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Hypoglycaemic Potential of *Ziziphus Spina-Christi* Fruit on Alloxan Induced Hyperglycaemic Rats

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Abstract

Hyperglycaemia is a key symptom in diabetes mellitus associated with long term damages, dysfunction and eventually failure of organs, especially the eyes, kidneys, nerves, heart and blood vessels. This study was to evaluate the hypoglycaemic potential of orally administered aqueous and ethanol extracts of *Zyziphus spina-christ* fruit on alloxan induced hyperglycaemic Wistar rats.

The plant was extracted using maceration using aqueous and 80% ethanol as extraction solvents. Qualitative phytochemical analysis was done. An *in vitro* assessment of both aqueous and ethanol extract to demonstrate hypoglycaemic activity via inhibition of alpha amylase enzyme and alpha glucosidase enzymes were done. Total of 45 albino rats were used in the study of both sexes divided in 9 groups. Group 1: normal control group, group 2: diabetic control group, group 3: positive control group (metformin 300mg/kg), group 4-6: (150mg/kg, 300mg/kg and 600mg/kg) aqueous extract and group 7-9: (150mg/kg, 300mg/kg and 600mg/kg) ethanol extract. Groups were compared using one way ANOVA for significant differences and Dunnet's posthoc test was deployed were differences exit. Data were represented as mean ± SEM and p value <0.005

The aqueous and ethanol extract yielded 35.59%% and 46.68% respectively. Phytochemical analysis revealed the presence of alkaloids, flavonoids, terpenoids, tannins, Saponins, Saponins glycosides, steroids, Phytosteroids, carbohydrate and volatile oil. An *in vitro* assessment of aqueous and ethanol extract demonstrated hypoglycaemic activity via inhibition of both alpha amylase enzyme and alpha glucosidase enzymes. The percentage inhibition of alpha amylase was presented as IC_{50} of 0.14, 0.19 and 0.58 for the acarbose, ethanol extract and aqueous extract respectively. Alpha glucosidase inhibition was represented by the IC_{50} of 0.7mg/ml, 0.9mg/ml and 0.7mg/ml for acarbose, ethanol extracts and aqueous extracts. The aqueous and ethanol extracts significantly decrease the blood glucose level. Elevation of cholesterol and LDL was seen in diabetic control group.

The results from the studies showed that *Ziziphus spina-christi* fruit aqueous and ethanol extracts has an anti-hyperglycaemic potential which is not a dose dependent both *in Vitro* and *in Vivo*. *Ziziphus spina-Christi* fruit aqueous and ethanol extract also causes a significant reduction in cholesterol level.

Keywords: Hyperglycaemia, *Ziziphus spina-Christi*, Phytochemicals

Introduction

Diabetes mellitus (DM) is globally defined as a group of metabolic disorders associated with deficiency of insulin or insulin inactivity which results in hyperglycaemia or impaired glucose tolerance [1]. Insulin is a pancreatic hormone produced by the β-cell of the islet cells of Langerhans. The hormone facilitates the movement of glucose into the body's cells where it is converted in to energy in the form of ATP needed by muscles and tissues to function especially the brain cells that depend solely on glucose [2, 20]. A person with DM does not absorb glucose properly and higher concentration of glucose remains circulating in the blood (hyperglycaemia) damaging body tissue over time. Thus, hyperglycaemia is due to deficiency of insulin secretion or resistance of the body cells to the action of circulating insulin, often associated with carbohydrate, protein and lipid metabolism [3]. These metabolic disturbances result in acute and long-term diabetic complications, including polyuria, polyphagia, ketosis, retinopathy as well as cardiovascular disorder responsible for premature death and disability [4, 5]. Over a decade ago (2004) the World Health Organization reported, that more than 100 million peoples have diabetes mellitus worldwide [6]. Attention to diabetes mellitus is necessary as the number of diabetic patients is increasing resulting to an increase in morbidity and mortality from the disease. Cardiovascular related events are the leading cause of mortality in diabetic patients [7].

Diabetes Mellitus has been classified into three types: Type I, Type II and Gestational diabetes mellitus [8,9]. Type I DM is an insulin dependent diabetes mellitus affecting lowest percentage of the diabetic population. Type II, the non-insulin dependent diabetes mellitus, occurs as a result of insulin resistance or abnormality in insulin secretion and accounting for higher cases of diabetes. Type II DM is commonly seen in adults over the age of 30 years. Gestational diabetes is another type of diabetes that occurs during pregnancy due to glucose intolerance. It can complicate pregnancy leading to prenatal or postnatal morbidity and mortality.

