

ANTIDIABETIC ACTIVITY OF BENZYL TETRA ISOQUINOLINE ALKALOID BERBERINE IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS

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Key words: berberine, streptozotocin-nicotinamide induced diabetes, antidiabetic activity, carbohydrate metabolism, antioxidants

SUMMARY

The antidiabetic potential of berberine was evaluated in a streptozotocin-nicotinamide induced type 2 diabetic rat model. On administration of graded doses of berberine to normal and experimental diabetic rats for 12 days, a significant ($p < 0.05$) reduction was observed in fasting blood glucose levels. However, serum insulin levels failed to be stimulated on treatment with berberine. Significant changes were observed in serum lipid profiles, thiobarbituric acid reactive substance levels, glycosylated hemoglobin and liver glycogen levels in berberine treated diabetic rats as compared with diabetic control and normal animals. The effect of berberine was also studied for its carbohydrate metabolism and antioxidant status in streptozotocin-nicotinamide induced type 2 diabetic rats. Oral administration of berberine caused a significant increase in both enzymatic and nonenzymatic antioxidants. Studies of the effect of berberine on glycolytic enzymes showed a significant increase in their levels whilst a significant decrease was observed in the levels of the gluconeogenic enzymes in treated diabetic rats. Serum creatinine and urea levels also declined significantly.

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INTRODUCTION

Berberine is a quaternary protoberberine alkaloid commonly found in a number of clinically important medicinal plants (1). Berberine containing plants are used medicinally in virtually all traditional medicinal systems and have a history of usage in Ayurvedic and Chinese medicine dating back for at least 3000 years. This alkaloid has demonstrated significant antimicrobial activity against bacteria (2), fungi (3), protozoa (4), viruses (5), helminths (6) and chlamydia (7). In addition, its actions include antagonism of the effects of cholera and *Escherichia coli* heat stable enterotoxin (8), inhibition of intestinal ion secretion (9), inhibition of smooth muscle contraction (10), inhibition of ventricular tachyarrhythmias (11), reduction of inflammation (12), elevation of platelet count in patients with primary and secondary thrombocytopenia (13), and stimulation of bile secretion and bilirubin discharge (14). Evidence also suggests that intravenous berberine administration can play a role in preventing the onset of ventricular tachyarrhythmia and sudden coronary death after myocardial ischemic damage (15).

Studies have been carried out on its role in the treatment of diabetes mellitus but its mechanism of action has not been elucidated (16-18). Leng *et al.* (19) studied the lipid lowering action of berberine in a murine diabetic model induced by streptozotocin

(STZ) and high fat laboratory chow. However, there are no data available on the glucose lowering effect of berberine in STZ-nicotinamide induced diabetic rats; hence the present study is an attempt in this direction.

MATERIALS AND METHODS

Chemicals and instruments used

The following chemicals were used in the study: glucose-6-phosphate dehydrogenase, glucose-6-phosphate, lactate dehydrogenase, streptozotocin (Sigma Aldrich Co., Germany), ascorbic acid, metaphosphoric acid, O-phosphoric acid, magnesium chloride, EDTA, sodium citrate (NICE Chemicals Pvt Ltd., Cochin, India), phenazine methosulfate, nitroblue tetrazolium chloride, NADH, NADPH, ATP, glutathione, 5, 5' dithio nitro bis benzoic acid, tocopherol (Himedia Laboratories Ltd., Mumbai, India), disodium hydrogen phosphate, potassium hydrogen phosphate, (E. Merck (India) Ltd., Mumbai, India), 2,4 dinitro phenylhydrazine (Sarabhai M. Chemicals, Baroda, India), sodium pyruvate (S.D Fine-Chem Ltd., Mumbai), Tris buffer (SISCO Research Laboratories Ltd., Mumbai, India), sodium pyrophosphate (Thomas Baker Chemicals Ltd., Mumbai, India), and nicotinamide (Qualigens Fine Chemicals, Division of Glaxo, Mumbai, India).

An UV spectrophotometer (Shimadzu 160 IPC), homogenizer, centrifuge and pH meter were the instruments used in the study.

Animals

Healthy adult male Wistar albino rats weighing about 250-300 g were used for the study. The animals housed in polypropylene cages, maintained under standard conditions (12-h light/12-h dark cycle; 25 ± 3 °C; 35%-60% humidity) were fed standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. The study was approved by the Institutional Animal Ethics Committee of KMC, Manipal, India (IAEC/KMC/03/2003-04).

Acute toxicity studies

Healthy adult Wistar albino rats of either sex, starved overnight, were divided into four groups (n=6) and were orally fed berberine in increasing doses of 100,

500, 1000 and 3000 mg/kg body weight (20). The rats were observed continuously for 2 h for behavioral, neurological and autonomic profiles, and then at 24 h and 72 h for any lethality (21).

