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Protective Effects of *Zanthoxylum chalybeum* in Diabetes-induced Myocardial Dysfunction in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author MSA has made significant contribution throughout the study starting from collection of Z. chalybeum roots to the completion of the study. Author AMN participated in drafting the manuscript and author PCV participated in revising it critically for important intellectual content. Both authors AMN and PCV obtained funding for the study and were involved in the study supervision. All authors read and approved the final manuscript.

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ABSTRACT

Background: Control of glycaemia and hyperlipidemia are important objectives in preventing diabetes induced cardiac dysfunction.
Objective: The aim of the study was to examine the effects of *Zanthoxylum chalybeum* in myocardial dysfunctions associated with type-1 diabetes.
Place and Duration of the Study: The histopathological analysis was done in the pathology Laboratory College of Veterinary Medicine and Biosecurity, Makerere University while the biochemical studies were carried out in biochemistry laboratory Natural Chemotherapeutics Research Institute, Ministry of Health, Kampala, Uganda. The study was done between January and December, 2014.
Materials and Methods: Diabetes was induced by single intravenous injection of alloxan monohydrate (150 mg/kg, i.p.). *Zanthoxylum chalybeum* root bark aqueous extract was administered daily at two doses (200 and 400 mg/kg p.o.) for 4 weeks. Blood samples were collected before treatment, 14 and 28 days after repeated treatment and analyzed for various

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biochemical parameters. Thin blocks of cardiac tissue were taken at the end of the experiment for histology evaluation.
Results: Injection of alloxan produced marked hyperglycemia and altered several biochemical parameters. Treatment with *Zanthoxylum chalybeum* significantly lowered ($p<0.05$) fasting glucose level in a dose-dependent manner. There was no significant difference in total cholesterol levels between the control groups and the *Zanthoxylum chalybeum* treated groups. *Zanthoxylum chalybeum* root bark extract (400 mg/Kg body weight) significantly increased ($p<0.05$) HDL-Cholesterol after 28 days of repeated treatment, which was comparable to the normal control. *Zanthoxylum chalybeum* root bark extract (400 mg/Kg body weight) also significantly decreased ($p<0.05$) the levels of triglycerides after 28 days of repeated treatment, which was comparable to the normal control. There was no significant difference in LDL-Cholesterol levels between the control groups and the *Zanthoxylum chalybeum* treated groups. In addition, histopathological changes also revealed the protective nature of the aqueous roots extract of *Z. chalybeum* against alloxan induced necrotic damage of cardiac tissues.
Conclusion: The results of this study suggest that *Zanthoxylum chalybeum* is beneficial for the prevention of myocardial damage associated with type-1 diabetes.

Keywords: Zanthoxylum chalybeum; dyslipidemia; cardiomyopathy; antihyperglycemic and antihyperlipidemic activity.

ABBREVIATIONS

HDL-Cholesterol: High Density Lipoprotein Cholesterol; LDL-Cholesterol: Low Density Lipoprotein Cholesterol; CVD: Cardiovascular disease, TC: Total Cholesterol; TG: Triglycerides

1. INTRODUCTION

The prevalence of diabetes mellitus is growing rapidly. It is estimated that the number of adults with diabetes in the world will increase from 135 million in 1995 to 300 million by 2025 [1]. Patients with diabetes mellitus are at an increased risk for cardiovascular diseases. Cardiovascular disease (CVD) is the cause of death in 65% of patients with diabetes [2]. There is substantial evidence that dyslipidemia is an important modifiable risk factor for CVD in these patients. Diabetic patients are at an increased risk of congestive heart failure. Several factors probably underlie diabetic cardiomyopathy: severe coronary atherosclerosis, prolonged hypertension, chronic hyperglycemia, microvascular disease, glycosylation of myocardial proteins and autonomic neuropathy. Improved glycemic control, better control of hypertension, and prevention of atherosclerosis with cholesterol-lowering therapy may prevent or mitigate diabetic cardiomyopathy [3]. Hyperlipidemia and hypercholesterolemia are the key risk factors for Cardiovascular Disorders [4]. The cluster of lipid abnormalities associated with diabetes is defined by a high concentration of Triglycerides, low concentration of HDL cholesterol and small dense LDL [5]. Plasma LDL cholesterol levels are generally normal. The HDL particles induce the removal of cholesterol from cells, including those in atherosclerotic plaques, and carry them to the liver. Several mechanisms by which HDL confer protection from atherosclerosis have been suggested and these include; reversal of cholesterol transport, anti-inflammatory and antioxidant properties, inhibition of oxidation of LDL cholesterol, expression of cellular adhesion molecules and monocyte recruitment, reduction of the risk of thrombosis by inhibition of platelet activation and aggregation [5].