Hyperglycaemia is associated with long term damages, dysfunction and eventually failure of organs, especially the eyes, kidneys, nerves, heart and blood vessels [10]. In spite of newer agents' introduction, modifications and extensive utilization of hypoglycaemic agents, diabetes and its associated complications continue to be a major health problem worldwide, [11]. The disease is considered a major economic burden and can hinder the development of nations [12].

Environmental factors such as diet, obesity and sedentary life style ranked high among the risk factors that predisposes to the development of diabetes mellitus [9]. Other important risk factors include high family aggregation, insulin resistance, nutritional status, age and lifestyle change due to urbanization [13]. Management of diabetes is a global problem until now and successful treatment is yet to be discovered [14]. Insulin and oral hypoglycaemic agents such as Sulfonylureas (Tolbutamine, Chloropropamide and Gilbenclamide), the Biguanides (Phenformin and Metformin) and the β-glucosidase inhibitors (Acarbose and Miglitol) are pharmacologically used in the treatment of diabetes [9]. Dietary modifications, life style changes, exercises and proper education are the non-pharmacological means of diabetic management.

About 463 million people worldwide were estimated to have DM and more than 19 million in Africa, this figure is projected to rise to about 47 million by 2045 [15]. Treatment of diabetes is both expensive and tedious [14], multidisciplinary approach that included physical exercise, dietary modification, use of insulin and chemicals such as sulfornylureas is used [9]. Current system of diabetic treatment comes along with shortcomings limiting the usage and success of available treatment modules. These chemicals when administered in high doses leads to hypoglycaemia and may cause liver damage, lactic acidosis, loss of appetite and abdominal pain [9]. Chronic disease like diabetes and its cardiovascular complication are becoming endemic to even the developing countries adding to the burden on the already existing communicable diseases [12].

Almost a thousand plant species have been reported to possess antidiabetic properties [16]. Several plant species have been used for the prevention or management of diabetes by the Native Americans, Chinese, South Americans and Asian Indians [17]. The pharmacological actions of these plants are related to their chemical composition. Those rich in phenolics, alkaloids, flavonoids, terpenoids, coumarins, and glycosides usually show positive effects [18]. The consumption of moringa oleifera leaves powder appears to be effective in reducing blood glucose level in diabetic patients [19]. This anti-diabetes effect of moringa has been linked to the presence

of certain polyphenols. According to certain scientific research, moringa leaves significantly decrease blood glucose concentration in whister rats and goto-kakizaki (GK) rats modelled type 2 diabetes [19].

Extracted the polyphenolic components of Roselle and studied their effect in a type II diabetic rat model (high fat diet model) [20]. The study revealed anti-insulin resistance properties of the extract at a dose of 200mg/kg, and reduction in hyper glycaemia and hyper insulinemia. Other studies indicated that oral administration of ethanolic extract of *Ziziphus maur*itiana leaves has a dose dependent blood glucose level reduction in diabetic rats, it also causes a significant increase in glucose tolerance in diabetic rats. Additionally, the extract lowers cholesterol level without producing toxicity to liver and kidney up to a period of 4 weeks study [21, 22].

Ziziphus spina-christi commonly known as Christ's Thorn Jujube, is a deciduous or evergreen trees and shrubs throughout the world and native to the warm-temperate and subtropical regions, including North Africa, South Europe, Mediterranean, Australia, tropical America, South and East of Asia and Middle East [23, 24, 25]. *Ziziphus spina-christi* locally known as "Kurna" (Hausa), kurnahi (Fulani), Korna (Kanuri) or Eakannase-adie (Yoruba) is a member of the Rhamnaceae family in the order of Rosales that contains about 60 genera and more than 850 species, the genus Ziziphus consists of about 100 species [26, 27]. Majority of the rural populace in Northern Nigeria use *Ziziphus spina-christi* extensively for its medicinal and economic importance [28].

Methodology

All chemicals used in this study were of highest analytical grade. Metformin was a Product of AVRO Pharma Limited, Nigeria. Alloxan monohydrate (inducing agent) was purchased from Sigma-Aldrich Co. LLC respectively. All reagents used for the Biochemical analysis were of analytical grade purchased as kits produced by Randox Laboratories Limited, United Kingdom.