Oral glucose tolerance test

The oral glucose tolerance test (22) was performed in overnight fasted (18-h) normal animals. Rats divided into four groups (n=6) were administered 2% gum acacia solution, berberine (75 mg/kg), berberine (150 mg/kg) and glibenclamide (0.25 mg/kg), respectively. Glucose (2 g/kg) was fed 30 min after the administration of berberine. Blood was withdrawn from the retro-orbital sinus at 0, 30, 60, 90 and 120 min of berberine administration. Fasting blood glucose levels were estimated by glucose oxidase-peroxidase reactive strips (Accu-check, Roche Diagnostics, USA).

Normoglycemic study

For normoglycemic study, rats were divided into four groups (n=6) and were administered 2% gum acacia solution, berberine (75 mg/kg), alcoholic extract (150 mg/kg) and glibenclamide (0.25 mg/kg), respectively. Blood glucose levels were estimated on days 0, 5 and 12.

Induction of experimental diabetes

The animal model of type-2 diabetes mellitus (NIDDM) was induced (23) in overnight fasted animals by a single intraperitoneal injection of 60 mg/kg STZ, 15 min after the i.p. administration of 120 mg/kg nicotinamide. Hyperglycemia was confirmed by the elevated blood glucose levels determined at 72 h and then on day 7 of the injection. Only rats confirmed with permanent NIDDM were used in the antidiabetic study.

Experimental design

The animals were divided into four groups (n=6) of normal rats administered 2% gum acacia solution, diabetic rats administered gum acacia 2% solution, diabetic rats administered berberine 75 mg/kg, and

diabetic rats administered berberine 150 mg/kg for 12 days by oral route. Fasting blood glucose levels were estimated on days 0, 5 and 12.

Sample collection

Blood sampling

At the end of day 12, blood samples were collected retro-orbitally from the inner canthus of the eye under light ether anesthesia using capillary tubes (Micro Hemocrit Capillaries, Mucaps). Blood was collected into fresh vials containing anticoagulant and serum was separated in a centrifuge at 2000 rpm for 2 min.

Collection of organs

The animals were euthanized by an overdose of intraperitoneal anesthesia and tissue samples were collected for the assessment of biochemical parameters. Serum insulin levels, serum lipid profiles, liver glycogen levels (24), glycosylated hemoglobin levels, thiobarbituric acid reactive substance levels (TBARS) (25) and changes in body weight assessed in the diabetic animals treated with berberine were compared with diabetic control and normal animals.

Serum insulin levels were estimated by the Radio Immuno Assay Kit issued by the Board of Radiation and Isotope Research, Bhaba Atomic Research Centre (BARC), Mumbai, India. Serum lipid profiles and glycosylated hemoglobin levels (turbidimetric inhibition immunoassay) were estimated on an autoanalyzer (Hitachi 912).

Estimation of enzymes in carbohydrate metabolism

The following enzymes were evaluated: hexokinase EC 2.7.1.1 (26), glucose-6-phosphate dehydrogenase EC 1.1.1.49 (27), lactate dehydrogenase EC 1.1.1.27 (28), glucose-6-phosphatase EC 3.1.3.9 (29), and alanine aminotransferase EC 2.6.1.2 (30).

Urea and creatinine

Serum urea was determined by the urease enzyme kit modified Berthelot method (Agappe Diagnostics, Thane, India). Creatinine level in serum was estimated by the alkaline picrate - modified Jaffe's method using creatinine kit (Agappe Diagnostics, Thane, India).

Estimation of antioxidant parameters

The enzymatic antioxidants glutathione synthetase and glutathione peroxidase (31), catalase EC.1.11.1.6 (32,33), peroxidase EC.1.11.1.7 (34), superoxide dismutase EC.1.15.1.1 (35), and nonenzymatic antioxidants ceruloplasmin (36), tocopherol (37) and ascorbic acid (38) were determined. The protein content in tissue homogenate was estimated by use of standard methodology (39).

Statistical analysis

Data were statistically evaluated by use of one-way ANOVA, followed by post hoc Scheffe's test using 7.5 version of SPSS computer software. The values were considered significant when $p < 0.05$.

RESULTS

Acute toxicity studies revealed the non-toxic nature of berberine at the two dose levels tested. No lethality or toxic reactions were observed until the end of the study. In oral glucose tolerance test the berberine treated animals showed a significant reduction in blood glucose levels from 30 min onwards (Table 1). Normal animals also exhibited significant reduction in the blood glucose level as compared to controls (Table 2). The effect of berberine on fasting blood glucose levels in diabetic animals is presented in Table 3. Serum insulin levels were not stimulated; these results are presented in Table 4. A significant ($p < 0.05$) difference was observed in glycosylated hemoglobin levels (Table 4), serum lipid profiles (Table 5), liver glycogen levels, thiobarbituric acid reactive substance levels (Table 7) and changes in body weight (Table 6) between the berberine treated diabetic animals and either diabetic control or normal animals.