The frequency of cardiovascular disease is two to five times higher in diabetic patients as compared to non-diabetics [6]. The mechanisms underlying the development of cardiovascular dysfunction are unclear and appear to have a complex etiology [4]. Various hypotheses such as low insulin levels, impaired carbohydrate and lipid metabolism, formation of advanced glycosylated end products, and oxidative stress have been suggested to explain the relationship between diabetes and the occurrence of cardiovascular disease [7]. The occurrence of hyperglycemia and hyperlipidemia has been extensively documented, and is implicated in the pathogenesis of various cardiovascular complications including cardiomyopathy [8]. Thus, among the various therapeutic strategies, antihyperglycemic and antihyperlipidemic activity can be useful in the prevention of

cardiomyopathy in alloxan or streptozotocin induced diabetes in rats. Although there is treatment for diabetes mellitus, the entire drugs available generally have inadequate efficacy, a number of serious adverse effects and high costs of treatment. Thus there is a need for a variety of newer therapeutic agents for the management/treatment of diabetes [9].

Due to the side effects associated with synthetic anti-diabetic drugs, clinical importance of herbal drugs has received considerable attention in recent years. Several medicinal products of herbal origin have been reported to have hypolipidemic and hypocholesteremic properties [10]. *Zanthoxylum chalybeum* is an important medicinal plant that is widely used in traditional medicine for treatment of several diseases which include; malaria, diabetes, fevers, wounds and sickle cell disease [11,12]. Root or stem bark decoctions are widely taken to treat diabetes and root bark decoctions are considered to be stronger than stem bark decoctions [13]. Studies have shown *Z. chalybeum* to be safe for human use [14]. Although *Zanthoxylum chalybeum* is reported to be used in treatment and management of diabetes, no scientific study has been done to evaluate the cardioprotective effect of the aqueous roots bark extract.

Thus, this study investigated the antihyperglycemic and antihyperlipidemic effects of *Zanthoxylum chalybeum* on diabetes induced myocardial dysfunction in alloxan-induced type-1 diabetic rats. In this study, oral administration of *Zanthoxylum chalybeum* at different doses for 4 weeks was evaluated for effects of *Z. chalybeum* in myocardial dysfunctions associated with type-1 diabetes.

2. MATERIALS AND METHODS

2.1 Sample

Zanthoxylum chalybeum root bark was collected from Usuk a local area in Katakwi district, Eastern Uganda where it is abundant and locally called Eusuk (in Ateso local language). The plant was authenticated by a plant taxonomist at Natural Chemotherapeutics Research Institute-Ministry of Health. After collection a voucher specimen was deposited at the National herbarium, Makerere University, Kampala. The root was washed, debarked and dried in an air oven at 50℃ for 48 h. The bark was then pulverized in to powder using a grinder. The powder was extracted by boiling in water for 30 minutes and allowing cooling to room temperature. The extract was then filtered, concentrated using an air oven at 50℃ to obtain the crude extract. The crude extract was reconstituted in distilled water and used to evaluate the cardioprotective property in rats.

2.2 Experimental Animals

Twenty four male and twenty four female Wistar rats (aged 12–14 weeks, weighing 180–240 g) were obtained from the animal facility at the College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB), Makerere University, Kampala and used for evaluation of the effects of Z. *chalybeum* in myocardial dysfunctions associated with type-1 diabetes.