Sample Collection and Preparation

Ziziphus Spina Christi fruit was purchased dried from Muda Lawan market Bauchi local government area of Bauchi state. The plant was taken to the Herbarium in the Department of Biological Science Bauchi State University Gadau, and was authenticated by a Taxonomist voucher number of the specimen (0028) was deposited. The *Ziziphus Spina-christi* fruits were washed to remove dust and then drain and It was then grinded into powder using Mortar and pestle and then subjected to extraction.

Extraction of Ziziphus Spina-christi Fruit

About 100g of *Ziziphus Spina-christi* fruit powder was weighed with an analytical weighing balance and transferred to a 1000ml beaker to which 1000ml of 80%ethanol was measured and transferred into, another 100g of the sample was weights and dissolved in 1000ml of distilled water in a 1000ml beaker, the two substances were allowed to dissolve for 72hrs and then filtered using filter paper and the filtrate was concentrated at 50°C on water bath. The percentage yield was calculated as follows:

$$\text{Percentage yield} = \frac{Weight\ of\ extract}{Original\ weioght} \times 100\%$$

The portion of the two extracts were used for the qualitative phytochemical analysis of the following: test for alkaloids, test for flavonoid, test for tannins, test for terpenoids, test for chalcones, test for phlobatannins, test for anthraquinone, test for steroids, test for phytosterol, test for reducing sugar and test for cardiac glycoside. Another portion from the two extracts was used for *in vitro* anti diabetic study for: alpha amylase and alpha glucosidase inhibition.

The extract was given to the rats at different concentrations.

Phytochemical Analysis

The phytochemical analysis was done for qualitative determination. The analyzed include; Alkaloids, Flavonoids, Tannins, Terpenoids, Saponins, Chalcones, Phlobatannins, Anthraquinones, Steroids, Phyto steroids, reducing sugars and Cardiac glycosides according the method outlined by Association of Analytical Chemist (AOAC 1984), National Committee on Clinical Laboratory Standards (NCCLS, 2011), El-Olemy et al; (2000) [29].

In Vitro Anti Diabetic Study

Inhibition of α amylase Enzyme: A 500µl of test samples and acarbose (standard drug) (0.125, 0.25, 0.5, 1.00 and 2.00mg/ml) were added to 500µl of 0.02 M phosphate buffer (pH 6.9) containing 6 mM sodium chloride and α-amylase (0.5 mg/ml; 0.04 units Porcine Pancreatic α-amylase (PPA)) solution and incubated at 37 °c for 10 min. after which, 500µl of a 0.5% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube and incubated at 37 °C for 10 min. The reaction was stopped by adding 1 ml of 3,5-dinitrosalicyclic acid (DNSA) colour reagent. The test tubes were incubated in a boiling water bath for 5 min, cold to room temperature. The reaction mixtures were diluted with 10 ml of distilled water and absorbance was measured at 540 nm. Control represents 100% enzyme activity and was conducted in similar way by replacing fraction with vehicle [30].

Calculation: % inhibitory activity of test = $\frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} X\ 100$

Inhibition of α glucosidases Enzyme: α glucosidases inhibitory activity was determined by incubating 1 ml solution of starch substrate (2% w/v maltose) with 1 mn 0.2 M Tris buffer pH 8.0 and various concentratios extracts fractions and acarbose (standard drug) for 5 min at 37 °C. The reaction was initiated by adding 1 ml of α glucosidase enzyme (IU/ml) to it followed by incubation for 10 min at 37 °C. The reaction mixture was heated for 2 mn in boiling water bath to stop the reaction. The amount of liberated glucose wasmeasured by glucose oxidase peroxidase method [31, 30].

Calculation: % inhibitory activity of test = $\frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} X\ 100$

Experimental Animals

Total of 45 rats were utilised and grouped into nine (9) groups of five (5) rats each.

Group I: Normal control: Rats were not induced and untreated. They were given normal feed and parameters from this group served as a base line data (normal control).

Group II: Negative control: Rats were induced with Alloxan monohydrated (150 mg/kg b.w.) and were untreated, this served as the hyperglycaemic control group and in addition they were given normal feed /water *ad libitum*.

Group III: Positive control: Rats were induced with Alloxan monohydrate thereafter they were treated with Glucophage hydrochloride tablet B.P (Metformin tablet), this group served as the test Group A.