Table 1. Effect of berberine on oral glucose tolerance test in glucose loaded rats

Group	Treatment	Blood glucose concentration (mg/dL)				
		0 min	30 min	60 min	90 min	120 min
I	Control (vehicle)	88.6 ± 9.8	110 ± 2.3.	104 ± 11.8	101.5 ± 13.4	97.5 ± 6.2
II	Berberine (75 mg/kg)	84.1 ± 2.5	96.5 ± 8.5 ^a	90.1 ± 7.4 ^a	86.4 ± 5.5 ^a	83.8 ± 9.3 ^a
III	Berberine (150 mg/kg)	81.3 ± 6.3	74.1 ± 4.8 ^a	77.8 ± 3.5 ^a	78.6 ± 8.3 ^a	76.5 ± 4.9 ^a
IV	Glibenclamide (0.25 mg/kg)	79.9 ± 4.8	70.7 ± 6.1 ^a	73.3 ± 2.3 ^a	75.5 ± 4.4 ^a	72.9 ± 3.2 ^a

Values are expressed as mean ±SE; n=6; ^a p 0.05 vs. control; one-way ANOVA followed by post hoc Scheffe's test.

Table 2. Effect of berberine on normal animals

Group	Treatment	Blood glucose concentration (mg/dL)		
		Day 0	Day 5	Day 12
I	Control (vehicle)	74.3 ± 1.7	74.8 ± 3.6	67.2 ± 5.9 ^a
II	Berberine (75 mg/kg)	73.9 ± 4.6	65.9 ± 9.3 ^a	61.8 ± 6.9 ^a
III	Berberine (150 mg/kg)	71.1 ± 1.3	63.8 ± 3.2 ^a	52.5 ± 7.2 ^{a,b}
IV	Glibenclamide (0.25 mg/kg)	74.7 ± 5.7	67.2 ± 5.9 ^a	59.8 ± 4.0 ^a

Values are expressed as mean ±SE; n=6; ^astatistical significance vs. control (p<0.05); ^bstatistical significance vs. standard (p<0.05); one-way ANOVA followed by post hoc Scheffe's test.

Table 3. Effect of berberine on diabetic animals

Group	Treatment	Blood glucose concentration (mg/dL)		
		Day 0	Day 5	Day 12
I	Control (vehicle)	223.7 ± 18.6	230 ± 11.6	231.4 ± 14.3
II	Berberine (75 mg/kg)	248.0 ± 20.6	154.9 ± 14.3 ^a	89.4 ± 12.6 ^a
III	Berberine (150 mg/kg)	236.4 ± 12.3	129.4 ± 22.6 ^a	78.5 ± 17.2 ^{a,b}
IV	Glibenclamide (0.25 mg/kg)	187.7 ± 15.7	126.2 ± 13.9 ^a	103.8 ± 14.0 ^a

Values are expressed as mean ±SE; n=6; ^a statistical significance vs. control (p<0.05); ^b statistical significance vs. standard (p<0.05); one-way ANOVA followed by post hoc Scheffe's test.

Table 4. Effect of berberine on serum insulin and glycosylated hemoglobin levels

Treatment	Insulin	Glycosylated hemoglobin
	Serum (μU/mL)	Whole blood (%)
Normal	125.7 ± 15.3	3.2 ± 0.5
Diabetic control	95.4 ± 9.8	7.1 ± 0.2
Berberine (75 mg/kg)	104.6 ± 11.3	4.3 ± 0.7 ^{a,b}
Berberine (150 mg/kg)	97.1 ± 6.1	3.9 ± 0.9 ^{a,b}

^ap<0.05 vs. control; ^bp<0.05 vs. normal; one-way ANOVA followed by post hoc Scheffe's test; mean ±SE; n=6; insulin levels were not increased significantly and the levels of glycosylated hemoglobin were decreased in the treated diabetic rats as compared with diabetic rats.

Table 5. Effect of berberine on serum lipid profile

Treatment	Triglyceride	Cholesterol	HDL-Cholesterol
	Serum (mg/dL)	Serum (mg/dL)	Serum(mg/dL)
Normal	92.4 ± 1.7	59.3 ± 2.6	53.0 ± 3.2
Diabetic control	183 ± 13.2	123.9 ± 15.2	35.2 ± 2.7
Berberine (75 mg/kg)	57.3 ± 1.8 ^{a,b}	53.2 ± 3.8 ^{a,b}	54.9 ± 3.9 ^{a,b}
Berberine (150 mg/kg)	45.5 ± 2.3 ^{a,b}	45.7 ± 7.3 ^{a,b}	58.7 ± 4.5 ^{a,b}

^ap<0.05 vs. control; ^bp<0.05 vs. normal; one-way ANOVA followed by post hoc Scheffe's test; mean ±SE; n=6; cholesterol and triglyceride levels were decreased in the treated diabetic rats as compared with diabetic rats whilst HDL cholesterol levels were improved in the treated rats.

