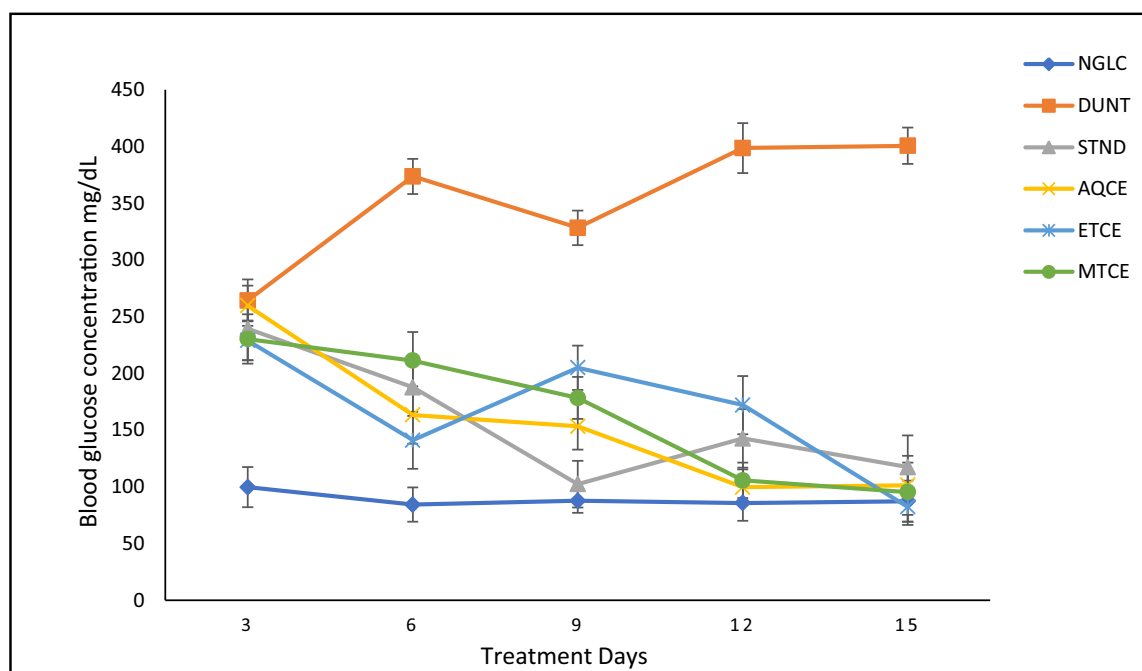


Fig. 1. Blood glucose of diabetic rats treated with *C. equisetifolia* leaf extracts

Key: NGLC; normoglycemic rats, DUNT; Diabetic untreated rats, STND; Diabetic rats treated with 15 mg/kg bodyweight standard drug (Glidazide), AQCE; Diabetic rats treated with 150 mg/kg bodyweight aqueous extract, ETCE; Diabetic rats treated with 150 mg/kg bodyweight ethanol extract, MTCE; Diabetic rats treated with 150 mg/kg bodyweight methanol extract.