Group IV: Extract low dose: Rats were induced with Alloxan monohydrate thereafter they were treated with low dose of the water extract.

Group V: Extract moderate dose: Rats were induced with Alloxan monohydrate thereafter they were treated with moderate dose of the water extract.

Group VI: Extract high dose: Rats were induced with Alloxan monohydrate thereafter they were treated with high dose of the water extract.

Group VII: Extract low dose: Rats were induced with Alloxan monohydrate thereafter they were treated with low dose of the ethanol extract.

Group VIII: Extract moderate dose: Rats were induced with Alloxan monohydrate thereafter they were treated with moderate dose of the ethanol extract.

Group IX: Extract high dose: Rats were induced with Alloxan monohydrate thereafter they were treated with high dose of the ethanol extract.

Induction of Diabetes

Diabetes was induced by subjecting the animals to 12 h fasting overnight, followed by a single injection of 150 mg/kg alloxan monohydrate dissolved in ice cold normal saline [32] intra peritoneal. Fasting blood glucose concentration was determined after 72hrs. Periodic blood glucose concentration was determined using Accu-check glucometer.
Rats with fasting blood glucose of 140 mg/dl and above were used for the study [33].

Determination of Biochemical Parameters

At the end of the experiment, the animals were sacrificed to collect blood and some organs for analysis. The blood was allowed to clot and serum separated and the following investigations were carried out in the serum sample.

1. Total cholesterol [34]
2. LDL [35]
3. HDL [35]
4. Triglycerides [34]
5. VLDL [35]

Statistical Analysis

Statistical analysis was carried out using Mean ± standard error of mean (SEM) and analyse with standard statistical software package for social science (SPSS) using one-way Analysis of variance (one-way ANOVA) from which (P) value was derived. The P value <0.05 were considered significant.

Result

The percentage yield of aqueous extract is:

$$\%yield=\frac{35.585}{100}\times 100 = 35.59\%$$

The percentage yield of ethanol extract is:

$$\%yield = \frac{46.68}{100}\times 100 = 46.68\%$$

Phytochemical analysis carried out of the aqueous and ethanol extract of fruits of *Ziziphus spina-christ* revealed presence of Alkaloids, Flavonoids, Tannins, Terpenoids, Saponins, Saponins glycoside, Steroids, Phytosteroids, Cardiac glycosides, Volatile oil and Carbohydrate in different proportion. Detailed of the phytochemicals obtained is shown in the table 1

Phytochemicals	Aqueous extract	Ethanol extract
Alkaloids	+	++
Flavonoids	++	+++
Terpenoids	+	-
Tannins	+	+++
Saponins	+++	++
Saponins glycoside	+	++
Steroids	+	+++
Phytosteroids	+	+++
Cardiac glycoside	-	+
Volatile oil	-	+
Carbohydrate	++	++

Key; += present and - = absent

Table 1: Qualitative Phytochemical contents of *Zizipus spina-christi* fruit

An *in vitro* assessment of the two extract to see their hypoglycaemic activity was done using alpha amylase enzyme. A standard hypoglycaemic agent called Acarbose was equally used and it was compared with the two extracts. The percentage inhibition of the enzyme by the ethanol extraction in all concentration tested was found to be almost similar to the standard agent used. Aqueous extract demonstrated a lower inhibition compared to the acarbose. An IC50 of 0.14, 0.19 and 0.58 was recorded for the acarbose, ethanol and aqueous extract respectively.

ALPHA AMYLASE			
Conc (mg/mL)	Acarbose	Ethanol	Aqueous
0.125	78.08±0.96	73.09±0.79	51.41±7.34
0.250	72.03±0.67	72.17±0.22	50.01±2.39
0.500	74.58±0.65	71.14±0.36	48.22±6.06
1.000	75.18±1.55	69.96±1.03	48.22±2.55
2.000	71.57±0.44	71.42±1.55	46.09±1.75
IC_{50}	0.14	0.19	0.58

α-Amylase Inhibition Activities of Ethanol and Aqueous extract. Values are expressed as the mean±SEM for triplicate determination and were analyse using one-way ANOVA. Differences with $p<0.05$ are considered statistically significant

Table 2: *In vitro* inhibitory activity of *Ziziphus spina-christi* fruit aqueous and ethanol extracts on alpha amylase

Alpha glucosidase inhibition was determined *in vitro* for the aqueous and ethanol extracts of *Ziziphus spina-christi* fruit using acarbose, as the control drug. The IC50 revealed a similar inhibitory activity. Detail display in Table 3.