2.3 Induction of Diabetes in Rats

The animals were allowed to acclimatize for 2 weeks in the animal facility at the Natural Chemotherapeutics research Institute, Ministry of Health, Kampala, Uganda. Diabetes mellitus was induced in the rats by intraperitoneal injection of freshly prepared solution of alloxan monohydrate in distilled water at a dose of 150 mg kg^{-1} body weight. Since alloxan caused fatal hypoglycemia due to massive insulin release by the pancreas, six hours after induction of diabetes, the rats were in addition orally (gavage) given 20% glucose solution (5–10 ml). They were further kept for 24 h on 5% glucose solution to prevent hypoglycemia. Rats which developed Diabetes mellitus observed by glycosuria and hyperglycemia (i.e., blood glucose concentration >250 mg dl^{-1}) were selected for the subsequent experimental tests. This study was approved by Graduate Research Committee, College of Natural Sciences, Makerere University, Kampala.

2.4 Experimental Design

Animals were kept under standard laboratory conditions (25±3℃, 12-h light/dark cycle), fed on pelleted food and clean tap water *ad libitum* for 28 days of experimental period. The procedures were of animal experiments in accordance with the Organization for Economic Cooperation and Development (OECD) guidelines for testing of chemicals. All the procedures were in accordance with ICLAS Ethical Guideline for Researchers. Experimental study was conducted on four groups of animals each with twelve rats. Six male and six female Wistar albino rats were randomly allocated to each of the 4 groups. The groups were treated as follows: Group I: consisted of diabetic rats orally given water, food

and metformin by gavage (10mg/Kg body weight) once daily for 28 days. Group II: consisted of diabetic rats orally given water, food and *Z. chalybeum* extract by gavage (200 mg/Kg body weight) once daily for 28 days. Group III: consisted of diabetic rats orally given water, food and *Z. chalybeum* extract by gavage (400 mg/Kg body weight) once daily for 28 days. Group IV: consisted of normal rats, orally given water and food with no treatment administered i.e., the normal control group.

2.5 Biochemical Measurements Following Treatment of Alloxan- induced Diabetic Rats with *Zanthoxylum chalybeum* Root Bark Extract

Two male and two female Wistar albino rats were randomly selected from each of the 4 groups and blood samples collected by cardiac puncture at day, 0 i.e., before start of treatment; days, 14 and 28 after consecutive treatment. The blood obtained by cardiac puncture was used for estimation of the following biochemical parameters; glucose, total cholesterol, HDL-Cholesterol, LDL-Cholesterol and triglycerides. All the biochemical assays were carried out spectophotometrically in the Clinical Chemistry department, Mulago National Referral Hospital, Kampala.

2.6 Histopathological Analysis of Rat Tissues Following Treatment

The animals were anesthetized using diethyl ether and the heart removed and fixed in a 10% solution of formaldehyde. The tissues were dehydrated because the reagents used at a later stage were not miscible with water. Varying concentration of isopropyl alcohol i.e. 70%, 80%, 90%, 96% and 100% were used. The minimum time for dehydration between two concentrations was 1 h.

The fixed tissues were then cleared in xylene and embedded in paraffin wax. The sections (5 µm) from each of the tissues were examined using a microscope (x40) after staining with hematoxylin and eosin dye.

2.7 Statistical Data Analysis

The data was analyzed with Graph pad prism 5 (Inc, USA) software. The glucose concentration and Lipid profile results were expressed as mean value ±Standard Error of the Mean (SEM). The mean and SEM of the treatment groups were generated by use of the analysis of variance (ANOVA) test. The significant difference between and within the treatment groups was considered significant at set $P<0.05$ by use of dunnet multiple comparison tests.

3. RESULTS

3.1 General Characteristics of the Animals

A day after intraperitoneal injection of the rats with alloxan monohydrate (150 mg/kg body weight), the rats were lethargic, displayed polyuria and polydipsia. However the control group in which diabetes was not induced remained active throughout the study period.