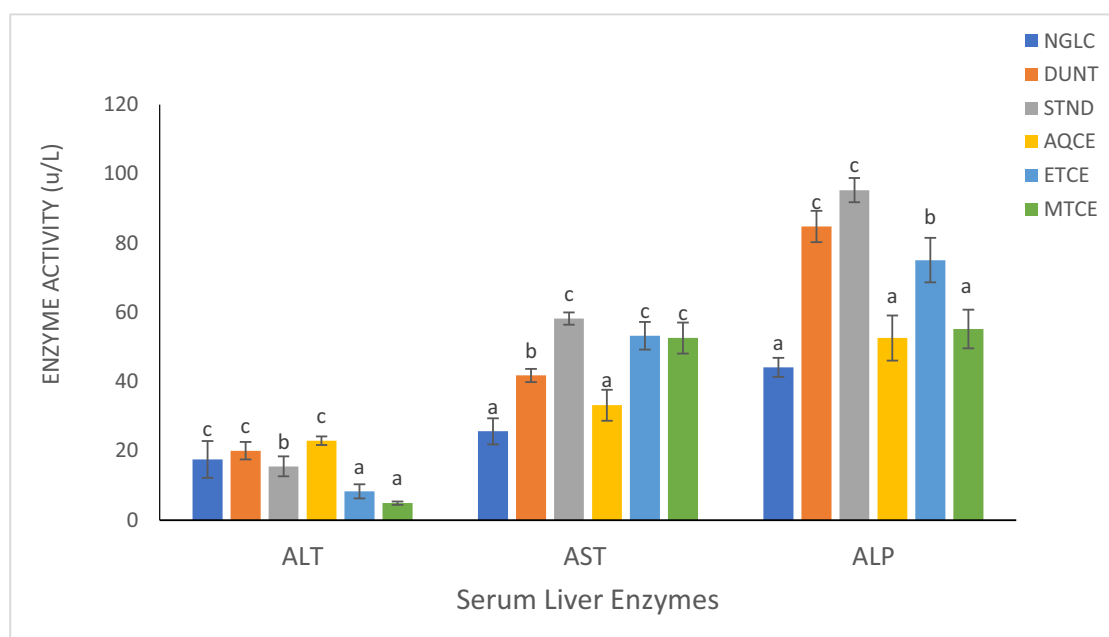


Fig. 2. Effect of *C. equisetifolia* extracts on some liver biomarker enzymes

Key: NGLC; normoglycemic rats, DUNT; Diabetic untreated rats, STND; Diabetic rats treated with 15 mg/kg bodyweight standard drug (Glidazide), AQCE; Diabetic rats treated with 150 mg/kg bodyweight aqueous extract, ETCE; Diabetic rats treated with 150 mg/kg bodyweight ethanol extract, MTCE; Diabetic rats treated with 150 mg/kg bodyweight methanol extract. ALT; Alanine aminotransferase, AST; Aspartate aminotransferase, ALP; Alkaline phosphatase.

should be able to effectively control hyperglycemia. In line with this, there was significant reduction ($p < 0.05$) in the blood glucose levels of all diabetic rats upon oral administration of the methanol, ethanol and aqueous extracts of the leaf of *C. equisetifolia* in the order; ethanol extract>methanol extract>aqueous extract with values 64%, 59% and 58% respectively. This is similar to the work reported by Sriram [13] and Kantheti et al., [14] on the effect of ethanol extracts of various parts of

C. equisetifolia plant on Streptozocin induced diabetic rats. The ethanolic extract demonstrated the most significant anti-diabetic activity than the other extracts. This observation is in line with the work of Muthuraj et al., [10] who reported that the ethanol extract of *C. equisetifolia* seed showed good antidiabetic activity when compared to other extracts used in their research. Studies have postulated that various plant extracts could elicit their glucose lowering effect in experimental rats

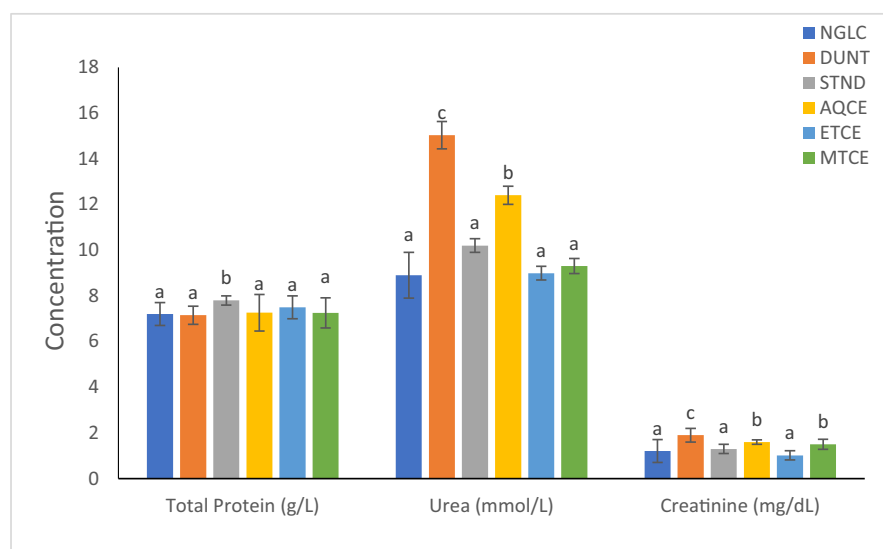


Fig. 3. Effect of *C. equisetifolia* extracts on total protein, urea and creatinine