ALPHA GLUCOSIDASE			
Conc (mg/mL)	Acarbose	Ethanol	Aqueous
0.125	91.17±0.63	83.67±2.80	88.84±1.06
0.250	88.78±2.20	87.42±0.65	89.19±3.84
0.500	86.66±1.97	87.31±0.55	83.24±1.11
1.000	83.65±0.81	84.06±1.86	78.43±2.44
2.000	84.65±0.83	79.45±3.40	74.99±0.38
IC_{50}	0.07	0.09	0.07

α-Glucosidase Inhibition Activities of Ethanol and Aqueous extracts. Values are expressed as the mean±SEM for triplicate determination and where analyze using one-way ANOVA. Differences with $p<0.05$ are considered statistically significant

Table 3: *In vitro* inhibitory activity of *Zizipus spina-Christi* fruit aqueous and ethanol extracts on alpha glucosidase

Body weight of hyperglycaemic rats treated with aqueous and ethanol extracts of *Zizipus spina-christi* fruit for 14 days. Values were recorded on day 0, day 7 and day 14. There is a significant different between group 4: 150mg/kg aqueous extracts and the normal control group on day and14, as presented in figure 1

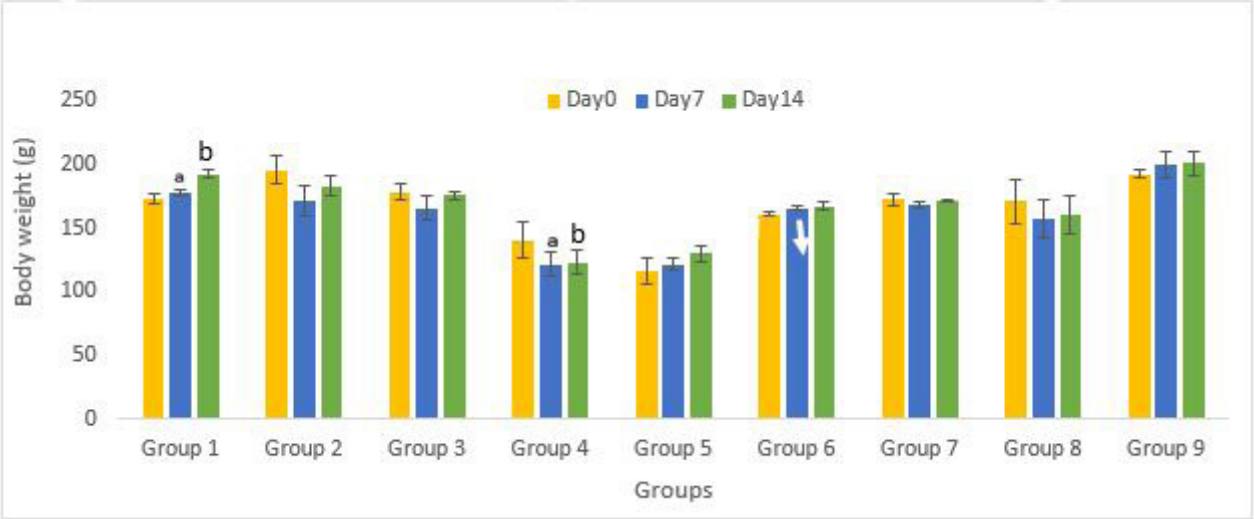

Figure 1: Effect of *Ziziphus spina-christi* fruit extracts on the body weight of hyperglycaemic Rats recorded on day 0, 7 and 14. Differences with $p<0.05$ are considered statistically significant. Values with the same superscript (a, b) are significantly different

Figure 2 showed anti-hyperglycaemic potential of single dose of aqueous and ethanol extracts of *Ziziphus spina-christi* fruit of 150mg/kg, 300mg/kg and 600mg/kg. The positive control group receive standard drug, Metformin at 300mg/kg body weight, each group containing 5 rats. A significant blood glucose elevation was seen in the treatment group when compared with the normal control group on day 0 of the experiment. And a noticeable and statistically significant different glucose level on day 14 between the normal control and negative control groups. All the treated group presented with sustain decrease in blood sugar after receiving the extracts, though the effect were not dose dependent in most groups.

