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Antihyperglycemic activity of *Zanthoxylum chalybeum* stem bark extract in diabetic rats

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Abstract

Zanthoxylum chalybeum Engl. (Rutaceae) stem bark is used by communities in Africa and Asia to manage diabetes mellitus. This study determined the anti-hyperglycemic effect of *Z. chalybeum* aqueous stem bark extract in streptozotocin-induced diabetic rat. The plant was obtained from Machakos County, Kenya and a stem bark extract prepared. Rats were divided into experimental (n=5), negative control (n=5) and positive control groups (n=5). Diabetes was induced in each rat in experimental groups by a single dose intraperitoneal injection of Streptozotocin at 45mg/kg body weight. The plant extract was administered orally to the experimental rats at dosages of 10, 100 and 1000mg/kg body weight for 14 days. The negative control group was left untreated while the positive control group was treated orally with glibenclamide (10mg/kg body weight). The effect of the extract on blood glucose, body weight, food and water intake and oral glucose tolerance were determined in all rats in the experimental and control groups. The aqueous stem bark extract exhibited significant antidiabetic activity compared to the untreated diabetic controls ($P < 0.05$). Additionally, there was no significant difference between the extract fed diabetic rats and the normal controls. Furthermore, extract treated diabetic animals recorded a comparatively decreased weight loss which was dose dependent. These results suggest that the aqueous stem bark extract of *Z. chalybeum* possesses significant antihyperglycemic activity. This study thus corroborates the traditional use of the plant for the management of diabetes. However, further studies are required to identify the active ingredient(s) and determine the mode of action.

Keywords: Diabetes mellitus, *Zanthoxylum chalybeum*, Streptozotocin.

Introduction

Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both^[1]. The twenty first century has seen a rise in diabetes and its complications in Africa and it is estimated that from 2007 to 2025, the prevalence of diabetes in the continent will increase from 3.1% to 3.5% which is an increase from 10.4 to 18.7 million people^[2-4]. The most affected are between the ages of 35 and 64 years, impacting people in their most economically productive years^[3]. According to the World Health Organization (WHO), the global prevalence was estimated at 2.8% in 2000, with projections of 4.8% in 2030. The total number of persons affected will rise from 171 million in 2000 to 366 million in 2030 if no action is taken^[5]. However, recent data from the International Diabetes Federation (IDF) revealed that this number had already been reached in 2011. The IDF expects an even higher number of 552 million affected persons in 2030^[6,7].

The only available pharmacological intervention for Type 1 diabetes is insulin^[8]. Conversely, therapeutic strategies for type 2 diabetes involve insulin and four main classes of oral antidiabetic agents that stimulate pancreatic insulin secretion (sulphonylureas and rapid-acting secretagogues/insulinotropics like glibenclamide, glipizide, rapaglinide), reduce hepatic glucose production (biguanides like metformin), delay digestion and absorption of intestinal carbohydrate (α -glucosidase inhibitors like acarbose) or improve insulin action (thiazolidinediones like pioglitazone, rosiglitazone)^[9, 10]. Each of above agents suffers from generally inadequate efficacy and a number of serious adverse effects^[11]. With regard to these limitations and the high cost of medication, especially the high cost of insulin, there is needed to explore other treatment and management strategies^[12-15]. This high cost of anti-diabetic drugs has seen these populations turn to traditional medicines and natural products to treat diabetes^[16, 17]. The available literature shows that there are more than 800 plant species showing antidiabetic activity^[18-21]. Plant drugs^[22] and herbal formulations^[23, 24] are frequently considered to be less toxic and have fewer side effects compared to synthetic ones. Based on the WHO recommendations, hypoglycemic agents of plant origin used in traditional medicine are important^[25]. The antihyperglycemic effects of these plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin

output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Although some of these plants have great reputation in traditional medicine, for a majority, the pharmacology and efficacy has not been studied using scientific methods^[16, 17]. There is, therefore, a need to generate efficacy and safety data for these herbal remedies. This information will inform the development of low cost, anti-diabetic drugs with fewer side effects that will find greater affordability with diabetes patients, particularly those in Africa.

