ORIGINAL ARTICLE

Antidiabetic potential of *Zanthoxylum armatum* bark extract on streptozotocin-induced diabetic rats

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Objective: Hydromethanolic extract of the bark of *Zanthoxylum armatum* (HMZA) was evaluated for its antidiabetic and antioxidant activity in streptozotocin-induced diabetic rats. **Materials and Methods:** HMZA was evaluated in normal and diabetic rats. Diabetes was induced by streptozotocin (60 mg/kg i.p.). Normal and diabetic rats were divided into different groups and orally administered with HMZA (200 and 400 mg/kg) and glibenclamide (5 mg/kg) for 21 days. Blood samples were collected from overnight-fasted rats on at 7, 14 and 21 days of treatment and analysed for blood glucose level and lipid profile. On day 21, rats were sacrificed and liver and kidney tissues were excised to measure their antioxidant status. **Results:** Oral administration of HMZA for 21 days (200 and 400 mg/kg) resulted in significant reduction in blood glucose, total cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and significant increase in high density lipoprotein and body weight of streptozotocin diabetic rats. In addition to that, significant decrease in lipid peroxidation and increase in catalase, superoxide dismutase and reduced glutathione were observed in streptozotocin diabetic rats. **Conclusion:** From the present study, it is evident that, the bark of *Z. armatum* possesses significant antidiabetic and antioxidant effect on diabetic rats and suggests that the plant may have therapeutic value in diabetes and related complications.

Key words: Antidiabetic, antioxidant, glibenclamide, streptozotocin, *Zanthoxylum armatum*

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterised by hyperglycaemia due to either insufficiency of insulin or inability of cells to respond to insulin. Diabetes, by itself, increases the production of tissue damaging reactive oxygen species (ROS)[1] by glucose autoxidation and/or non-enzymatic protein glycosylation.[2] There are many clinical and experimental evidences indicating the involvement of oxidative stress in the pathogenesis of diabetes mellitus and more importantly in complications such as diabetic neuropathy, retinopathy and nephropathy.[3] Increased oxidative stress in diabetics is because of excessive ROS production due to persistent hyperglycaemia[4] as well as inadequate antioxidant defences.[5] Therefore, efforts are being made to reduce the oxidative stress in these patients by use of antioxidants which may reduce the severity of the disease. An antidiabetic drug with antioxidant potential is therefore considered to be very useful and valuable for treatment of diabetes. Due to undesired side effects of insulin and oral hypoglycaemic, continuous efforts are being made to develop new compounds or combinations for treatment of diabetes, especially of herbal origin.

Zanthoxylum armatum belongs to family Rutaceae. Commonly known as 'timur' or 'Nepali Dhania' it is a shrub or tree common in the temperate Himalaya, in Bhutan and Khasi hills. It is found in the hot valleys of Himalaya at an altitude of 1000-2000 meters. *Z armatum* is commonly used in the Indian system of medicine, as carminative, stomachic and anthelmintic.[6] The essential oil of fruits shows good antibacterial and antifungal activities.[7] However, to the best of our knowledge, no data are available for its used as antidiabetic. The present study was designed to evaluate antidiabetic activity of this plant.

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MATERIALS AND METHODS

Chemicals and Reagents

Agappe diagnostics kits (Ernakulan, Kerala, India) were used for the estimation of blood glucose, blood cholesterol and serum triglycerides while kit manufactured by Piramal Healthcare Limited (Navi

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Mumbai, India) was used for the diagnosis of HDL. STZ was bought from Sigma Co. (USA). Glibenclamide was obtained from Inga Laboratories Pvt. Ltd, Mumbai. DTNB [5, 5-dithiobis (2- nitrobenzoic acid)], TBA (Thio barbituric acid) and NBT (Nitro-blue tetrazolium) were also obtained from Sigma chemicals Co. (USA). All solvents used in this study were of analytical reagent grade.

Collection of Plant Material

Stem bark of *Z. armatum* were collected in the month of September-October 2010 from Bhimtal, Nainital District, Uttarakhand, India. The plant material was taxonomically identified by the N.B.P.G.R. Niglat Bhawali, Nainital Uttrakhand. Voucher specimen of plant was deposited in the RSB herbarium under the number RSB/Tech. Corrsp./2011-12/525.