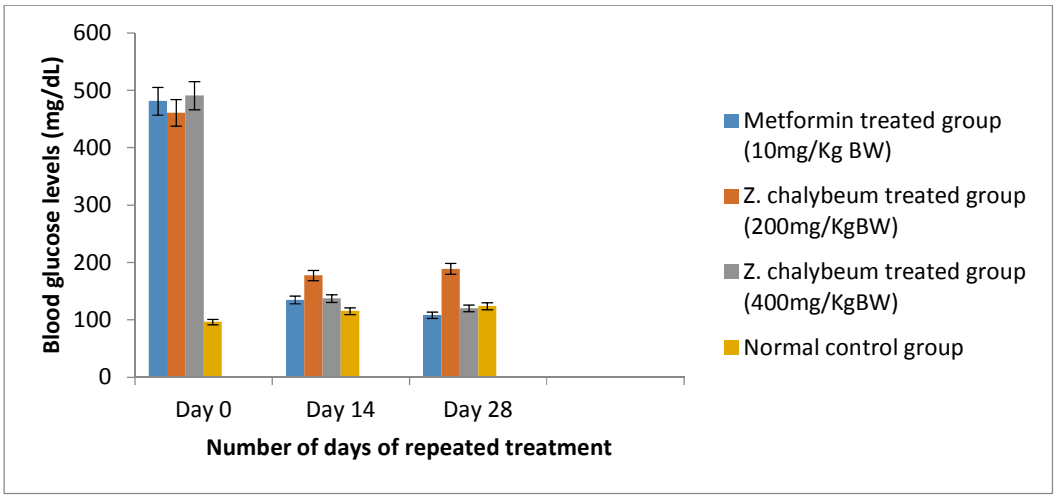

Fig. 1. Effect of aqueous root extract of *Z. chalybeum* for 4 weeks on plasma glucose levels in alloxan induced diabetic rats

Table 1. Effect of aqueous root extract of *Z. chalybeum* for 4 weeks on plasma glucose levels in alloxan induced diabetic rats

Parameters	Metformin treated group (10 mg/Kg BW)			*Z. chalybeum* treated group (200 mg/KgBW)			*Z. chalybeum* treated group (400 mg/KgBW)			Normal control group		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
Glucose (mg/dL	481.3±68.89	134.8±24.94*	108.3±3.75*	461.0±108.5	177.3±34.22*	189.0±46.16*	490.8±73.30	137.3±16.52*	120.3±15.42*	96.25±2.78	115.3±5.19	123 .8±7.122

Table 2. Effect of *Z. chalybeum* on lipid profiles of diabetic rats

Parameters	Metformin treated group (10 mg/Kg BW)			*Z. chalybeum* treated group (200 mg/KgBW)			*Z. chalybeum* treated group (400 mg/KgBW)			Normal control group		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
CHOL (mmol/l)	2.13±0.17	2.03±0.30	1.77±0.05	1.975±0.12	2.41±0.11	2.15±0.18	2.32±0.31	2.63±0.21	1.58±0.09	2.04±0.07	1 .68±0.11	2.00±0.17
HDLC (mmol/l)	1.47±0.24	1.40±0.08	1.63±0.06	0.98±0.23	1.38±0.03	1 .71±0.06*	0.64±0.18	1.29±0.06*	1.84±0.11*	1.41±0.22	1.47±0.06	1.59±0.19
TRIGL (mmol/L)	7.27±1.71	1.99±0.66*	0.89±0.09*	6.80±3.05	1.69±0.56*	1.53±0.26*	5.81±1.27	2.13±0.56*	0.73±0.11*	0.89±0.13	0.60±0.04	0.65±0.07
LDLC (mmol/l)	0.15±0.08	0.35±0.08	0.37±0.04	0.45±0.12	0.38±0.03	0 .48±0.05	0.34±0.19	0.60±0.07	0.31±0.01	0.46±0.04	0. 32±0.02	0.51±0.08