The study investigated the effect of *Z. chalybeum* stem bark extract on Streptozotocin-induced type 1 diabetes in Wistar rats. *Z. chalybeum* Engl., family: Rutaceae, is a deciduous spiny shrub or tree up to 12 m, crown rounded but open, bark pale grey; smooth dark with scales and prickles. *Z. chalybeum* is a tree of medium to low altitudes in dry woodland or grassland, often on termite mounds. It is native to Burundi, Democratic Republic of Congo, Ethiopia, Kenya, Lesotho, Malawi, Mozambique, Namibia, Rwanda, Somalia, South Africa, Swaziland, Tanzania, Uganda, Zambia and Zimbabwe. Decoctions of the stem bark are used to treat diabetes mellitus related symptoms in Kenya, Tanzania and Asia^[26]. The plant is also used for treatment of malaria, sickle cell disease, measles, skin infections and coughs^[27]. However, the anti-diabetic potential of *Z. chalybeum* has not been determined experimentally.

Materials and methods

Plant collection and identification

The plant was identified at the University of Nairobi herbarium, in the Department of Botany, where a voucher specimen was also deposited (NK2011/1).

Preparation of plant extract

Harvesting was done on a dry day and plants harvested manually and washed thoroughly in running water. Cleaned plant materials were dried in the shade for one week, weighed and ground into powder using an electric mill. For aqueous extraction, 100 g of powder was extracted in 1 litre distilled water for 25 minutes using a hot plate. The decoction was filtered and centrifuged at 5000rpm for 10 min and the supernatant collected. The supernatant was freeze dried (Christ Beta 336, Martin Christ Freeze Dryers, Osterode, Germany) and stored at 4°C awaiting phytochemical screening and efficacy and toxicity evaluation.

Phytochemical screening

The extract was analyzed for principle chemical groups using standard procedures^[28].

Determination of antidiabetic efficacy

(i) Ethical Approval

The efficacy study was conducted at the rodent facility, Institute of Primate Research (IPR), Karen, Kenya. Approval for the study was obtained from the Institutional Review Committee, Institute of Primate Research.

(ii) Study design and treatment regimen

Thirty five, 10 week old, male Wistar rats were purchased from the University of Nairobi. The rats were housed in plastic cages with stainless steel covers in groups of five. They were acclimatized for 3 weeks at room temperature (20–25°C) under a 12/12 h light/dark cycle. All rats received standard rat chow (Ungafeeds™) and distilled water *ad libitum* during acclimatization and also throughout the experimental period. The rats were randomized using a table of random numbers and assigned to seven experimental groups in sets of five animals per group as follows; Group 1- Normal controls, Group 2-

Normal rats treated with 1000mg/kg bwt of extract, Group 3- Untreated diabetic, Group 4- Diabetic treated with 10mg/kg bwt of extract, Group 5- Diabetic treated with 100mg/kg bwt of extract, Group 6- Diabetic treated with 1000mg/kg bwt of extract and Group 7- Diabetic treated with 10mg/kg bwt Glibenclamide.

Z. chalybeum extract, doses 10mg/kg bwt, 100mg/kg bwt and 1000mg/kg bwt were reconstituted in distilled water and administered daily at 0900 hrs via stomach tube. This was done for fourteen days. Rats in groups 1 and 2 received distilled water, while rats in group 7 received Glibenclamide (10mg/kg bwt) at a concentration of 200µg/ml for fourteen days. Rats were weighed on a weekly basis using a weighing balance (Sartorius, GMBH GOTTINGEN, Type L2200P Germany). Weights were recorded in grams.