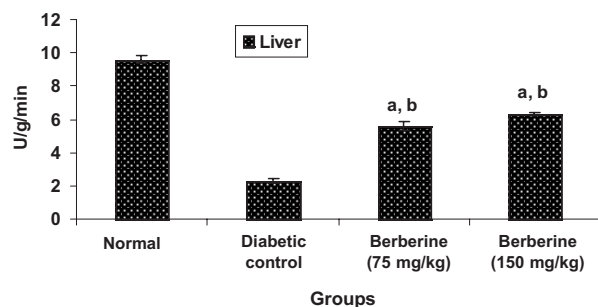
Table 6. Effect of berberine on changes in body weight in diabetic rats

Group	Treatment	Initial (g)	Final (g)
I	Diabetic control	273.3 ± 14.2	201.9 ± 13.2
II	Berberine (75 mg/kg)	219.4 ± 10.1	184.4 ± 14.8
III	Berberine (150 mg/kg)	237.8 ± 9.6	196.2 ± 12.5

Values are expressed as mean ± SE; n=6; the body weight of the treated animals did not improve significantly

Figures 1, 2 and 3 represent the activity of different key enzymes such as hexokinase, glucose-6-phosphate dehydrogenase and lactate dehydrogenase. Diabetic animals treated with berberine 150 mg/kg showed better enzyme activity than those treated with a lower berberine dose of 75 mg/kg. The treated groups exhibited a significant decrease in the levels of glucose-6-phosphatase (Fig. 4), alanine aminotransferase (Fig. 5), serum urea (Fig. 6) and serum creatinine levels (Fig. 7) as compared to the diabetic control animals. Figures 8, 9, 10, 11 and 12 represent the activity of enzymatic antioxidants such as catalase, superoxide dismutase, peroxidase, glutathione synthetase and glutathione peroxidase, which all increased significantly in the extract treated animals ($p < 0.05$). The effect of berberine treatment on nonenzymatic antioxidants such as ceruloplasmin, ascorbic acid and tocopherol is presented in Figures 13, 14 and 15, respectively. Significant results were observed in the treated groups as compared to the diabetic control and normal animals.

Figure 1. Hexokinase



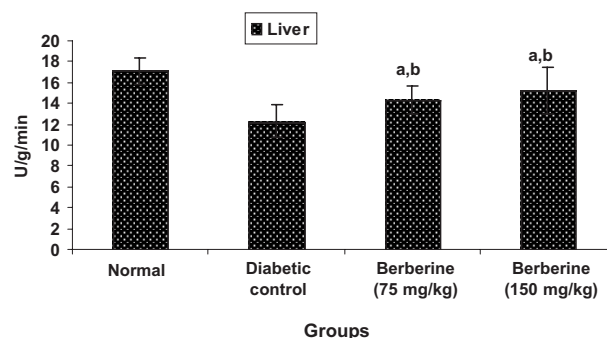
Berberine significantly increases hexokinase levels in diabetic rats. Each value represents mean ± SE, n=6; a, statistical significance vs. control ($p < 0.05$); b, statistical significance vs. normal ($p < 0.05$); U, μmol reduction of NAD+ per minute.

Table 7. Effect of berberine on liver glycogen and thiobarbituric acid reactive substance TBARS levels

Treatment	Glycogen		TBARS
	Liver (mg/g)	Liver (nM/mg)	Pancreas (nM/mg)
Normal	3.9 ± 0.6	0.144 ± 0.01	0.078 ± 0.05
Diabetic control	1.2 ± 0.1	0.304 ± 0.03	0.177 ± 0.003
Berberine (75 mg/kg)	2.4 ± 0.5 ^{a, b}	0.179 ± 0.01 ^{a, b}	0.105 ± 0.06 ^{a, b}
Berberine (150 mg/kg)	2.9 ± 0.1 ^{a, b}	0.165 ± 0.03 ^{a, b}	0.099 ± 0.01 ^{a, b}

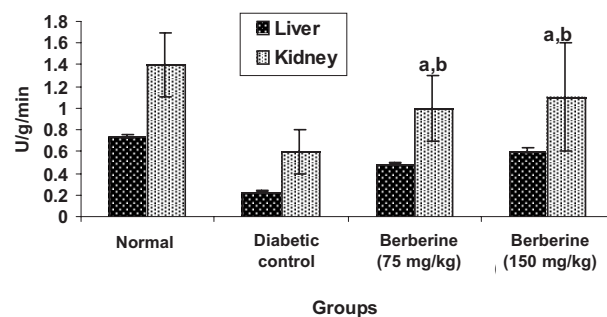
^a $p < 0.05$ vs. control; ^b $p < 0.05$ vs. normal; one-way ANOVA followed by post hoc Scheffe's test; mean ± SE; n=6; liver glycogen levels were increased significantly and the levels of TBARS in the liver and pancreas were decreased in the treated diabetic rats and compared with diabetic rats.

