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Full Length Research Paper

Effect of 3-hydroxymethyl xylitol on hepatic and renal functional markers and protein levels in streptozotocin-diabetic rats

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Oral administration of 3-hydroxymethyl xylitol at 40 mg/kg body weight to diabetic rats for 45 days resulted in a significant reduction in blood glucose and significant increase in plasma insulin levels. In addition, the diabetic rats had decreased levels of plasma total protein, albumin, globulin and albumin/globulin ratio as compared to control rats. After treatment with 3-hydroxymethyl xylitol and glibenclamide total protein, albumin, globulin and albumin/globulin ratio were brought back to near normal. The activities of hepatic and renal markers were significantly elevated in diabetic rats as compared to control rats. Treatment with 3-hydroxymethyl xylitol and glibenclamide reversed these parameters to near normal levels. In diabetic rats, the decreased levels of urea, uric acid and creatinine with increased levels of albumin and urine volume was observed, and treatment with 3-hydroxymethyl xylitol and glibenclamide reversed these parameters to near normal. These results indicate that 3-hydroxymethyl xylitol, a compound isolated from *Casearia esculenta,* has beneficial effect on hepatic and renal functional markers.

Key words: *Casearia esculenta,* 3-hydroxymethyl xylitol, glibenclamide, diabetes, liver, kidney, functional markers.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from the defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (American Diabetes Association, 2008). The liver and kidney play a major role in the pathogenesis of type 2 diabetes. Nephrotoxicity is one of the major side effects of drug therapy in clinical practice, frequently leading to acute renal failure. Many physiological mechanisms have been implicated in streptozotocin-induced renal injury in diabetes (Babu and Srinivasan, 1998). In diabetes mellitus, a variety of proteins is subjected to non-enzymatic glycation and is thought to contribute to the long-term complication of the disease (Vlassara et al*.,* 1981). Renewed attention to alternative medicines and natural therapies has stimulated new wave of research interest in traditional practices, and there is a need to look for more efficacious agents with lesser side effects. Recently there is a growing interest in herbal remedies due to the side effects associated with the available oral hypoglycemic agents for the treatment of diabetes mellitus (Kim et al., 2006).

Casearia esculenta Roxb. (Flacourtiaceae) is one such plant in Indian traditional medicine has a popular remedy for the treatment of diabetes (Yoganarasimhan, 2000). Preliminary research revealed a significant blood glucose lowering effect (Prakasam et al*.,* 2002) and antihyperlipidemic activity (Prakasam et al. 2003) after oral administration of *C. esculenta* root extract in normal and streptozotocin-diabetic rats. The active compound, 3-hydroxy-

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methyl xylitol was isolated from *C. esculenta* root and at an optimum dose of 40 mg it decreased blood glucose level and improved body weight in a 15-day study (Chandramohan et al.., 2007). The compound exhibited antihyperglycemic activity in STZ-rats in a long-term study (45 days) (Chandramohan et al., 2008). In this study, we have investigated the effect of 3-HMX on hepatic and renal functional markers and protein level status in streptozotocin-diabetic rats.

MATERIALS AND METHODS

Plant material

The root of *C. esculenta* was collected from Kolly Hills, Namakkal District, Tamil Nadu, India. The plant was botanically identified and authenticated in the Department of Botany, Annamalai University, Annamalainagar, Tamil Nadu, India and a voucher specimen (No. AU 2145) was deposited at the herbarium of Botany. The root of the plant was air dried at 25–28°C and the dried root was ground into fine powder with auto-mix blender.

Isolation and identification of the active compound (Chandramohan et al., 2007, 2008)

Using percolation method, 2 kg of *C. esculenta* root powder was extracted with 6 L of benzene (1:3 w/v). The residue left after extraction with benzene was further extracted with alcohol (1:3 w/v) for 72 h. The filtrate was concentrated using a rotary evaporator at room temperature (32±2°C) and centrifuged. The solid matter obtained was washed with diethyl ether, dried at room temperature (5.7 g) and labeled as fraction 1. The remaining alcohol portion was labeled as fraction 2. Fractions 1 and 2 were tested for plasma glucose lowering activity in normal and streptozotocin-diabetic rats, over a period of 2 h that is fasting and postprandial glucose level. Fraction 1 exhibited a significant reduction in plasma glucose while fraction 2 showed no activity. Then fraction 1 was treated with hot water (65 ± 5°C) and filtered (Fraction 1a). The remaining residue was labeled as fraction 1b. Fraction 1a was freeze-dried and lyophilized to obtain a white amorphous compound (1.6 g), with a sweet taste and a melting point of 128°C, which exhibited significant reduction of plasma glucose. Fraction 1b did not show any significant reduction on plasma glucose. Fraction 1a was spotted on a precoated silica gel 60 F254, 0.25 mm thick TLC plate (Merck) and run in acetonitrile and water (8.5:1.5) system. A single spot was obtained confirming the purity of the compound. The structure of the active principle was determined on the basis of FT-IR, ^{1}H NMR, ^{13}C NMR and MS.