Preliminary Phytochemical Screening

The hydromethanolic extract was qualitatively tested for the presence of phytochemical constituents.[8]

Preparation of Extract

The hydromethanolic extract of the bark of *Z. armatum* (HMZA) was prepared by cold maceration of the shade-dried bark powder in methanol: Water (4:1) for 24 hours. The extract was filtered, concentrated, dried and the residue stored in a refrigerator at 2-8 °C for use in subsequent experiments.

Animal

Wistar rats of either sex (200-300 g) were used for the study maintained on standard laboratory diet and tap water *ad libitum*. They were housed in departmental animal house 12 hr light and 12 hr dark cycle. The animal house and breeding facility have been registered by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the protocol number is 06/2011.

Induction of Diabetes

Diabetes was induced in overnight-fasted rats by intraperitoneal injection of STZ at a dose of 60 mg/kg body weight dissolved in freshly prepared 0.01 M citrate buffer, pH 4.5.[9] STZ can induce fatal hypoglycaemia as a result of massive pancreatic insulin release and to avoid this hypoglycemic effect, the rats were provided with 5% dextrose solution after 6 h of STZ administration for next 24 h. Induction of diabetes was verified after 72 h and the animals having a blood glucose level higher than 200 mg/dl were considered diabetic and used for the experiments.

Experimental Design

Animals were divided into five groups of five rats each.

Group 1 (Normal Control) - 1% CMC (1ml/kg/day, p.o.)
Group 2 (Diabetic control) - Streptozotocin + 1% CMC (1ml/kg/day, p.o.)
Group 3- Streptozotocin + Glibenclamide (5mg/kg/day, p.o.) for 21 days.
Group 4- Streptozotocin + HMZA (200mg/kg/day, p.o.) for 21 days.
Group 5- Streptozotocin + HMZA (400mg/kg/day, p.o.) for 21 days.

The test drug (*Z. armatum*) and the reference drug (Glibenclamide) were administered orally as a suspension in a 1% w/v CMC.

Collection of Blood and Tissue Samples

Blood samples were collected from the retro-orbital plexus on 0, 7th, 14th and 21st day of test drug and standard drug administration. Blood was collected in centrifuged tube serum was separated by centrifugation (for 20 min at 2000 rpm) and used for the estimation of blood glucose, total cholesterol, serum triglyceride, HDL, LDL and VLDL according to the instructions given in kit. On day 22nd rats were sacrificed by cervical dislocation under light anaesthesia, liver and kidney tissues were isolated, washed with saline, homogenised by Tris–HCl buffer. Supernatant fractions of their homogenate were used for measuring the activity of SOD, Catalase, GSH and Lipid peroxidation level.

Biochemical Estimations

Fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL) were estimated by commercially available kits on 0,7,14 and 21 day of experiment. Their levels were expressed as mg/dl. LDL and VLDL were estimated by using Freidewald's Formula.[10] The peroxidation level was estimated as thiobarbituric acid reactive substances (TBARS) in liver and kidney.[11] Reduced glutathione (GSH) was estimated by the method of Ellman's (1959).[12] The antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were estimated by using the methods described by Kakkar *et al*., (1984) and Luck (1963) respectively.[13,14] Body weight was estimated on 0,7,14 and 21 day of experiment.

Statistical Analysis

All the data are expressed as mean ± standard error of mean (SEM) for five animals in each group. Evaluation done by one-way analysis of variance (ANOVA), employing Tukey's test for multiple comparisons and values of $P < 0.05$ were considered as statistically significant.

RESULTS

Phytochemical Screening

The phytochemical studies revealed the presence of

alkaloids, tannins, phlobatannin, terpenoids, fixed oil, phenolic compounds and flavonoids in HMZA [Table 1].