(iii) Induction of diabetes

Rats in groups 3-7 were fasted overnight and injected intraperitoneally with streptozotocin (STZ, Sigma Aldrich, USA) at a dose of 45mg/kg body weight to induce diabetes. The streptozotocin powder was reconstituted in sterile 0.9% sodium chloride, at a concentration of 7.5mg/ml. A drop of blood was collected from the tail vein on day 3, 7 and 12 after injection and glucose levels determined using a glucometer (Softstyle®, Chemlabs, Kenya) to confirm stable hyperglycemia. Rats with glucose levels greater than 14mmol/L were considered diabetic and used for the efficacy study.

(iv) Determination of plasma glucose

Blood was obtained by a prick on the lateral tail vein and blood glucose determined using a glucometer (SoftStyle® Chemlabs, Kenya). Results were expressed in mmol/L.

(v) Oral glucose tolerance test (OGTT)

The OGTT was performed to determine the short-term effect of the extract on glucose control at the end of extract administration (day 14). Rats in all groups were fasted overnight and then administered 2 g glucose kg⁻¹ body weight orally. A drop of blood was withdrawn from the tail vein before (0 min) and 30, 60, 90 and 120 min after administration of glucose solution. Blood glucose was measured using a glucometer and results expressed in mmol/L.

(vi) Biochemical parameters

Blood for biochemical evaluation was collected via cardiac puncture at euthanasia. Samples of 5mls were collected into serum tubes. The blood was allowed to clot and left for 10 minutes at room temperature for serum to form. Serum was separated by centrifugation at 3000rpm for 10 minutes and stored at -20°C until required for analysis. Liver enzymes; glutamic oxaloacetic transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase, creatinine and blood urea nitrogen were determined using commercial kits (Humalyzer 2000, Human Diagnostics®, Germany) according to the manufacturers' protocol.

(vii) Statistical analysis

Data was expressed as mean ± standard error of mean (SEM). Two-way analysis of variance (ANOVA, GraphPad Prism 5) was used to determine differences in means between groups. Values were considered significantly different at the level of P<0.05.

Results

(i) Phytochemical analysis

The aqueous stem bark extract of *Z. chalybeum* was found to contain several secondary metabolites as shown in Table 1.

Table 1: Secondary metabolites from *Z. chalybeum* crude aqueous stem bark extract

Compound	Present(+)/Absent(-)
Alkaloids	+
Flavonoids	+
Steroids	-
Terpenoids	+
Saponins	+
Tannins	+
Phenols	+
Glycosides	+

(ii) General characteristics of the animals

Three days after administration of streptozotocin (45mg/kg bwt IP) the rats appeared lethargic and displayed restricted movement, however their demeanor improved in the weeks during and after treatment. Rats classified as diabetic had hyperglycemia ≥ 14 mmol/L. Diabetic rats also displayed polyuria, polydipsia and weight loss three days after induction. Normal control rats were active throughout the study period.

(iii) Effect of *Z. chalybeum* stem bark extract on fasting blood glucose levels

At baseline, before induction of diabetes, blood glucose levels mean 3.65mmol/L \pm 0.145. Three days after administration of streptozotocin the rats exhibited hyperglycemia (mean 19.33mmol/L \pm 1.349). Administration of the extract of *Z. chalybeum* stem bark caused significant decreases in fasting blood glucose in diabetic rats at the three dose levels of 10mg, 100mg and 1000mg per kg body weight compared to untreated diabetic rats (mean 13.08mmol/L \pm 1.65). There was no significant difference between the three doses of the extract and the rats given Glibenclamide (10mg/kg bwt) ($P > 0.05$). Similarly, there was no significant difference between the extract fed diabetic rats and the normal controls or the normal rats that were given 1000mg/kg bwt of the extract ($P > 0.05$). However, blood glucose levels of the rats given 10mg/kg bwt of the extract were significantly higher ($P < 0.05$) compared to both the normal controls and the normal rats given *Z. chalybeum* at 1000mg/kg body weight. There was no significant difference ($P > 0.05$) in blood glucose levels at different doses of the extract for the diabetic rats. The blood glucose levels of normal rats were not changed. Untreated diabetic rat blood glucose levels were significantly ($P < 0.05$) and continuously elevated throughout the experimental period (Figure 1).