The compound was identified based on the following evidences: MS: m/z=182 [M] +, [M+1] 183, 133, 115, 103, and 85. Figure 2 shows the IR (neat) max/cm: 3369, 2942 and 1455 indicating the presence of corresponding functional groups (–OH) (–$CHCH_2$) and (–CH). $^{1}HNMR$ (400 MHz, D_2O) δ/ppm: 3.5 (d, –CH_2–CHOH, J=6.78 Hz), 3.8 (t, 2H, CHOH, J =6.32 Hz). ^{13}C NMR (400 MHz D_2O) δ/ppm: 63 (CH_2OH), 70 (CHOH), 70.8 (CHOH).

Animals

Male Wister albino rats of strain with body weight ranging from 180 to 200 g were procured from Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. The animals were maintained in an air conditioned room (25 ± 1°C) with a 12 h light: 12 h dark cycle. Feed and water were provided *ad libitum*. Studies were carried out in accordance with Indian National Law on Animal Care and Use. Committee for the Purpose of Control and Supervision of Experiments on Animals of Rajah Muthiah Medical College and Hospital (Pro. No. 282, Reg No.160/1999/CPCSEA), Annamalai University, Annamalainagar, provided ethical clearance.

Chemicals

Streptozotocin was purchased from Sigma-Aldrich, St. Louis, USA. All other chemicals were of analytical grade and obtained from E. Merck or Himedia, Mumbai, India.

Experimental induction of diabetes

The animals were rendered diabetes by a single intraperitoneal injection of streptozotocin (40 mg/kg body weight) in freshly prepared citrate buffer (0.1 M, pH 4.5) after an overnight fast (Ramesh and Pugalendi, 2006). Streptozotocin injected animals were given 20% glucose solution for 24 h to prevent initial drug induced hypoglycemic mortality. Streptozotocin injected animals exhibited massive glycosuria (determined by Benedict's qualitative test) and diabetes in streptozotocin rats was confirmed by measuring the fasting blood glucose concentration, 96 h after injection. The animals with blood glucose above 240 mg/dl were considered to be diabetic and used for the experiment.

Experimental design

The animals were randomly divided into five groups of six animals each. 3-HMX or glibenclamide were dissolved in water and administered orally using intragastric tube at 10.00 a.m. The duration of treatment was 45 days.

Group I : Normal (1 ml of water)
Group II : Normal + 3-HMX (40 mg/kg BW.) in 1 ml of water
Group III: Diabetic control (1 ml of water)
Group IV: Diabetic + 3-HMX (40 mg/kg BW.) in 1 ml of water
Group V: Diabetic + glibenclamide (600µg/kg BW.) in 1 ml of water (Ramesh and Pugalendi, 2006)

Sample collection

After 45 days of treatment, the animals were fasted for 12 h, anaesthetized using ketamine (24 mg/kg body weight, intramuscular injection), and sacrificed by decapitation. Blood was collected in tubes with a mixture of potassium oxalate and sodium fluoride (1:3) for the biochemical analysis. Animals were placed in individual metabolic cages for 24 h urine collection and measured to determine the urea, uric acid, creatinine and albumin. Urine samples were acidified with 2 ml 1 mol/1 HCL and centrifuged at 1200 ×g for 10 min at 4°C to contaminating and aliquots were protected from light and stored at 20°C until they were assayed. Tissues of liver and kidney were immediately dissected out, washed in ice-cold saline to remove the blood and were sliced into pieces and homogenized in an appropriate buffer (pH 7.0) in cold condition to give 20% homogenate (w/v). The homogenates were centrifuged at 1000 rpm for 10 min at 0°C in cold centrifuge. The supernatants were separated and used for various biochemical estimations.