Effect of Extract on Fasting Blood Glucose

The effect of HMZA on the fasting blood glucose levels of diabetic rats is given in Figure 1. The fasting blood glucose levels of diabetic untreated rats were significantly higher than those of normal untreated rats. There was a significant improvement in the blood glucose levels after the treatment with HMZA. A significant ($P < 0.05$) decrease of 17.30% and 22.70% in blood glucose levels was observed in diabetic treated group by 200 mg/kg dose of extract on 14th and 21st day, respectively. While significant ($P < 0.01$) reduction in blood glucose of 22.70% and 43.14% was observed from dose 400mg/kg on day 14th and 21st, respectively. No significant reduction on blood glucose level by plant extract was observed on 7th day of study.

Table 1: Phytochemical screening of extract

Phytochemical constituents	Result
Alkaloids	+
Proteins	–
Saponins	–
Tannins	+
Phenolic compounds	+
Flavonoides	+
Phlobatannin	+
Fixed oil	+
Terpenoid	+

+ – Present, – – Absent

Effect of the Extracts on Lipid Profile

Total Cholesterol

The effect of *Z. armatum* treatment on the total cholesterol level of the tested rat groups is given in Table 2. The enhanced level of TC was reduced significantly ($P < 0.05$) 21.02% by 200 mg/kg and ($P < 0.01$) 28.23% by 400 mg/kg after 21 days of treatment.

Triglyceride

A significant ($P < 0.05$) fall of 21.69% in TG after 21 days treatment period with 200 mg/kg dose was observed, while 400mg/kg dose reduces the enhanced TG level ($P < 0.05$) 21.69% and ($P < 0.01$) 28.89% after 14 and 21 days, respectively [Table 2].

HDL

In diabetic rats, the level of HDL-c was significantly lower ($P < 0.01$) than those of the NC group, shown in Table 2. Extract do not show any significant increment in HDL-c level by dose 200mg/kg. While dose 400 mg/kg shows significant increase 57.92% ($P < 0.05$) after 14 days and 88.11% ($P < 0.01$) in HDL-c after 21 days.

Table 2: Effect of extract on lipid profile (mg/dl)

Groups	Parameter	0 day	7th day	14th day	21st day
Normal control	TC	80.41±4.164	82.92±3.903	81.12±3.898	81.46±2.577
	TG	76.42±5.711	75.41±6.044	81.99±7.696	77.90±5.801
	HDL	42.01±5.244	41.35±5.418	41.36±3.756	40.69±4.417
	LDL	23.12±4.694	26.49±6.018	23.36±2.336	25.19±3.462
	VLDL	15.28±1.142	15.08±1.209	16.40±1.539	15.58±1.160
Diabetic control (STZ)	TC	155.72±7.787	165.64±7.640^{p}	171.7±11.73^{p}	164.48±7.738^{p}
	TG	164.76±9.430	172.04±8.103^{p}	184.22±9.951^{p}	191.68±11.429^{p}
	HDL	35.15±2.793	28.04±2.696	22.34±1.888^{q}	20.01±1.583^{p}
	LDL	87.62±4.347	103.2±7.039^{p}	112.5±11.44^{p}	108.1±6.981^{p}
	VLDL	32.95±1.886	34.41±1.621^{p}	36.84±1.990^{p}	38.34±2.286^{p}
STZ+5 mg/kg Glibenclamide	TC	176.02±9.263	147.64±9.876	124.88±7.505^{c}	115.06±7.602^{a}
	TG	187.24±7.720	162.47±7.742	144.2±7.549^{c}	134.3±10.60^{b}
	HDL	32.26±2.532	36.72±3.120	38.28±3.306^{b}	42.32±2.132^{a}
	LDL	106.3±6.805	78.43±8.152	57.76±6.033^{b}	45.88±5.683^{a}
	VLDL	37.45±1.544	32.49±1.548	28.84±1.510^{c}	26.86±2.120^{b}
STZ+200 mg/kg HMZA	TC	165.09±10.887	153.06±10.75	142.18±10.497	129.9±9.558^{c}
	TG	173.12±6.009	165.42±8.055	157.99±7.749	150.1±8.183^{c}
	HDL	25.15±2.193	25.17±1.582	26.52±2.218	27.62±2.885
	LDL	105.3±7.861	94.81±8.628	84.06±9.258	72.30±8.002^{b}
	VLDL	34.62±1.202	33.08±1.611	31.60±1.550	30.02±1.637^{c}
STZ+400 mg/kg HMZA	TC	159.42±12.259	149.62±11.889	129.47±14.163	118.04±7.201^{b}
	TG	185.1±7.565	162.02±9.861	147.19±9.194^{c}	136.3±7.800^{b}
	HDL	29.12±2.992	32.01±2.105	35.28±3.103^{c}	37.64±3.590^{b}
	LDL	93.28±8.016	85.21±8.114	64.75±10.03^{b}	53.14±5.091^{a}
	VLDL	37.02±1.513	32.40±1.972	29.44±1.839^{c}	27.26±1.560^{b}