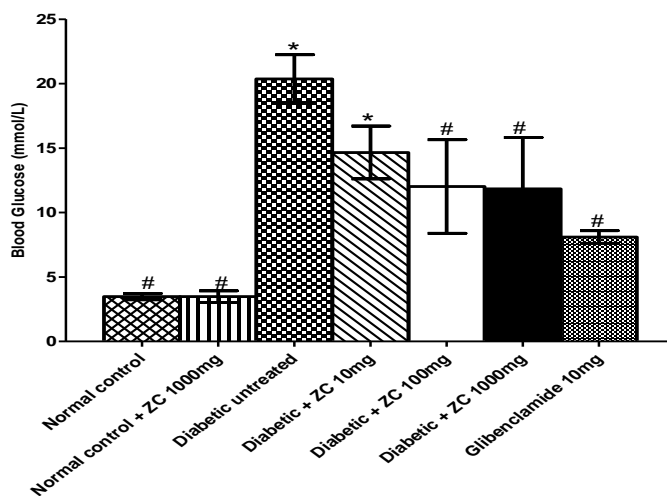


Figure 1: Effect of *Z. chalybeum* stem bark extract on fasting blood glucose levels in normal and diabetic rats following treatment for two weeks

Normal control rats, (diagonal squares); Normal control + ZC 1000mg (non-diabetic rats treated with 1000mg if extract (black vertical lines); Diabetic untreated(diabetic control rats)(black and white squares); Diabetic + ZC 10mg (diabetic rats given 10mg of the extract for two weeks)(black diagonal lines); Diabetic + ZC 100mg (diabetic rats given 100mg of the extract for two weeks) (white bar); Diabetic + ZC 1000mg (diabetic rats given 1000mg of the extract for two weeks)(black bar); Glibenclamide 10mg (diabetic rats given 10mg glibenclamide for two weeks)(small black and white boxes); Diabetic control rats showed a marked increase of blood glucose level compared with non-diabetic control rats. n=5 rats. Values are shown as means \pm SEM. * $P < 0.05$ (compared to normal control rats); # $P < 0.05$; (compared to diabetic control rats).

(iv) Effect of *Z. chalybeum* stem bark extract on oral glucose tolerance

Z. chalybeum administration 30 minutes prior to glucose loading resulted in a gradual reduction in glucose levels, but this was not statistically significant compared to diabetic controls ($P > 0.05$). Results of the OGTT are shown in Figure 2. Response to Glibenclamide was not significant compared to untreated diabetic controls. The results were comparable to those of the three dose levels of the extract. The gradual decrease in blood glucose levels was not dose dependent. There was no significant difference in the normal controls compared to extract treated normal rats ($P > 0.05$). Data not presented.

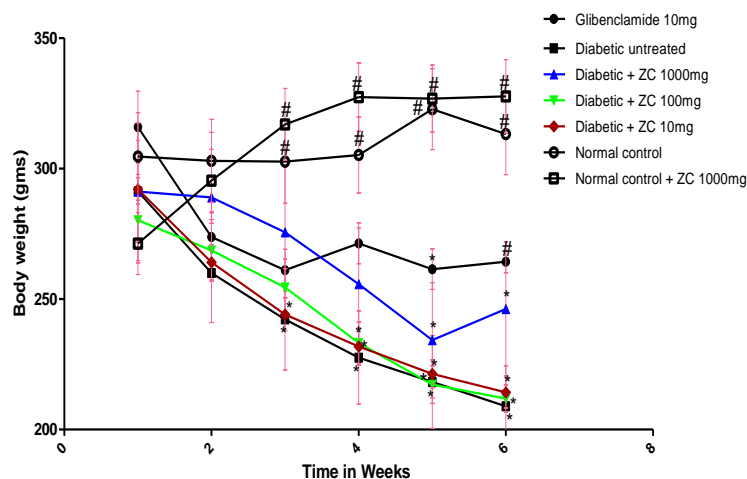


Figure 2: Effect of *Z. chalybeum* stem bark extract on body weight in normal and diabetic rats