Key: NGLC; normoglycemic rats, DUNT; Diabetic untreated rats, STND; Diabetic rats treated with 15 mg/kg bodyweight standard drug (Glidazide), AQCE; Diabetic rats treated with 150 mg/kg bodyweight aqueous extract, ETCE; Diabetic rats treated with 150 mg/kg bodyweight ethanol extract, MTCE; Diabetic rats treated with 150 mg/kg bodyweight methanol extract.

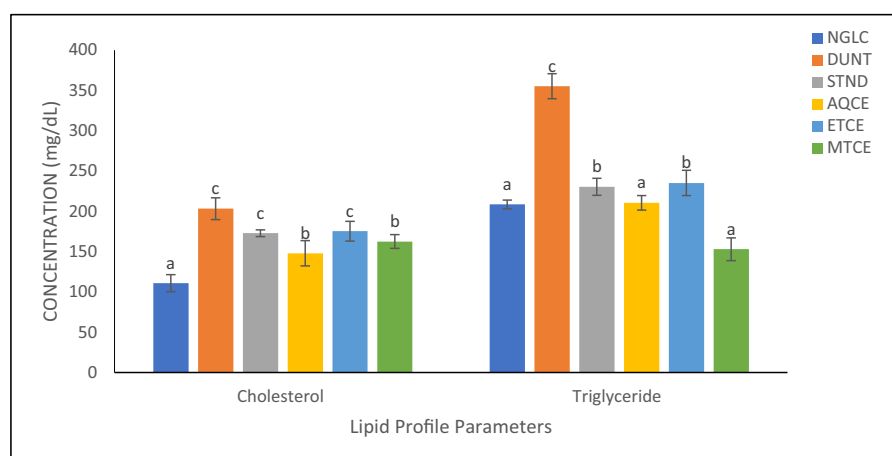


Fig. 4. Effect of *C. equisetifolia* extracts on serum cholesterol and triacylglycerol levels

Key: NGLC; normoglycemic rats, DUNT; Diabetic untreated rats, STND; Diabetic rats treated with 15 mg/kg bodyweight standard drug (Glidazide), AQCE; Diabetic rats treated with 150 mg/kg bodyweight aqueous extract, ETCE; Diabetic rats treated with 150 mg/kg bodyweight ethanol extract, MTCE; Diabetic rats treated with 150 mg/kg bodyweight methanol extract.

induced with diabetes by; stimulating insulin secretion [26], inhibiting intestinal α -amylase activity [27], increasing muscle basal glucose uptake [28] as well as possessing antioxidant activity [29], which might have been possibly due to the variety of biologically active chemicals present in the plants used [30]. The glucose lowering effect observed to a large extent by the ethanol extract in this study could be attributed to phenols and flavonoids which possess antioxidant properties. Furthermore, flavonoids have been reported to inhibit sodium-dependent vitamin C transporter 1 (SVCT 1) and glucose transporter Isoform 2 (Glut 2) which are the intestinal transporters for vitamin C and glucose and consequently, resulting to a decrease in the intestinal absorption of glucose, hence decrease in the blood glucose concentration [31].