Biochemical analysis

Plasma glucose was estimated by the method of Trinder using a reagent kit (Trinder, 1969). The plasma insulin was measured by the method of Burgi et al., (1988). The activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) were es-

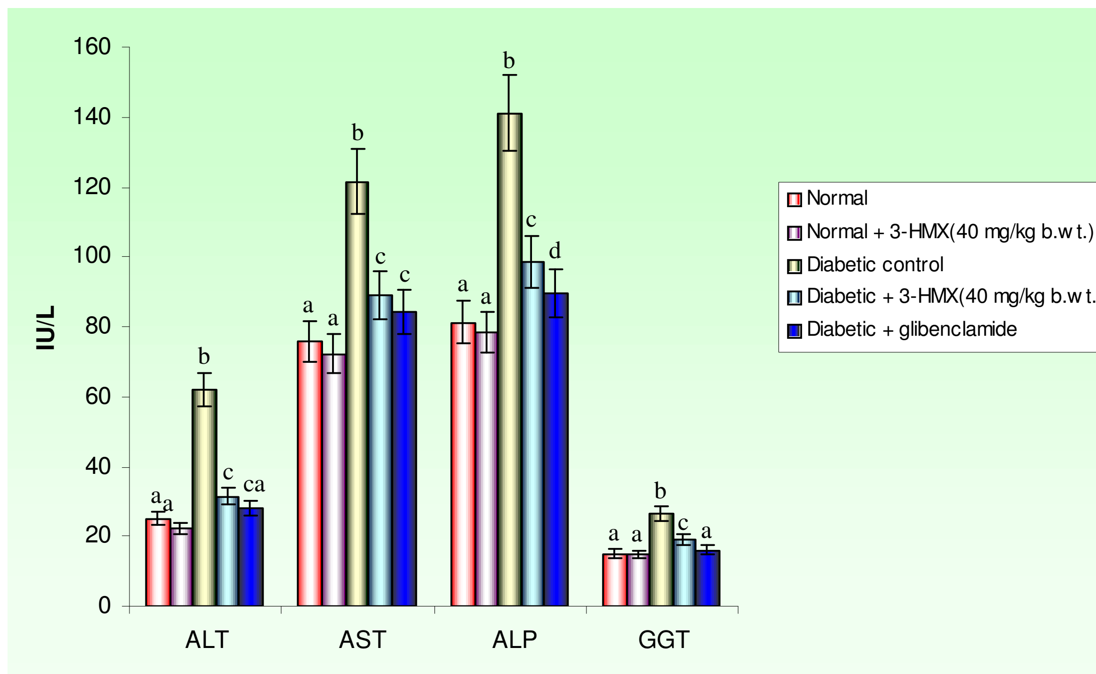


Figure 1. Effect of 3-HMX on serum ALT, AST, ALP and GGT activities in the normal and STZ- diabetic rats.

Values are means ± S.D for six rats.
Values not sharing a common superscript differ significantly at *P*<0.05 (DMRT).
ALT and AST:IU[@]- µmol of pyruvate liberated per hour
ALP: IU[$] - µmol of phenol liberated per minute
GGT:*-µmol of p-nitroanilide liberated per minute.

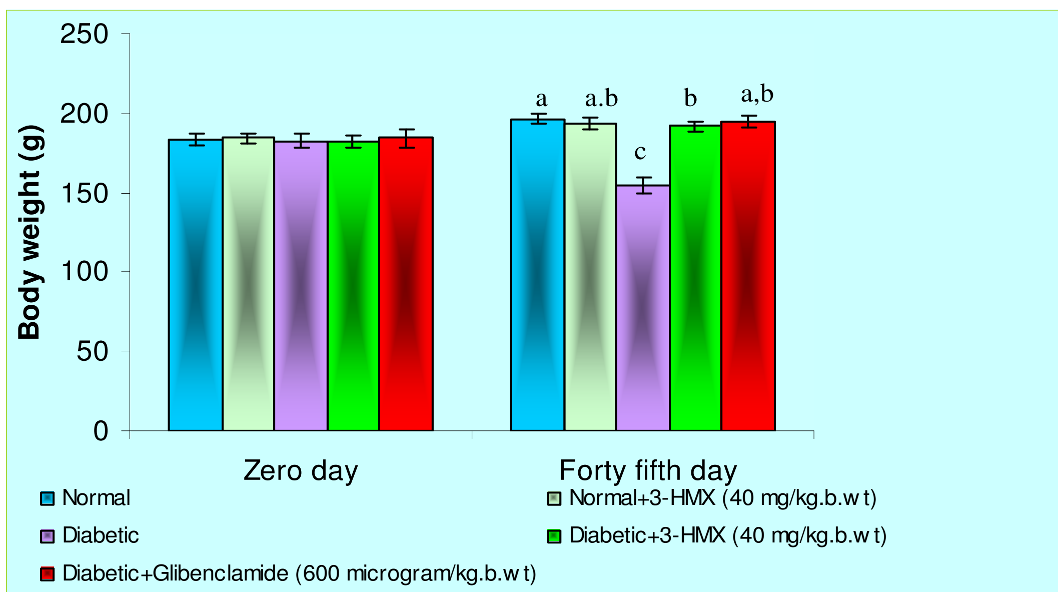


Figure 2. Effect of 3-HMX on body weight changes in the normal and STZ- diabetic rats.