Normal control rats, (diagonal squares); Normal control + ZC 1000mg (non-diabetic rats treated with 1000mg if extract (black vertical lines); Diabetic untreated(diabetic control rats)(black and white squares); Diabetic + ZC 10mg (diabetic rats given 10mg of the extract for two weeks)(black diagonal lines); Diabetic + ZC 100mg (diabetic rats given 100mg of the extract for two weeks) (white bar); Diabetic + ZC 1000mg (diabetic rats given 1000mg of the extract for two weeks)(black bar); Glibenclamide 10mg (diabetic rats given 10mg glibenclamide for two weeks)(small black and white boxes); Diabetic control rats showed a marked increase of blood glucose level compared with non-diabetic control rats. n=5 rats. Values are shown as means \pm SEM. * $P < 0.05$ (compared to normal control rats); # $P < 0.05$; (compared to diabetic control rats).

(v) Effect of *Z. chalybeum* stem bark extract on body weight

There was a significant loss ($P < 0.05$) in the body weight of treated and untreated diabetic rats 1 week after induction of diabetes. This decrease was greatest in the untreated diabetic group (36.44%). *Z. chalybeum* extract treated diabetic animals experienced a comparatively decreased weight loss which was dose dependent at 15.45%, 24.38% and 26.63% for 1000mg/kg, 100mg/kg and 10mg/kg treated animals respectively, compared to the untreated diabetic rats at 36.44% four weeks after the start of treatment (Figure 2). The weight of the Glibenclamide treated rats was not significantly different compared to the normal control rats 4 weeks post treatment, although this difference was significant when compared with the normal rats

that were given 1000mg/kg bwt of the extract. The weights of the extract treated diabetic rats, at all three doses were not significantly different compared to the Glibenclamide treated rats ($P>0.05$). Extract treated normal rats had a higher weight gain (20.79%) compared to the normal controls (2.82%).

(vi) Effect of *Z. chalybeum* stem bark extract on food intake

Diabetic rats consumed significantly higher amounts of food compared to non-diabetic rats ($P<0.05$). Administration of extract or Glibenclamide did not reduce these levels back to baseline or normal control levels. There was also no significant difference between the three extract groups. Extract treated normal rats (Normal + ZC 1000mg) had a higher food intake compared to normal controls but this difference was not statistically significant ($P<0.05$).

(vii) Effect of *Z. chalybeum* stem bark extract on water intake

Significant differences ($P<0.05$) in water consumption between diabetic and non-diabetic rats were recorded 72 hours post induction and throughout the 28 days thereafter. Extract administration did not significantly reduce the levels. Water intake in extract treated rats was not significantly different compared to normal controls ($P>0.05$). Diabetic rats treated with Glibenclamide had significantly elevated water intake throughout the study period ($P<0.05$).

(viii) Effect of *Z. chalybeum* stem bark extract on biochemical parameters (GOT, GPT and ALP)

All diabetic rats had significantly elevated alkaline phosphatase (ALP) levels compared to the normal controls ($P<0.05$). Diabetic rats treated with *Z. chalybeum* had significantly lower ALP levels compared to untreated diabetic rats ($P<0.05$). The levels of ALP were inversely proportional to dose of extract used with the difference between the doses being statistically significant ($P<0.05$). In contrast extract treated normal rats had significantly lower levels compared to normal controls ($P<0.05$).

Diabetic rats had elevated levels of serum glutamic oxaloacetic transaminase (GOT) and glutamate pyruvate transaminase (GPT) compared to normal controls, but these levels were not significant ($P>0.05$). Treatment did not have a significant effect on these levels in diabetic rats. However, extract treated normal rats had lower GOT and GPT levels compared to the normal controls although this difference was not significant (Figure 3).