Figure 2. Glucose-6-phosphate dehydrogenase



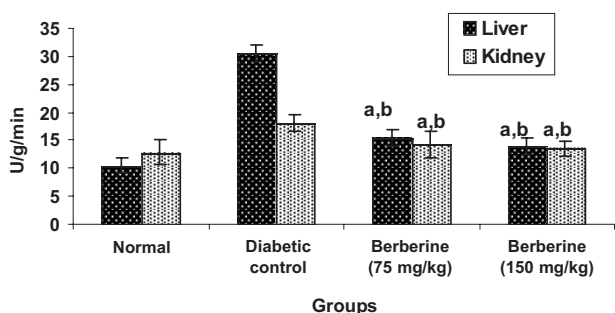
Berberine significantly increases glucose-6-phosphate dehydrogenase levels in diabetic rats. Each value represents mean ± SE, n=6; a, statistical significance vs. control ($p < 0.05$); b, statistical significance vs. normal ($p < 0.05$); U, μmol conversion of NAD to NADH per minute.

Figure 3. Lactate dehydrogenase



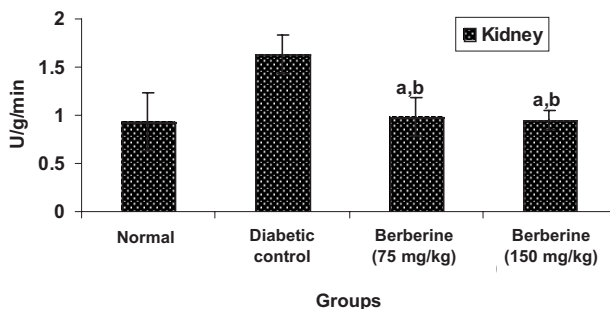
Berberine increases lactate dehydrogenase levels in diabetic rats. Each value represents mean ± SE, n=6; a, statistical significance vs. control ($p < 0.05$); b, statistical significance vs. normal ($p < 0.05$); U, μmol conversion of NAD to NADH per minute.

Figure 4. Glucose-6-phosphatase



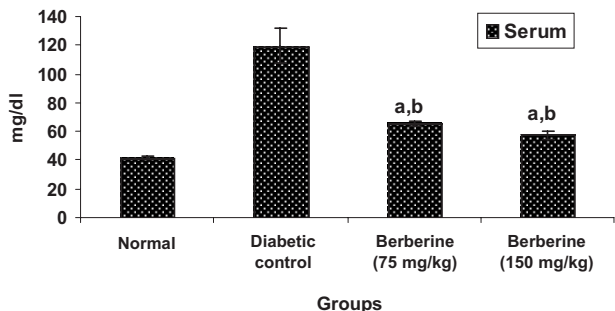
Berberine significantly decreases glucose-6-phosphatase levels in diabetic rats. Each value represents mean \pm SE, n=6; a, statistical significance vs. control ($p < 0.05$); b, statistical significance vs. normal ($p < 0.05$); U, μ mol conversion of glucose-6-phosphate to glucose.

Figure 5. Alanine aminotransferase



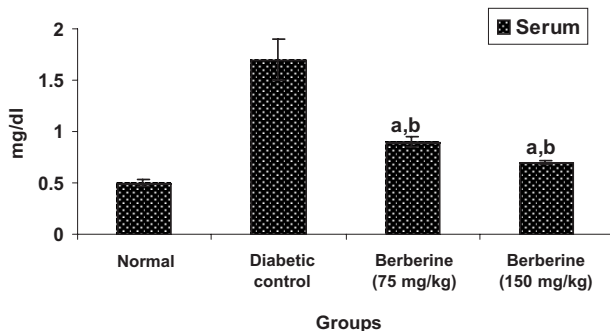
Berberine significantly decreases alanine aminotransferase levels in diabetic rats. Each value represents mean \pm SE, n=6; a, statistical significance vs. control ($p < 0.05$); b, statistical significance vs. normal ($p < 0.05$); U, μ mol conversion of NADH to NAD per minute.