At baseline, before induction of diabetes, there was no significant difference in blood glucose levels across the various experimental groups, with levels between 80 mg/dL and 120 mg/dL. A day after intraperitoneal injection of the rats with alloxan monohydrate (150 mg/Kg body weight), the rats developed diabetes characterized by hyperglycemia with glucose levels >250 mg/dL. Administration of Z. *chalybeum* root bark extract (400 mg/kg body weight) significantly reduced ($p<0.05$) the blood glucose levels from 490.8±73.30 before treatment to 120.3±15.42 after 28 days of treatment, which was comparable to the normal control, 123.8±7.122.

There was no significant difference in total cholesterol levels between the control groups and the *Z. chalybeum* treated groups (p= 0.27). *Z. chalybeum* root bark extract (400mg/Kg body weight) significantly increased ($p<0.05$) HDL-Cholesterol from 0.64±0.18 before treatment to 1.84±0.11 after 28 days of repeated treatment, which was comparable to the normal control, 1.59±0.19. *Z. chalybeum* root bark extract (400 mg/Kg body weight) significantly decreased ($p<0.05$) the levels of triglycerides from 5.81±1.27 before treatment to 0.73±0.11 after 28 days of repeated treatment, which was comparable to the normal control, 0.65±0.07. There was no significant difference in LDL-Cholesterol levels between the control groups and the *Z. chalybeum* treated groups (p= 0.13). The results are illustrated in the Fig. 2 and Fig. 3.

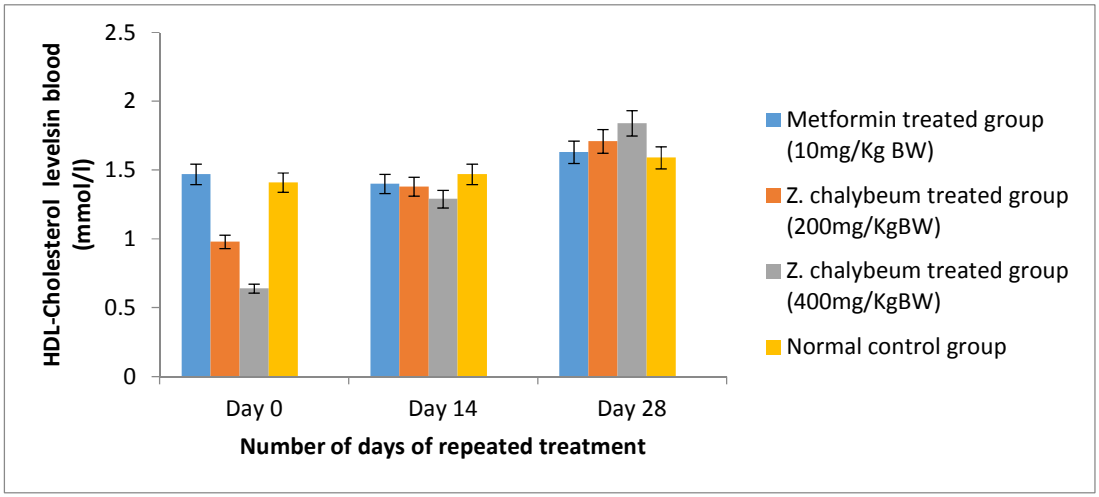

Fig. 2. Effects of aqueous roots extract of *Z. chalybeum* on levels of HDL-Cholesterol