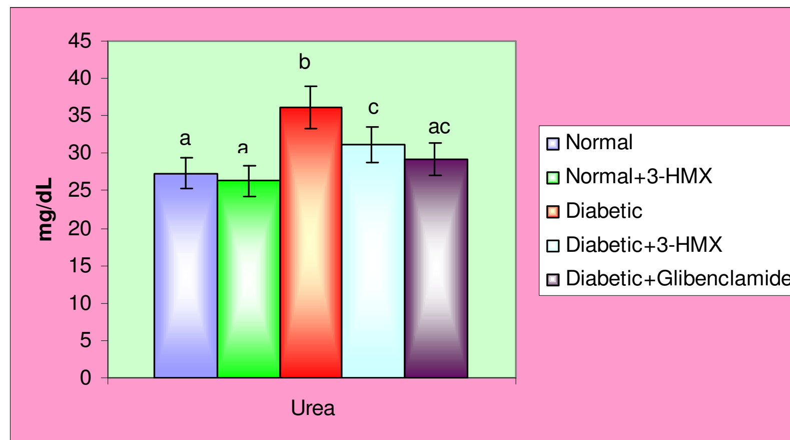
Values are means ± S.D for six rats.
Values not sharing a common superscript differ significantly at *P*<0.05 (DMRT).

Table 1. Effect of 3-HMX on glucose, insulin, total protein, albumin, globulin and A/G ratio in the plasma of normal and STZ- diabetic rats.

Name of the group	Plasma					
	Fasting Glucose (mg/dl)	Insulin (μU/ml)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
Normal	83.15 ± 6.33[a]	17.31 ± 0.83[a]	7.23 ± 0.55[a]	3.46 ± 0.26[a]	3.77 ± 0.28[a]	0.91 ± 0.06[a]
Normal + 3-HMX (40 mg/kg b.wt.)	69.83 ± 6.45[b]	18.23 ± 0.81[b]	7.47 ± 0.56[a]	3.83 ± 0.29[b]	3.64 ± 0.27[ab]	1.05 ± 0.07[b]
Diabetic control	292.45 ± 4.85[c]	5.88 ± 0.44[c]	4.14 ± 0.31[b]	1.81 ± 0.13[c]	2.33 ± 0.17[c]	0.71 ± 0.05[c]
Diabetic + 3-HMX (40 mg/kg b.wt.)	122.21 ± 6.05[d]	16.17 ± 0.48[d]	6.12 ± 0.46[c]	2.92 ± 0.21[d]	3.20 ± 0.24[d]	0.82 ± 0.06[d]
Diabetic + glibenclamide (600 μg/kg b.wt.)	117.39 ± 5.94[d]	16.49 ± 0.64[d]	6.58 ± 0.50[c]	3.15 ± 0.23[d]	3.43 ± 0.26[bd]	0.85 ± 0.06[ad]

Values are means ± S.D for six rats.
Values not sharing a common superscript differ significantly at *P*< 0.05 (DMRT).

Figure 3. Effect of 3-HMX on urea in the plasma of normal and STZ-diabetic rats.



Values are means ± S.D for six rats.
Values not sharing a common superscript differ significantly at *P*< 0.05 (DMRT).

timated (by using commercially available kits), by the method of Reitman and Frankel, (1957). The activities of serum alkaline phosphatase (ALP) and γ-glutamyl transferase (γ-GT) were estimated by the methods of Kind and King, (1954) and Rosalki and Rau, (1972), respectively. Total protein and albumin in the serum were estimated by Biuret method (Reinhold, 1953). Urea in the plasma and urine was estimated by using the diagnostic kit based on the method of Fawcett and Scott, (1960). Uric acid in the plasma and urine was estimated by using the diagnostic kit based on the enzymic method described by Caraway, (1955). Creatinine in the plasma and urine was estimated using the diagnostic kit based on the method of Tietz, (1987) using Jaffe's (1886) colour reaction.

Data were analyzed by one-way analysis of variance followed by Duncan's Multiple Range Test (DMRT) using SPSS version 10 (SPSS, Chicago, IL). The limit of statistical significance was set at *P*<0.05.