Figure 6. Urea



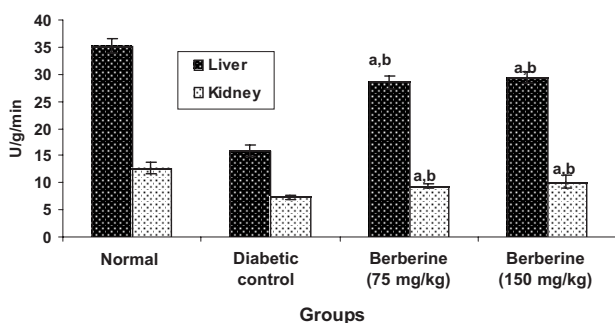
Berberine significantly decreases urea levels in diabetic rats. Each value represents mean \pm SE, n=6; a, statistical significance vs. control ($p < 0.05$); b, statistical significance vs. normal ($p < 0.05$).

Figure 7. Creatinine



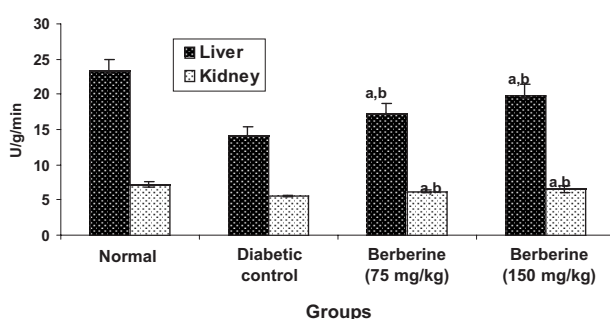
Berberine significantly decreases creatinine levels in diabetic rats. Each value represents mean \pm SE, n=6; a, statistical significance vs. control ($p < 0.05$); b, statistical significance vs. normal ($p < 0.05$).

Figure 8. Catalase



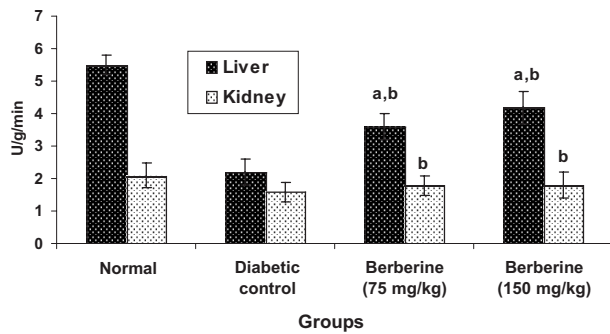
Berberine significantly increases catalase levels in diabetic rats. Each value represents mean \pm SE, n=6; a, statistical significance vs. control ($p < 0.05$); b, statistical significance vs. normal ($p < 0.05$); U, μ mol of hydrogen peroxide consumed per minute.

Figure 9. Glutathione peroxidase



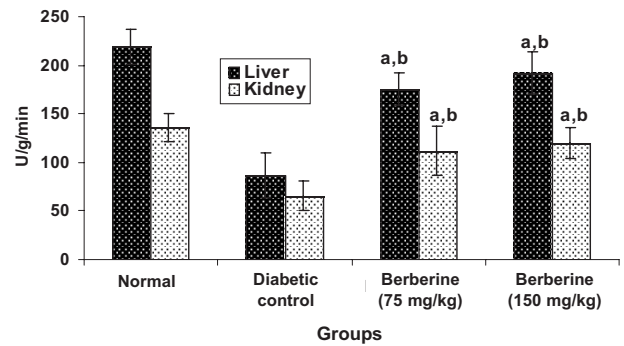
Berberine significantly increases glutathione peroxidase levels in diabetic rats. Each value represents mean \pm SE, n=6; a, statistical significance vs. control ($p < 0.05$); b, statistical significance vs. normal ($p < 0.05$); U, μ mol of glutathione consumed per minute.

Figure 10. Glutathione synthetase



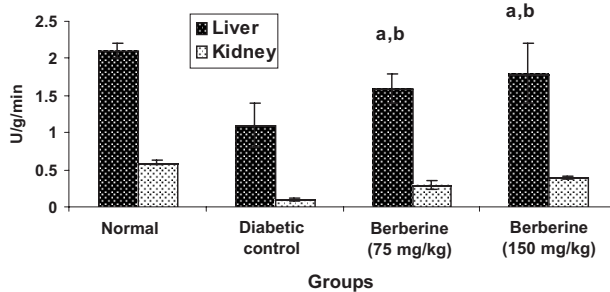
Berberine significantly increases glutathione synthetase levels in diabetic rats. Each value represents mean \pm SE, n=6; a, statistical significance vs. control ($p < 0.05$); b, statistical significance vs. normal ($p < 0.05$); U, μ mol of CDNB-glutathione synthetase conjugate formed *per* minute.