Value expressed as Mean±S.E.M (n=5); ^{p}=P<0.001, ^{q}=P<0.01, ^{r}=P<0.05 significant from normal control group; ^{a}=P<0.001, ^{b}=P<0.01 and ^{c}=P<0.05 significant from diabetic control group; TC – Total cholesterol; TG – Triglyceride; HDL – High density lipoprotein; LDL – Low density lipoprotein; VLDL – Very low density lipoprotein

LDL

The results in Table 2 showed that the levels of LDL-c in the diabetic control rats were significantly higher ($P < 0.001$) than those of the NC group. Level of LDL-c decreased significantly ($P < 0.01$) 33.11% by 200 mg/kg dose after 21 days. Four hundred mg/kg dose also reduces LDL-c level by 42.45% ($P < 0.01$) and 50.84% ($P < 0.001$), after 14 and 21 days, respectively.

VLDL

Level of VLDL also significantly increased in diabetic control group rats when compared to normal rats, result shown in Table 2. Both the doses causes significant reduction ($P < 0.05$) by 21.70% after 21 days of 200 mg/kg and 20.08% after 14 days of 400 mg/kg. 28.89% ($P < 0.01$) reduction in VLDL-c was observed after 21 days treatment with 400 mg/kg dose of extract.

Effect on Body Weight

The body weight of the animals in the normal control group increased significantly from 254 ± 16 g to 292 ± 12.41 g [Table 3], while the weight of the diabetic rats decreased remarkably from 272 ± 15.62 gm to 178 ± 11.58 gm. After the treatment of HMZA the body weight was significantly ($P < 0.001$) increased 43.82% and 60.67% compared to the diabetic control group by dose 200 and 400 mg/kg extract, respectively.

Table 3: Determination of body weight (gm)

Group	0 day	7th day	14th day	21st day
Normal control	254.0±16.00	266.0±15.36	274.0±13.64	292.0±12.41
Diabetic control (STZ)	272.0±15.62	236.0±16.91	212.0±10.20^{q}	178.0±11.58^{p}
STZ+5 mg/kg Glibenclamide	260.0±13.04	262.0±12.41	276.0±11.66^{b}	294.0±9.274^{a}
STZ+200 mg/kg HMZA	264.0±12.88	246.0±11.22	250.0±8.367	256.0±9.274^{a}
STZ+400 mg/kg HMZA	282.0±15.94	268.0±11.58	276.0±12.88^{b}	286.0±13.64^{a}

Value expressed as Mean±S.E.M (n=5); ^{p}=P<0.001, ^{q}=P<0.01 significant from normal control group; ^{a}=P<0.001 and ^{b}=P<0.01 significant from diabetic control group, STZ – Streptozotocin

Effect on Antioxidant Parameters in Liver and Kidney Tissue Homogenate

There is a widespread understanding that hyperglycaemia and hyperlipidaemia create excess reactive oxygen species, which are probably involved in some of the complications of diabetes. In diabetic rats treated with 400mg/kg extract, a significant increase was observed in liver SOD by 79.18% ($P < 0.05$), while in kidney SOD by 38.67% ($P < 0.01$) respectively, when compared with diabetic controls [Figure 2]. Two hundred mg/kg dose show significant increase only in kidney SOD by 26% ($P < 0.05$).