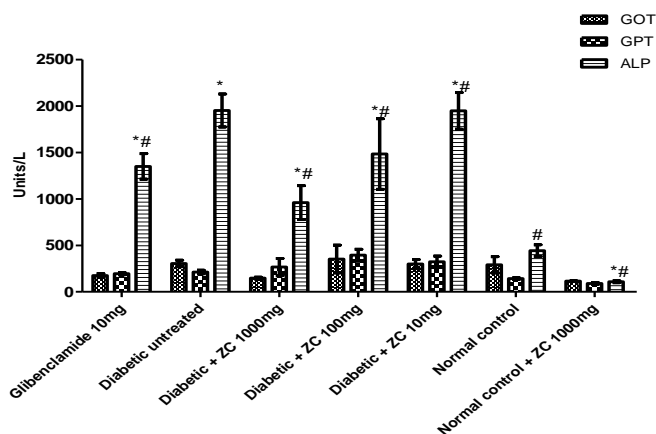


Figure 3: Effect of *Z. chalybeum* stem bark extract on serum GOT, GPT and ALP

Extract treated diabetic rats had alkaline phosphatase levels that were significantly higher compared to the normal controls and significantly lower compared to the diabetic controls. The decrease in ALP levels in extract treated diabetic rats was dose dependent. Diabetic rats had elevated levels of serum GOT and GPT compared to normal controls, but these levels were not significant. Values are shown as means \pm SEM. * $P<0.05$ (compared to normal control rats); # $P<0.05$ (compared to diabetic control).

(ix) Effect of *Z. chalybeum* stem bark extract on serum creatinine and bilirubin

Extract treated normal rats had lower creatinine levels, compared to normal controls, although the difference was not statistically significant. Additionally, extract treated normal rats had lower bilirubin levels compared to normal controls and diabetic rats, except for the diabetic rats that were treated with Glibenclamide. These differences were however not statistically significant. Creatinine and bilirubin levels were not significantly different in diabetic rats within the various treatment groups ($P>0.05$) (Figure 4).

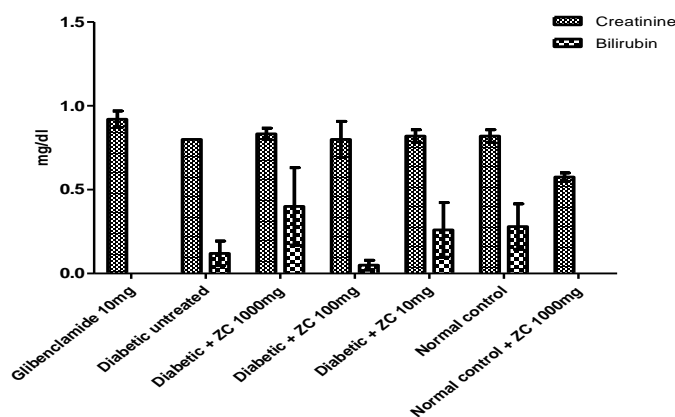


Figure 4: Effect of *Z. chalybeum* stem bark extract on serum creatinine and bilirubin levels

Creatinine (small black and white squares) and bilirubin (larger black and white squares) levels were not significantly different in diabetic rats within the various treatment groups ($P>0.05$)

(x) Effect of *Z. chalybeum* stem bark extract on serum urea

Extract treated normal rats had lower serum urea levels compared to normal controls but these levels were not significantly different. Serum urea levels were not significantly different between the different diabetic treatment groups ($P>0.05$).

(xi) Effect of *Z. chalybeum* stem bark extract on serum total protein and albumin

There was no significant difference between levels of total protein and albumin in serum across different extract treatments and diabetic and normal control groups $P>0.05$.