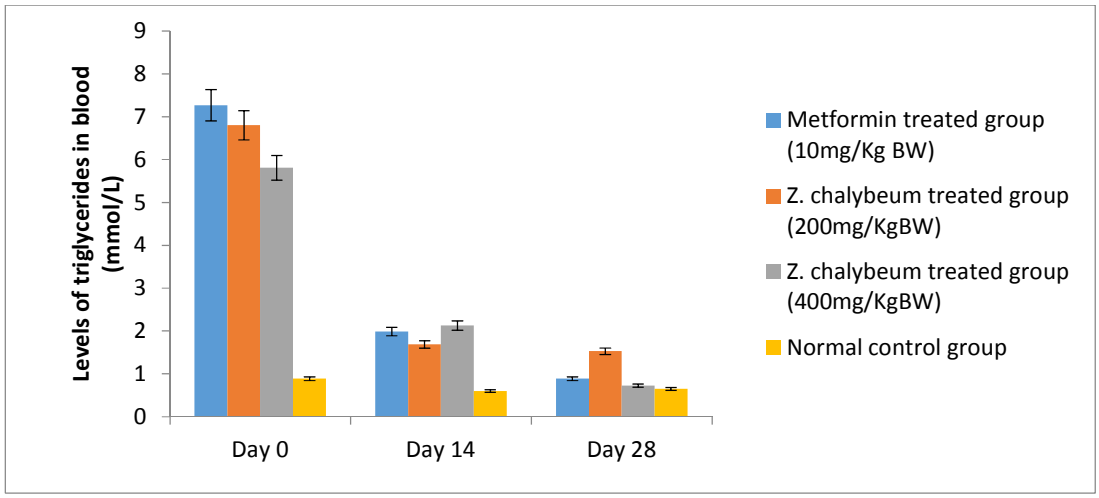

Fig. 3. Effects of aqueous roots extract of *Z. chalybeum* on levels of triglycerides

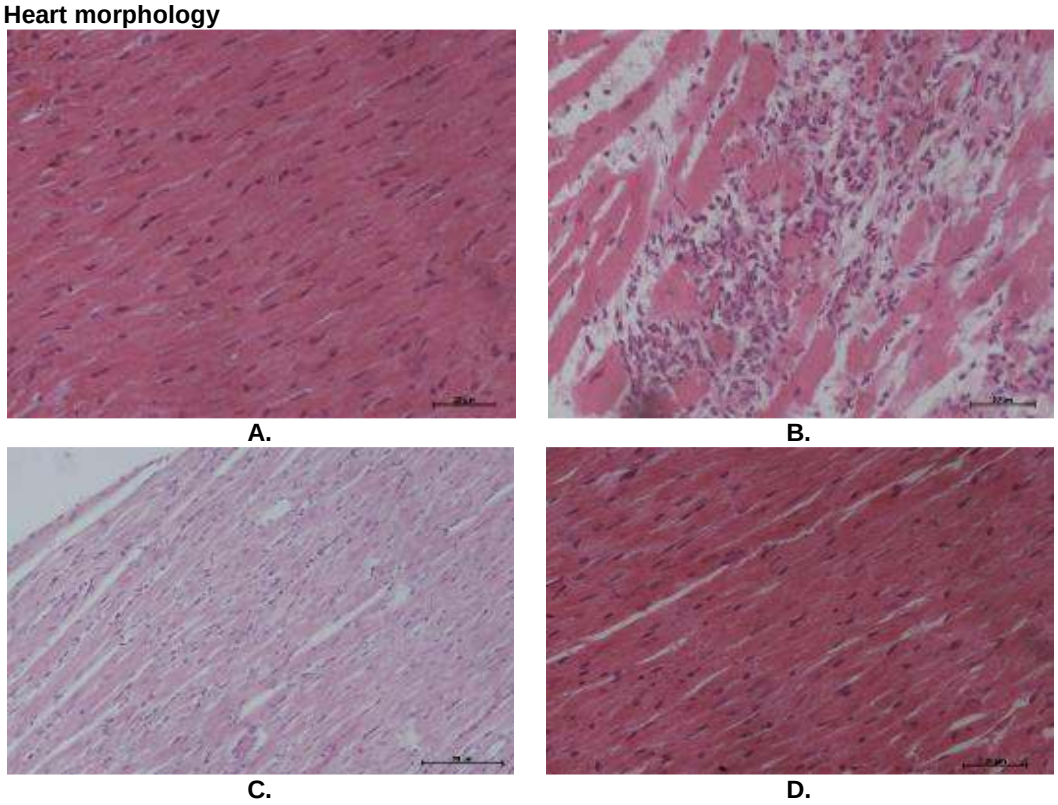

Fig. 4. Effect of *Z. chalybeum* aqueous root bark extract on the histopathology of the heart in diabetic rats at x40 magnification