Meanwhile, treatment with 400mg/kg extract caused a significant increase in liver and kidney GSH by 37.89% ($P < 0.05$) and 40.45% ($P < 0.01$), respectively. Whereas treatment with 200 mg/kg GSH level in the liver and kidney tissue were insignificantly increased by 9.18% and 8.56%, respectively, when compared with diabetic controls [Figure 3].

Treatment with extract at a dose of 400 mg/kg for 21 days showed significant reduction ($P < 0.001$) in LPO in liver by 61.23% and kidney by 61.14%, [Figure 4]. Dose 200 mg/kg insignificantly reduces liver LPO by 15.52% and kidney LPO by 33.22%, when compared to diabetic control group.

The CAT contents of liver and kidney tissues were augmented by the administration of HMZA at a dose of 400 mg/kg [Figure 5]. However, the most pronounced activity was observed in kidney GSH content, which was increased by 68.13% ($P < 0.001$), liver CAT was increased by 44.25% ($P < 0.01$). In contrast, CAT levels of both liver and kidney tissues were increased insignificantly when extract was given at a dose of 200 mg/kg under the same conditions, by 19.72% and 38.50%.

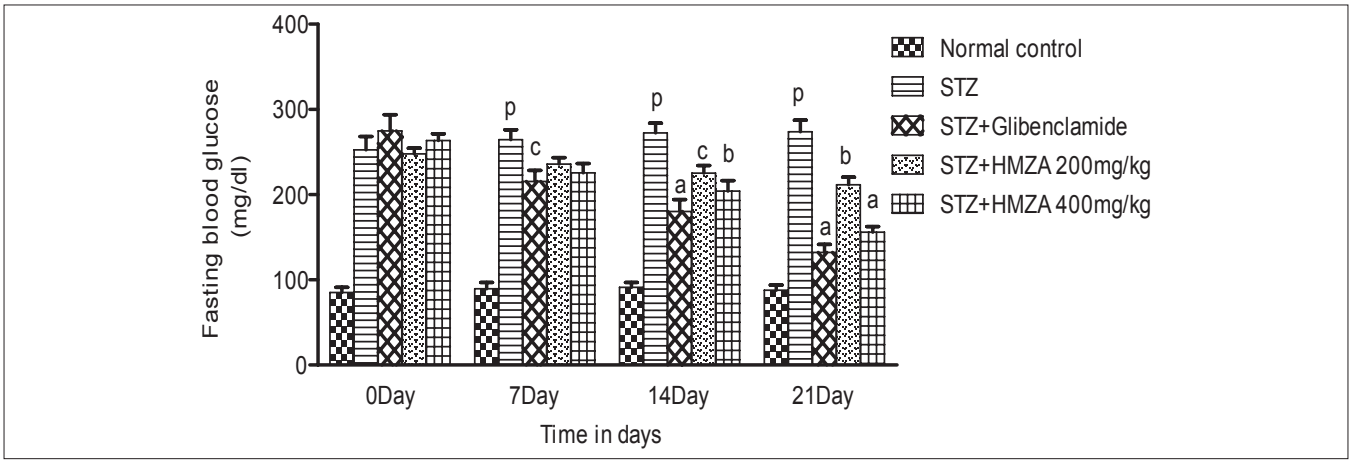

Figure 1: Effect of HMZA on fasting blood glucose. Value expressed as Mean ± S.E.M (n = 5); p= P<0.001 significant from normal control group; a=P<0.001, b=P<0.01 and c=P<0.05 significant from diabetic control group