Discussion

In the present study, *Z. chalybeum* extract was shown to have significant antidiabetic effects in streptozotocin- induced diabetic rats compared to untreated diabetic controls. Other species in the genus *Zanthoxylum* have also been studied experimentally, with significant antidiabetic activity reported. For example, various parts of *Z. zanthoxyloides* including the roots, bark and leaves have been used for medicinal purposes, including treatment of diabetes mellitus. Significantly lower blood glucose was observed in the treated animals in comparison to non-treated groups [29]. Other species in the genus that are used traditionally to treat diabetes are *Z. armatum* in Nepal, and *Z. nitidum* in India [30, 31]. The beneficial effect of *Z. chalybeum* treatment in diabetic rats was likely due to improved insulin release and glucose uptake in remnant β -cells [32]. Increased insulin secretion following *Z. chalybeum* could also increase conversion of blood glucose into glycogen by enhancing the glycolytic and glycogenic processes with concomitant decrease in glycogenolysis and gluconeogenesis [33].

The antihyperglycemic activity of *Z. chalybeum* observed in this study may be attributed to the secondary metabolites identified through phytochemical screening. These include alkaloids, saponins, glycosides, tannins, terpenoids and phenols. In general, there is very little biological knowledge on the specific modes of action in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids and flavonoids that are frequently implicated as having antidiabetic effects [34]. These phytochemicals possess wide therapeutic benefits and studies have demonstrated anti-diabetic, anti-oxidant, and anti-inflammatory activities with these compounds [35-37]. Medicinal properties of the genus *Zanthoxylum* have been attributed to the presence of secondary metabolites like alkaloids, sterols, flavonoids, aliphatic and aromatic amides, lignins, coumarins, sterols, carbohydrate residues [29]. Thus, combination of these compounds in *Z. chalybeum* may exert synergistic anti-diabetic effects in the diabetic rats in our study.

There was no significant difference in glucose tolerance between extract treated normal rats and untreated normal controls ($P < 0.05$). This may suggest that the antihyperglycaemic properties of the extract were not solely dependent on insulin action or secretion [24]. The different constituents of antidiabetic plants could have different sites of action in the body [38]. Other possible mechanisms of antidiabetic plants are adrenomimeticism, pancreatic β -cell potassium channel blocking, cAMP (2nd messenger) stimulation [39], inhibition in renal glucose reabsorption [40], inhibition of insulin degradative processes and reduction in insulin resistance [41], providing certain necessary elements like calcium, zinc, magnesium, manganese and copper for the β -cells [42], regenerating and/or repairing pancreatic β -cells [42], increasing the size and number of cells in the islets of Langerhans [42], stimulation of insulin secretion [43], stimulation and of glycogenesis and hepatic glycolysis [44], protective effect on the destruction of the β -cells [45], improvement in digestion along with reduction in blood sugar and urea [46], prevention of pathological conversion of starch to glucose [47], inhibition of β -galactosidase and α -glucosidase [48], cortisol lowering activities [49], inhibition of alpha-amylase [50] and preventing oxidative stress that is possibly involved in pancreatic β -cell dysfunction found in diabetes [51].

There was significant loss in body weight of diabetic rats compared to normal rats, a symptom synonymous with diabetes mellitus. The loss of body weight associated with STZ-induced diabetes could be due to dehydration and catabolism of fats or breakdown of tissue proteins, with consequent wasting of muscle [52]. Normal body weight gain is an indicator of efficient glucose homeostasis; but in diabetics, glucose is not available therefore the cells use alternatively proteins for energy; consequently due to excessive breakdown of tissue protein a loss in body weight occurs. Treatment with *Z. chalybeum* resulted in a reduction in body weight loss compared to untreated diabetic rats ($P < 0.05$). This can be attributed to the improvement in glycemic control [53]. Similar effect on body weight gain was previously reported with other plants, well known for their anti-diabetic activity [15, 54, 65]. This is also in agreement with the finding that normal rats that were given the extract had a higher weight gain compared to the normal controls. This could in part be explained also by the fact that extract treated normal rats had a higher food intake compared to normal controls.