Figure 11. Superoxide dismutase



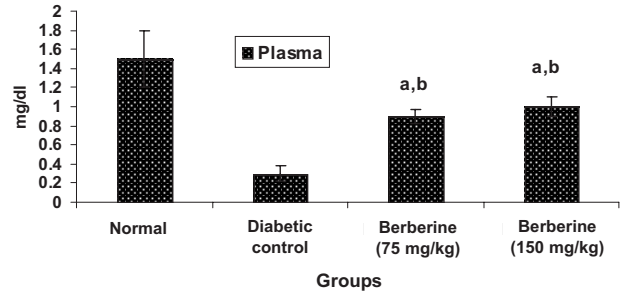
Berberine increases superoxide dismutase levels in diabetic rats. Each value represents mean \pm SE, n=6; a, statistical significance vs. control ($p < 0.05$); b, statistical significance vs. normal ($p < 0.05$); U, μ mol inhibition of NBT reduction *per* minute.

Figure 12. Peroxidase



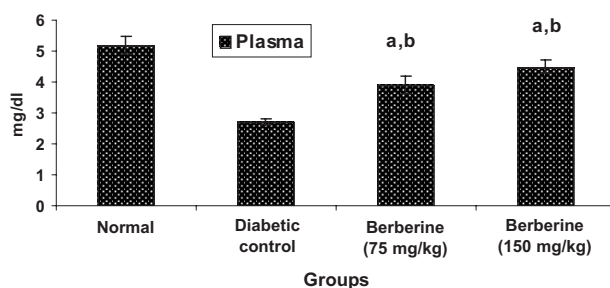
Berberine increases peroxidase levels in diabetic rats. Each value represents mean \pm SE, n=6; a, statistical significance vs. control ($p < 0.05$); b, statistical significance vs. normal ($p < 0.05$); U, μ mol of hydrogen peroxide consumed *per* minute.

Figure 13. Ascorbic acid



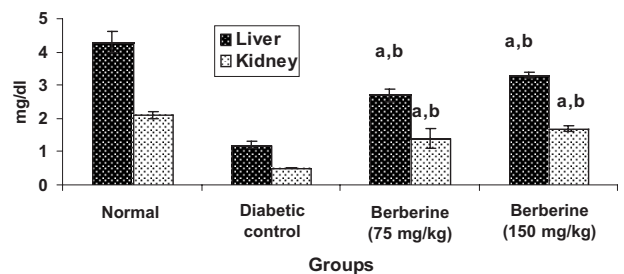
Berberine increases ascorbic acid levels in diabetic rats. Each value represents mean \pm SE, n=6; a, statistical significance vs. control ($p < 0.05$); b, statistical significance vs. normal ($p < 0.05$).

Figure 14. Ceruloplasmin



Berberine increases ceruloplasmin levels in diabetic rats. Each value represents mean \pm SE, n=6; a, statistical significance vs. control ($p < 0.05$); b, statistical significance vs. normal ($p < 0.05$).

Figure 15. Tocopherol



Berberine increases tocopherol levels in diabetic rats. Each value represents mean \pm SE, n=6; a, statistical significance vs. control ($p < 0.05$); b, statistical significance vs. normal ($p < 0.05$).

DISCUSSION

The present study was undertaken with the objective of exploring the antidiabetic potential of berberine in STZ-nicotinamide induced diabetic rats through carbohydrate metabolism, lipid metabolism and antioxidant status. No toxic reactions were observed, thereby suggesting the nontoxic nature of berberine at the selected doses of 75 and 150 mg/kg till the end of the experimental period. In the oral glucose tolerance test, berberine significantly reduced the blood glucose level in glucose-loaded rats at 30, 60, 90 and 120 min. At 30 and 60 min, a significant decrease was observed in the blood glucose level of treated glucose loaded rats as compared with control rats, loaded only with glucose. Normoglycemic studies further revealed the potential of berberine to decrease the blood glucose level in normal rats.

STZ causes diabetes by the rapid depletion of β -cells and thereby brings about a reduction in insulin release. Hyperglycemia causes oxidative damage by the generation of reactive oxygen species (40) and results in the development of diabetic complications (41,42), i.e. cardiovascular, gastrointestinal, nervous, vas deferens, kidney and urinary bladder dysfunctions (43). In our study, an increase in the blood glucose level in diabetic rats confirmed the induction of diabetes mellitus. A statistically significant ($p < 0.05$) decrease was observed in the blood glucose level of diabetic rats treated with berberine in the doses of 75 and 150 mg/kg when compared with diabetic control rats at the end of the 12-day experimental period. No significant change was noted in the serum insulin levels of the diabetic animals treated with berberine, thereby suggesting that berberine probably exerts antihyperglycemic activity by an extrapancreatic mechanism independent of insulin secretion.

In STZ induced diabetes, the characteristic loss of body weight caused by an increase in muscle wasting (44) showed no significant change upon treatment with berberine. The elevation of plasma lipid concentration has been well documented in diabetes (45), and the marked increase observed in serum triglycerides and cholesterol is in agreement with the findings of Nikkila and Kekki (46). While the serum triglyceride and cholesterol levels decreased significantly in berberine treated diabetic rats, the HDL cholesterol level was found to improve significantly. Hence, the weight loss associated with

treated diabetic rats may be attributed directly to the lipid lowering activity of berberine or indirectly to its influence on various lipid regulation systems.