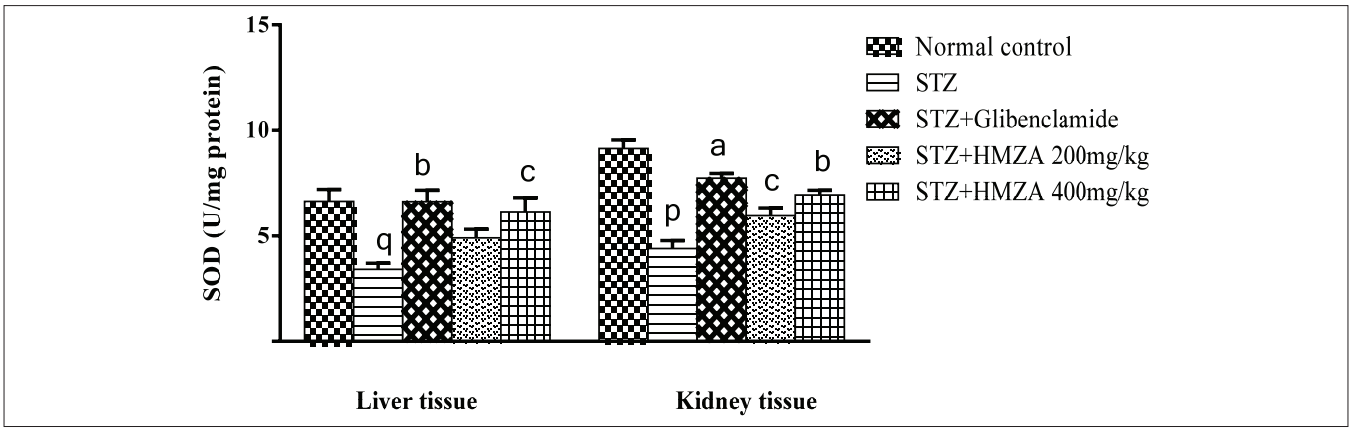

Figure 2: Effect of HMZA on Superoxide dismutase level of liver and kidney tissue Value expressed as Mean ± S.E.M (*n* = 5); *p*= *P*<0.001 significant from normal control group; a=*P*<0.001, b=*P*<0.01 and c=*P*<0.05 significant from diabetic control group

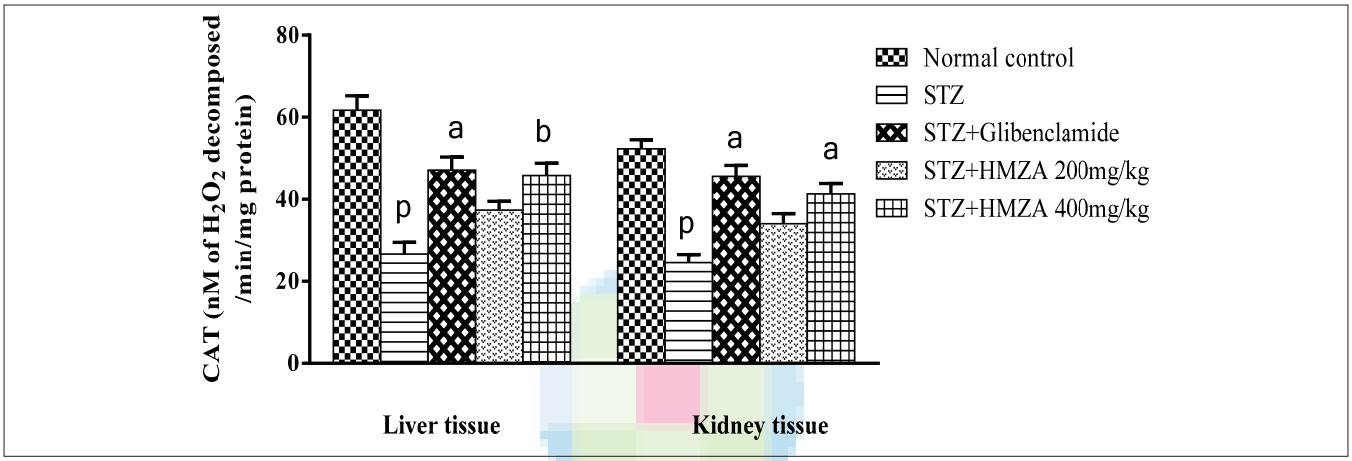

Figure 3: Effect of HMZA on catalase level of liver and kidney tissue Value expressed as Mean ± S.E.M (*n* = 5); *p*= *P*<0.001 significant from normal control group; a=*P*<0.001 and b=*P*<0.01 significant from diabetic control group