Diabetic rats had a significantly higher food intake compared to normal controls. This was not remedied by treatment either with extract or Glibenclamide. Polyphagia is a classic symptom of diabetes mellitus resulting from abnormalities in carbohydrate metabolism. Glucose entry into cells is dependent on insulin and therefore without insulin the cells cannot take up glucose from the blood stream and thus effectively start to starve [56]. In diabetes due to insulin resistance or absence of insulin, glucose cannot move into the satiety center thus the arteriovenous difference remains low and the feeding center is chronically active [56-58].

The diabetic rats significantly increased water intake compared to the normal controls. Treatment with extract or Glibenclamide did not reduce these levels back to normal control levels. The increased water intake is reported to be a result of prolonged hyperglycemia excessive leaking osmotically into the renal tubules and excretion along with the excessive glucose. The result is excessive intake of water to prevent dehydration and the consequent excessive passing of urine [56-58]. Ahmed and Urooj [53] also reported significantly increased water intake in *F. glomerata* extract treated diabetic rats compared to normal controls.

Diabetes mellitus is associated with high levels of circulatory cholesterol and other lipids and these accounts for the arteriosclerosis and severe coronary heart disease which leads to increase in levels of transaminases, marker enzymes important in heart and liver damage. Studies have reported that the liver is necrotized in diabetic patients [59, 60]. Therefore, the increment of the activities of GOT, GPT and alkaline phosphatase in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream, as a result of the hepatotoxic effect of STZ [61]. Other studies have also shown that STZ results in a significant increase in serum levels of GOT, GPT and ALP 1, 3 and 6 hours after treatment [27]. The study established that the level of increase of these enzymes was dependent on the dose of STZ administered with higher STZ doses resulting in a greater increase in enzyme levels. Ragbetli and Ceylan [27] also report that studies are needed to verify and clarify the relationship between different doses streptozotocin induced diabetes and biochemical parameters. The levels of GOT, GPT and ALP have been reported to be increased in alloxan-induced diabetic rats. In this study *Z. chalybeum* treated diabetic rats had significantly reduced levels of ALP suggesting improved renal and hepatic functions. This observation is consistent with earlier reports on hepatoprotective potentials of leaf extracts of *V. amygdalina* in mice [62]. In another study, treatment of diabetic rats with either onion or garlic was observed to cause a reduction in the activity of these enzymes in plasma compared to the untreated diabetic group [61]. Rawiet *al* [63] observed a highly significant decrease in activity of serum and liver ALP after four weeks of continuous treatment of diabetic rats with either glibenclamide, *Mangifera indica*, *Psidium guava* or the mixture of both *M. indica*, *P. guava* as compared with diabetic controls. The slightly elevated levels of GOT and GPT in diabetic rats compared to normal controls may be the result of the greater need for gluconeogenic substrate in the diabetic rats. The elevation of both enzymes may also reflect damage of the hepatic cells [63]. Treatment with the extracts and Glibenclamide in diabetic rats did not result in a significant decrease in the levels of these enzymes. The effect of the extract in lowering GOT and GPT levels was however observed in extract treated normal rats which were lower compared to normal controls. This decrease in serum GOT and GPT may be attributed to the presence of tannins and flavonoids in the plant extract [57]. The significant decrease in serum ALP activity indicates the protective effect of the extracts on the liver and improvement in liver function [63]. Serum levels of urea and creatinine in treated diabetic rats were not significantly different from those of normal rats. Extract treated normal rats however had lower levels of serum urea and creatinine compared to normal controls suggesting a possible improvement on renal function in extract treated normal rats. Elevated serum levels of urea and creatinine are significant markers of renal dysfunction. These results thus indicate that diabetic rats did not suffer any renal dysfunction [61].

Conclusion

Z. chalybeum aqueous stem bark extract possesses antihyperglycemic activity. The extract, however did not have any hypoglycemic effects in normal rats. The differential reduction in blood glucose and weight loss observed suggests dose related activity. Further research is required to explore different dosages and the possible mechanism of action of *Z. chalybeum* in the treatment of diabetes mellitus.

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Conflict of interest

None declared.

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