The significant decline in glycosylated hemoglobin indicates the efficiency of berberine in glycemic control. A marked increase in the concentration of TBARS has been observed in STZ diabetic rats (47). The increased susceptibility of the tissues of the diabetic animals may be due to the activation of the lipid peroxidation system. The decrease in the level of TBARS observed in the pancreas and liver of treated animals may be due to the inactivation of the lipid peroxidation system.

The significant increase in the levels of hexokinase, a key glycolytic enzyme known to decrease in the diabetic state (48), may be due to the direct stimulation of glycolysis in tissues with increased glucose removal from the blood. The significant reversal of diabetes induced decreased levels of glucose-6-phosphate dehydrogenase and lactate dehydrogenase may be attributed to an increase in glucose utilization through the pentose phosphate pathway (49). This interferes with the mitochondrial respiratory chain and promotes the peripheral glucose utilization by enhancing anaerobic glycolysis (50).

Glucose-6-phosphatase, a key enzyme in gluconeogenesis, plays an important role in glucose homeostasis in the liver and kidney (51). The decreased levels observed in treated diabetic animals may be due to the suppression of hepatic gluconeogenesis and glucose output from the liver. The elevation in alanine aminotransferase in the liver and kidney observed in STZ induced diabetic rats is consistent with earlier findings (52,53), which attribute the increased gluconeogenesis and ketogenesis observed in diabetes to the high activity of transaminases. In our study, the treatment with berberine was found to significantly decrease the enhanced transaminase activity.

Glycogen synthesis in the rat liver and skeletal muscle is impaired in diabetes (54). In earlier studies, a decrease was observed in the hepatic and muscle glycogen content of diabetic animals (55). The significant increase observed in the glycogen levels of treated groups in our study may be attributed to the reactivation of the glycogen synthase system.

Catabolism of the protein and nucleic acids results in the formation of non-protein nitrogenous compounds, urea and creatinine. In diabetes mellitus, the amino acid breakdown in the liver results in an increased production of urea and creatinine. Treatment with berberine was found to significantly decrease the creatinine and urea levels.

Catalase, superoxide dismutase, peroxidase, glutathione synthetase and glutathione peroxidase are examples of enzymatic antioxidants. Superoxide dismutase and catalase are considered as primary enzymes since they are involved in the direct elimination of reactive oxygen species (56). Superoxide dismutase is an important defense enzyme, which catalyzes the dismutation of superoxide radicals (57), and catalase is a hemoprotein, which catalyzes the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals (58). The reduced activity of superoxide dismutase and catalase in the liver and kidney observed in diabetes may pose deleterious effects as the result of the accumulation of superoxide anion radicals and hydrogen peroxide (59). Glutathione synthetase, the most important biomolecule protecting against chemical induced toxicity, participates in the elimination of reactive intermediates by reduction of hydroperoxide in the presence of glutathione peroxidase (60,61). The decreased level of glutathione synthetase observed in diabetic animals represents an increased utilization resulting from oxidative stress (62). Glutathione peroxidase, a selenium containing enzyme present in significant concentrations, detoxifies H_2O_2 to H_2O through the oxidation of reduced glutathione (63). Depression of glutathione peroxidase activity, observed in diabetic liver and kidney, has been shown to be an important adaptive response to increased peroxidative stress (64). In our study, the activity of enzymatic antioxidants was found to increase significantly upon the treatment with berberine ($p < 0.05$).

Antioxidants, i.e. tocopherol, ceruloplasmin and ascorbic acid, are nonenzymatic antioxidants. While α -tocopherol reduces lipid hydroperoxides generated during the process of peroxidation and protects cell structures against damage (65), the powerful nonenzymatic antioxidant ceruloplasmin inhibits lipid peroxidation by binding to copper (66). Vitamin C or ascorbic acid is an excellent hydrophilic antioxidant in plasma and disappears faster than other antioxidants upon exposure to reactive oxygen species (67). The decreased level of ascorbic acid in diabetic rats may be due either to increased utilization as an antioxidant defense against increased reactive oxygen species or to a decrease in glutathione level, since glutathione is required for the recycling of ascorbic acid (68,69).

The levels of both enzymatic and nonenzymatic antioxidants, which declined in the diabetic animals, were significantly restored upon the treatment with berberine. The overexpression of these antioxidant parameters in diabetic rats treated with berberine implies that this potential oxidant defense is reactivated by berberine with an increase in the capacity for detoxification through enhanced scavenging of oxy-radicals.

The present investigation shows that berberine has antidiabetic activity in STZ-nicotinamide induced type 2 diabetic rats. However, further studies on the structure activity relationship and its mode of action will provide deeper insights for the discovery of better and safer therapeutics.

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