Liver enzymes such as AST, ALT and ALP are biomarkers of injury to the liver resulting from diseases such as cirrhosis, necrosis, hepatitis, or exposure of the liver to toxic substances amongst other reasons. When any of this arises, the biomarker enzymes seep into the plasma and their concentrations become elevated. The measurement of serum liver biomarker enzyme levels serve as means for indirect assessment of liver function. Studies have demonstrated that aminotransferases (AST and ALT) levels independently predict type 2 diabetes [32]. They have been shown to be positively associated with indirect measurements of insulin resistance together with fasting blood glucose levels [33]. Elevated activities of ALT and AST have been reported in liver disease, obesity, diabetes and dyslipidemia [34, 35]. In this study, AST and ALP values in the diabetic untreated group and the groups treated with the

methanol and ethanol extracts for AST were significantly higher when compared to the normal control group. This is in line with the work of Muhammad *et al.*, [36] where the ethyl acetate fraction of *Ceiba pentandra* used in the treatment of diabetic rats elevated AST and ALT in all the extract treated groups. The observation in this study could be as a result of enhanced cellular damage in the case of diabetes and could suggest hepatotoxicity in the case of the plant extract. However, according to Muhammad *et al.*, [36], ALT is considered a more specific and sensitive indicator of hepatocellular injury than AST in rats.

Alkaline phosphatase (ALP) is a biomarker enzyme present in all body tissues with high amount in the liver and bone. The serum levels of ALP significantly increased in the group treated with the ethanol extract and the diabetic group compared to the normoglycemic. This agrees with the result obtained by Muhammad *et al.*, [37], stating that higher than normal values of ALP may be due to biliary obstruction and hepatitis however, increase in the activity of ALP may not be due to hepatic cell disruption, nor to a failure of clearance, but rather to increased synthesis of hepatic ALP.

Urea and creatinine levels in the blood are good pointers of kidney function, and any elevation in the two parameters is an indication of kidney function impairment [38]. The diabetic untreated group showed a remarkable derangement in urea and creatinine with the values deviating from the normal and diabetic treated groups. Extract treated groups had reduction in urea and creatinine levels to values either near or comparable to that of the normoglycemic group, suggesting improvement in

impaired kidney function. This observation is in line with the work of Atawodi *et al.*, [39] where the effect of methanol extract of *Tetrapleura tetraptera* on hyperglycemia and indices of diabetic complications were evaluated in alloxan-induced diabetic rats. The authors opined that their finding demonstrates the therapeutic importance of the plant used in the management of chronic glomerular disease, one amongst the complications of diabetes associated with elevated levels of blood urinary nitrogen and creatinine.

Complications associated with diabetes such as dyslipidemia are the major cause of death in diabetics [40]. Disturbances in lipid metabolism, hyperglycemia and insulin deficiency results to abnormal increase in triacylglycerol and cholesterol as seen in the diabetic untreated group. The observation is in agreement with the work of Abdulkareem *et al.*, [41] and Sharma *et al.*, [42] on effect of the leaf extract of *Morinda lucida* on pancreatic β -cell function of diabetic rats and the hypoglycemic potential of alcohol root extract of *Cassia occidentalis* Linn in diabetic mice. Insulin is a potent inhibitor of lipolysis and therefore during diabetes mellitus, activity of lipase enzyme increases lipolysis and release more free fatty acids in the circulation because of lack of insulin [43]. Increase in fatty acid concentration in turn increases the beta-oxidation of fatty acids by increasing the activity of β -Hydroxyl- β -methylglutaryl Coenzyme-A (HMG-CoA) reductase for production of more cholesterol [44, 45]. Insulin also increases the receptor mediated removal of low density lipoprotein-cholesterol (LDL-cholesterol) and decreased activity of insulin during diabetes mellitus causes hypercholesterolemia [43]. These anomalies were ameliorated in the extract treated groups and this observation could be attributed to the fact that as hyperglycemia reduced, insulin secretion improved and consequently lipolysis was inhibited. Also, phytochemicals such as saponins are known to interfere with fatty acid oxidation as a result of their antioxidant activity [46, 47]. Thus, indicating that the plant extract possesses hypolipidemic properties and this may help reduce the incidence of cardiovascular diseases like atherosclerosis associated with diabetes mellitus.

5. Conclusion

The extracts (aqueous, ethanol, methanol) from *Casuarina equisetifolia* were able to ameliorate changes observed in a diabetic state, such as hyperglycemia and dyslipidemia. Thus, it could be a novel source of a natural drug for the management of diabetes and its complications.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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