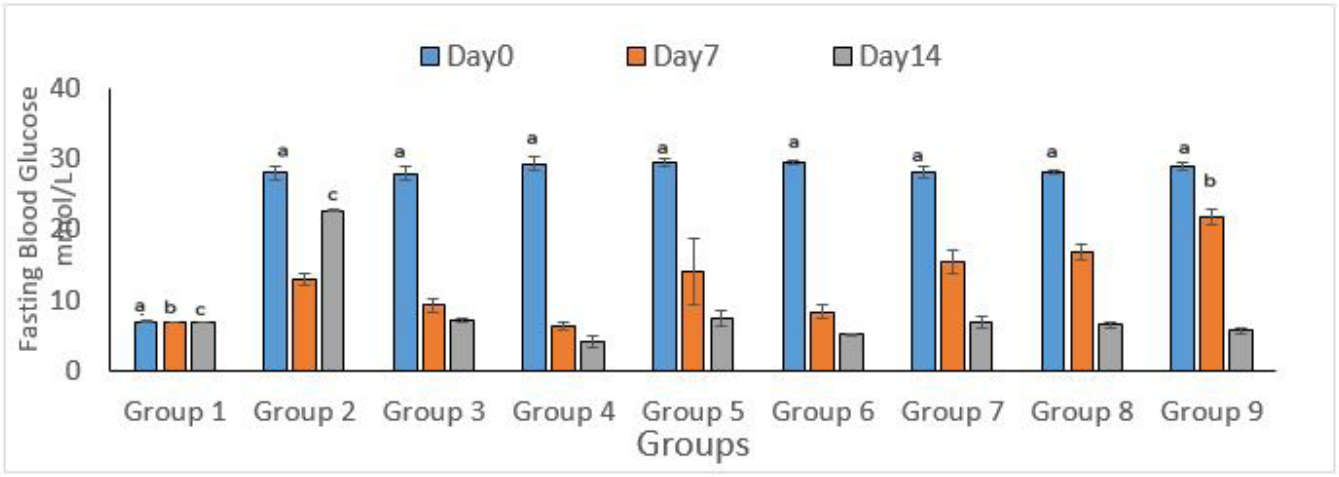

Figure 2: Effect of *Ziziphus spina-christi* fruit Aqueous and Ethanol extract on fasting blood glucose recorded at day 0, 7 and 14. Differences with $p<0.05$ are considered statistically significant. Values with the same superscript (a, b and c) are significantly different to the normal control group

The result in figure 3 showed effects of *Ziziphus spina-christi* fruit on cholesterol, triglycerides, high density lipoproteins (HDL), low density lipoproteins (LDL) and very low-density lipoproteins (VLDL) of hyperglycaemic rats treated for 14 days. A significant elevation of cholesterol and LDL level was seen in the negative control group when compared with the normal control group.