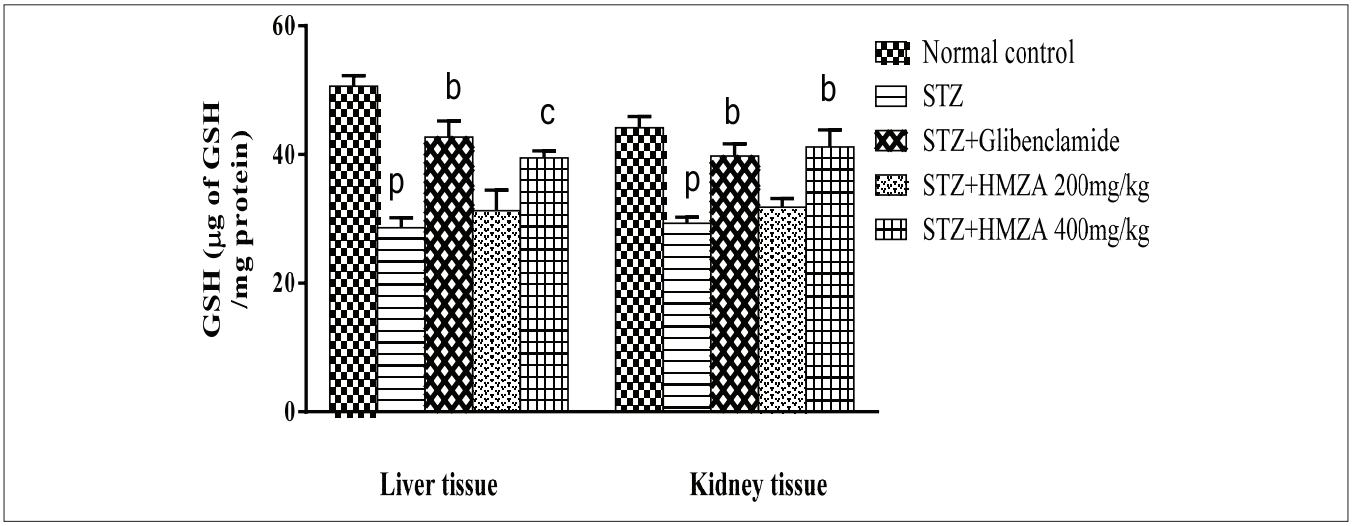

Figure 4: Effect of HMZA on reduced glutathione level of liver and kidney tissue Value expressed as Mean ± S.E.M (*n* = 5); *p*= *P*<0.001 significant from normal control group; b=*P*<0.01 and c=*P*<0.05 significant from diabetic control group

DISCUSSION

The present study was undertaken to evaluate the antidiabetic activity of HMZA on STZ induced diabetes. To the best of our knowledge this is the first report of antidiabetic activity of this plant. Hyperglycaemia, the most important feature of diabetes mellitus, is in itself very dangerous for diabetic patients. It impairs the