A. Cross-section of normal heart tissue B. Cross-section of untreated diabetic heart tissue C. Cross-section of heart tissue after 28 days of treatment with Metformin (10 mg/kg BW), showing reversal in the degenerated tissues and D. Cross-section of heart tissue after 28 days of treatment with Z. Chalybeaum (400 mg/kg BW), showing reversal in the degenerated tissues

After induction of diabetes with no treatment, there was focal myocardial necrosis (B). After 28 days of repeated treatment with *Z. chalybeum* (400 mg/Kg body weight), there was reversal of the degenerative changes and the heart looked normal (D). After 28 days of repeated treatment with metformin (10 mg/Kg body weight), there was reversal of the degenerative changes and the heart looked normal (C).

4. DISCUSSION

The fundamental mechanism in diabetes mellitus involves excessive hepatic glycogenolysis, gluconeogenesis and the decreased utilization of glucose by the tissues [15]. Alloxan induced diabetes in experimental rats develops through destruction of beta cells. It has been shown that alloxan has two distinct pathological effects: it selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta cell, and it causes a state of insulin-dependent diabetes through its ability to induce Reactive Oxygen Species (ROS) formation, resulting in selective necrosis of beta cells. These two effects can be assigned to the specific chemical properties of alloxan, the common denominator being selective cellular uptake and accumulation of alloxan by the beta cell leading to type 1 diabetes [16]. The aqueous root extract of *Z. chalybeum* significantly reduced ($P<0.05$) blood glucose level at a dose of 400mg/kg p.o. Diabetes is characterized by hyperglycemia which is associated with dyslipidemia, a risk factor for coronary heart diseases [17]. The abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots due to the actions of insulin. In normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency, resulting in hypertriglyceridemia [18]

and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities. The dyslipidemia is characterized by increase in, TC, LDL, TG, and fall in HDL. This altered serum lipid profile was reversed towards normal after treatment with aqueous root extract of *Z. chalybeum*. *Z. chalybeum* aqueous root bark extract has been shown to possess the following phytochemical compounds; tannins, reducing sugars, saponins, alkaloid salts, anthracenosides, coumarin derivatives, flavonosides, steroid glycosides, triterpenes and anthocyanosides [19]. These studies on hypoglycaemic activity of plants have identified compounds like, flavonoids [20], terpenoids and tannins [21], sterols [22] and alkaloids [23] to be responsible for this action.

The underlying mechanism of lipid lowering effect of the root extract may be by inhibition of lipid absorption due to the presence of saponins and tannins [24] or inhibition of cholesterol esterase, activation of fatty acid synthase, and production of triglyceride precursors such as acetyl-CoA and glycerol phosphate [25]. The mechanism of lipid lowering effect of *Z. chalybeum* aqueous root extract may be modulated by the flavonoid content [26]. Several authors have reported that plant derived flavonoids have an effect of lowering triglycerides and total cholesterol in diabetic rats [27,28] and variously implicated in the reduction of lipids by inhibiting hepatic HMG-CoA reductase. The decrease of LDL levels may occur due to the reduction of VLDL and increased hepatic depuration of LDL precursors [25]. Thus, the treatment with the *Z. chalybeum* extract resulted in the recovery of certain altered biochemical parameters and restored histology of the heart of diabetic rats.

5. CONCLUSION

The results of the present study indicate that the aqueous root extract of *Z. chalybeum* exhibits anti-hyperglycemic and anti-hyperlidemic properties and alleviates damage to the heart from diabetes. In conclusion, *Z. chalybeum* controls hyperglycemia, hyperlipidemia, improves cardiac dysfunction associated with alloxan induced diabetes and might be effective for prevention or delay of the progress of diabetic cardiomyopathy.

CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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