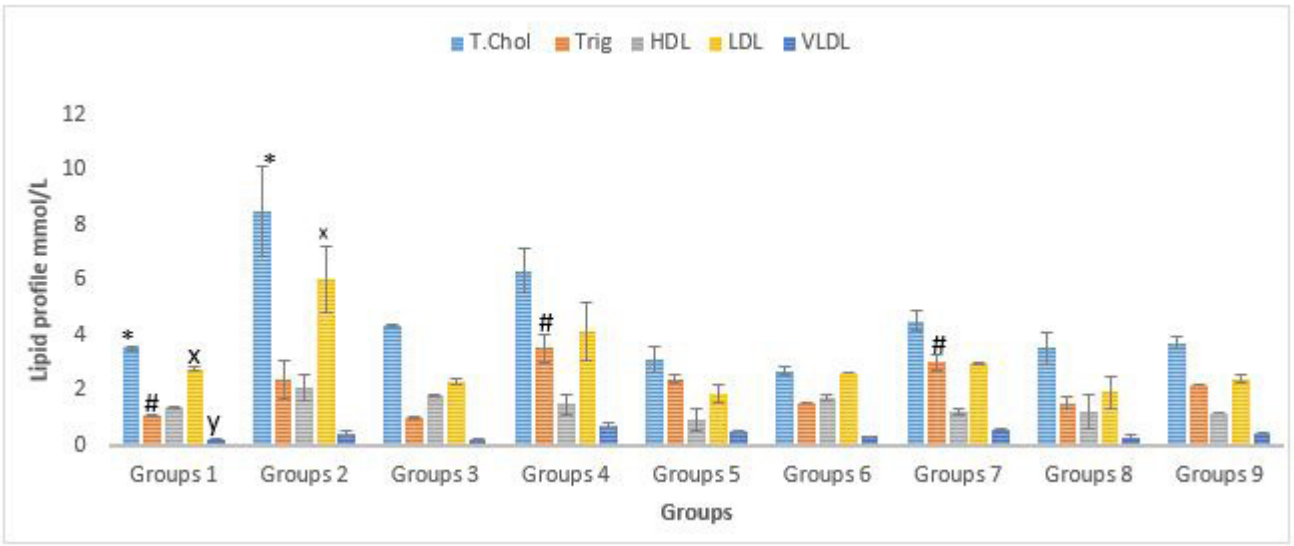

Figure 3: effect of *Ziziphus spina-christi* fruit Aqueous and ethanol extracts on Lipid profile (mmol/L) for total cholesterol, triglycerides, high density lipoproteins, low density lipoproteins and very low-density lipoproteins

Differences with $p<0.05$ are considered statistically significant. Values with the same superscript (*, # x, and y) are significantly different with the normal control group

Discussion

Plants as a whole and some of their essential parts including oils have been in use for many years, their nutritional and medicinal values make them part of food, pharmaceuticals, alternative medicine and natural therapies [23]. *Ziziphus spina-christi* fruits is an example of such plant with high nutritional and medicinal values [3, 38]. The curative properties of *Ziziphus spina-christi* fruits are due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides and tannins [36]. The phytochemical analysis revealed the presence of alkaloids, flavonoids, cardiac glycosides, tannins, terpenoids, Saponins, Saponins glycosides, carbohydrates, volatile oil, steroid and Phyto steroids in different concentration (Table 1). The values of alkaloids, tannins, steroids, cardiac glycosides and volatile oil were shown to be higher in ethanol extraction than the aqueous extract. While Saponins and terpenoids were more in aqueous than the ethanol extraction. The concentration of carbohydrate and flavonoids were shown to be equal in the two extraction solvents. This variation in secondary metabolite of aqueous and ethanol extract may be attributed to the polarity of the two solvents. This result is consistent with the finding of Truong *et al.,* (2019) [49] on *S. buxifolia* that showed differences due to differences in the polarity of the extracting solvents. Bukar *et al.,* (2015) and Mohammed et al. (2016) [13, 37] and did some studies on different part of the plants including leaves, stem bark, root and seed oil where they showed presence of most of these compounds in different solvents. However, no volatile oil was seen in their extraction which may be attributed to the volatile nature of the compound. The presence of Saponins in *Zizipus* fruit may have glucagon decreasing effect and may enhance glucose utilization and lower blood glucose. It is reported that Saponins stimulates insulin release from pancreas [38]. Saponins are a special class of glycosides which have soapy characteristics [38]. The presence of tannins in high amount suggests that the plant may be a good source of phenyl propane and flavone antioxidants. This may give the plant stimulating effect on the destructed beta cells to further release insulin [38].

Alpha amylase and alpha glucosidase are two important enzymes that are involved in the hydrolysis of alpha-1-4-bonds of alpha-linked polysaccharide to yield several monosaccharides. Both α-amylase and α-glucosidase can increase the postprandial glucose level in diabetic patients by digesting the complex carbohydrate taken. Inhibiting the activity of these two enzymes can control a form of hyperglycaemia called postprandial hyperglycaemia [39]. Acarbose is said to bind to the alpha-bond of polysaccharide and prevent its breakdown into mono and oligosaccharides [40]. An *in vitro* assessment of the two extract to see their hypoglycaemic activity via inhibition of both alpha amylase enzyme and alpha glucosidase enzymes were presented in table 2 and 3. The percentage inhibition of alpha amylase by the ethanol extraction in all concentration tested was found to be similar to the standard agent used. Aqueous extract demonstrated a lower inhibition compared to the acarbose. An IC_{50} of 0.14, 0.19 and 0.58 was recorded for the acarbose, ethanol extract and aqueous extract respectively. Alpha glucosidase inhibition showed an IC_{50} of 0.7mg/ml, 0.9mg/ml and 0.7mg/ml for acarbose, ethanol extracts and aqueous extracts. This result showed that aqueous and ethanol extracts are capable of inhibiting alpha amylase and alpha glucosidase activities effectively. Thus, they may reduce the formation of simple sugar from complex carbohydrate in rats leading to hypoglycaemic affect needed in the treatment of hyperglycaemia. Bitter gourd or balsam pear is one of such important plants used for controlling postprandial hyperglycaemia in diabetes patients. In a study of its protein extract it inhibited the activity of α-amylase and α-glucosidase via competitive inhibition, the inhibition was on par with Acarbose. Bitter gourd significantly reduced peak blood glucose in Streptozotocin-induced diabetic rats, which were orally challenged with starch and sucrose [39]. In another related evaluation of P. pavonica on glucose activity in rats, its extract inhibited α-amylase, but lower than acarbose. P. pavonica have higher inhibitory activity of α-amylase than α-glucosidase [41]. Hyperglycaemia is associated with excessive water lost in urine leading to dehydration and weight loss. Body weight of hyperglycaemic rats treated with aqueous and ethanol extracts of *Ziziphus spina-christi* fruit for 14 days recorded on day 0, day 7 and day 14 showed slight reduction in diabetic control group on day 7 but no significant different compared to other days. However, a significant different is seen in group 4 (150mg/kg) aqueous extracts and the normal control group on day 7 and14 which might be due to the low concentration of the extract.