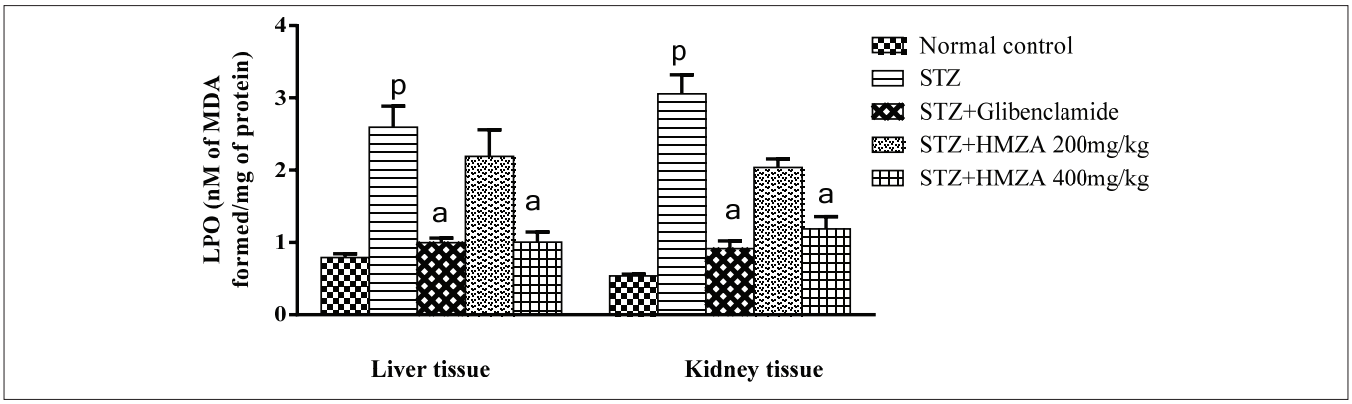

Figure 5: Effect of HMZA on lipid peroxidation level of liver and kidney tissue Value expressed as Mean ± S.E.M (*n* = 5); *p*= *P*<0.001significant from normal control group; a=*P*<0.001 significant from diabetic control group

pro-oxidant/antioxidant balance, reducing antioxidant levels and increasing free radicals,[15] which can damage the pancreatic β-cells and induce insulin resistance. The conclusions derived from the study of extract on diabetic rats revealed a defined role of the HMZA in controlling hyperglycaemia, the key feature of the diabetes and implied that *Z. armatum* probably could control the development of diabetes.

In addition to its hypoglycaemic effect, HMZA were also able to improve some lipid metabolites including TC, TG, HDL, LDL and VLDL cholesterol levels in diabetic rats. It is reported that diabetes are associated with profound alterations in lipid and lipoprotein profile.[16,17] Regulating the plasma or tissue lipid levels leads to a decrease in the risk of micro- or macro vascular disease and related complications.[18] Thus, this result suggested that *Z. armatum* would be helpful to the prevention of diabetic complications through improving dyslipidaemia.

Several workers have reported induction of diabetes with STZ is associated with the characteristic loss of body weight which is due to increased muscle wasting and loss of tissue proteins in diabetes.[19,20] Administration of HMZA to diabetic rats caused a significant increase in the body weight. The protective effect of plant on body weight of diabetic rats may be due to its ability to reduce hyperglycaemia.

Diabetics and experimental animal models exhibit high oxidative stress due to hyperglycaemia. Recently, much attention has been focused on the role of oxidative stress and it has been suggested that oxidative stress may constitute the key and common events in the pathogenesis of both micro vascular and macro vascular complications.[2] Diabetic subjects with such complications may have a defective cellular antioxidant response against the oxidative stress generated by persistent and chronic hyperglycaemia, which can predispose the patient to organ damage, and depletes the activity of antioxidative defence system and thus promotes de novo free radicals generation.[21,22]

Hyperglycaemia induces the generation of free radicals which can affect antioxidant defences thus leading to the disruption of cellular functions, oxidative damage to membranes and increased susceptibility to lipid peroxidation.[2] Higher MDA level has been observed in diabetic condition which is a measure of lipid peroxidation.[23] Treatment with HMZA lowered the level of MDA in kidney and liver of STZ-diabetic rats. In diabetic rats, decreased levels of glutathione in liver and kidney was observed [Table 2] probably due to its increased utilisation by the hepatic cells which could be result of decreased synthesis or increased degradation of GSH by oxidative stress in diabetes.[24] Treatment With *Z. armatum* extract increased the GSH level.

Antioxidant enzymes like superoxide dismutase and catalase were significantly reduced in diabetic rats.[25] Oral administration of HMZA increased the antioxidant enzymes level in liver and kidney of STZ-diabetic rats. Chronic oxidative stress is one of the major mechanisms responsible for glucose toxicity as the adverse effects of high glucose concentration on β-cell function are related to oxidative stress and shown to be prevented by antioxidants and over expression of antioxidant enzymes.[3,26]

CONCLUSIONS

In the previous studies most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc., that are frequently implicated as having antidiabetic effects.[27] As a conclusion, it could be speculated that the observed anti-hyperglycaemic activity of *Z. armatum* bark might be related to the presence of alkaloids, flavonoides, terpenoides and phenolic compounds. Antioxidant status of *Z.armatum*

might also be responsible for antidiabetic action of this plant. Further studies are needed to isolate the active components.

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