Diabetes mellitus is a metabolic heterogeneous disorder which is characterized mainly by hyperglycaemia or impaired glucose tolerance as a result of insulin resistance or inactivity [28]. The high level of fasting blood glucose in all the treatment groups at day 0 of the study may be due to possible damage to the pancreatic beta-cells by the effect of alloxan. Alloxan causes hyperglycaemia by partial degradation of islets cells of Langerhans and decreases insulin production [42].The positive control group receive standard

drug, Metformin, which is known to exerts it anti-hyperglycaemic activity by reducing hepatic glucose production and inhibiting glucose absorption and utilization there by improving glucose uptake and utilization through enhancing insulin sensitivity [43]. The noticeable reduction of blood glucose in day 7 and day 14 of the extracts group may be due to the presence of phytochemicals such as Saponins and tannins which are said to reduce blood glucose level by increasing the level of insulin as a result of the stimulating effect of the extracts on the remaining beta cells after the induction of hyperglycaemia with alloxan [5]. This result agreed with the findings of Abdel-Zaher *et al.,* (2005), Avizeh *et al.,* (2010), Hussein *et al.,* (2006), Adzu and Haruna (2007) [44, 45, 46, 38] where different part of plants extracted using many different solvents on different animal models demonstrated various level of anti-hyperglycaemic potential.

A significant elevation of cholesterol and LDL level were seen in the negative control group (8.52±1.64), (6.02±1.21) when compared with the normal control group (3.51±0.05), (2.74±0.07) and this might be as a result of disturbance in the regulation of the activity of the hormone sensitive enzyme; lipase. Alloxan induce destruction of beta islet cells causes deficiency of insulin [42]. This insulin deficiency induces the synthesis of lipase, which triggers lipolysis thereby increasing the concentration of free fatty acids in plasma and liver. High level of fatty acids in the serum results in their conversion to cholesterol and enhances the level of LDL [47]. The increase of insulin level and regression in blood glucose level in our group 3-9 may lead to the stimulation of fatty acid biosynthesis due to the ability of insulin to stimulate lipid synthesizing enzymes such as fatty acid synthase and acetyl-CoA carboxylase and also the incorporation of fatty acids into triglycerides [48]. Several reports showed the potential of plants extracts on hypolipidemic activity (Hussein et al., 2006; Adzu and Haruna 2007; Abubakar et al., 2018) [46,38,48].

Conclusion

The results from the studies showed that *Ziziphus spina-christi* fruit aqueous and ethanol extracts has an anti-hyperglycaemic potential which was not a dose dependent both in the *in Vitro* and *in Vivo* models. The aqueous extract possesses more of the anti-hyperglycaemic activity. *Ziziphus spina-christi* fruit aqueous and ethanol extract also causes a significant reduction in cholesterol level.

Recommendation

Further studies should be done on fractionation of the *Ziziphus spina-christi* fruit to isolate the actual component that has this hypoglycaemic potential.

Competing Interests

The authors declare that they have no competing interest.

Authors' Contributions

KAN performed the experiment, analysis and the initial write-up, HY, HT, UAG and AUK were involved with the conceptual design of the experiment drafting the manuscript, intellectual revision and gave approval for the final manuscript. All authors read and approved the final manuscript.

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