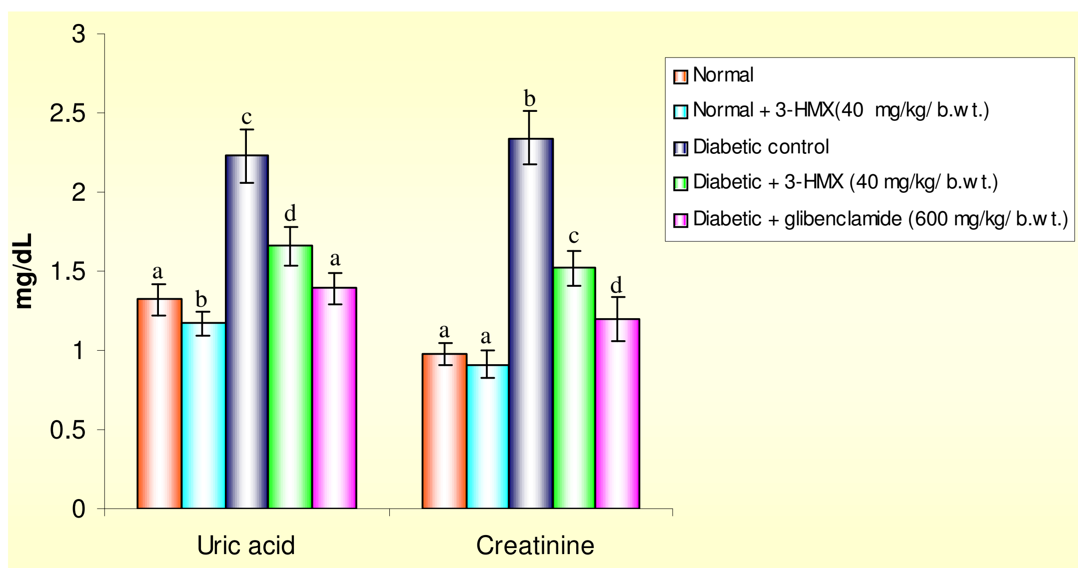
RESULTS

The activities of serum liver enzymes in normal and diabetic rats are shown in Figure 1. Increased activities of AST, ALT, ALP and GGT were observed in diabetic's rats. Oral administration of 3-HMX and glibenclamide reversed these parameters towards normalcy.

The body weight changes in normal and diabetic rats are shown in Figure 2 and the levels of glucose, insulin, total proteins, albumin, globulins and albumin/globulin ratio in the plasma of normal and diabetic rats are presented in the Table 1. The diabetic rats had decreased the body weight, increased glucose and decreased levels of plasma insulin, total proteins, albumin, globulins and albumin/globulin ratio when compared with normal rats. After treatment with 3-HMX or glibenclamide showed reversal of these parameters towards normalcy.

The urea, uric acid and creatinine in the plasma of normal and diabetic rats are shown in Figures 3 and 4 respectively. In our study, the levels of urea, uric acid and creatinine elevated markedly in the plasma of diabetic rat

Figure 4. Effect of 3-HMX on uric acid and creatinine in the plasma of normal and STZ-diabetic rats.



Values are means ± S.D for six rats.
Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 2. Effect of 3-HMX on urea, uric acid, creatinine and albumin in the urine of normal and STZ-diabetic rats.

Name of the group	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Albumin (µg/dl)	Urine volume (ml/d)
Normal	148.28 ± 11.28[a]	7.68 ± 0.58[a]	2.64 ± 0.20[a]	149.52 ± 11.38[a]	8.46 ± 0.64[a]
Normal + 3-HMX (40 mg/kg b.wt.)	146.87 ± 11.18[a]	7.82 ± 0.59[ab]	2.76 ± 0.21[a]	144.58 ± 11.00[a]	8.26 ± 0.57[a]
Diabetic control	109.41 ± 8.37[b]	5.89 ± 0.45[c]	1.69 ± 0.12[b]	313.27 ± 23.97[b]	19.54 ± 1.49[b]
Diabetic + 3-HMX (40 mg/kg b.wt.)	127.43 ± 9.70[ac]	6.58 ± 0.501[d]	2.25 ± 0.17[c]	198.90 ± 15.14[c]	12.61 ± 0.95[c]
Diabetic + glibenclamide (600 µg/kg b.wt.)	139.18 ± 10.59[a]	7.12 ± 0.54[ad]	2.38 ± 0.17[c]	171.31 ± 13.04[d]	10.23 ± 0.77[d]

Values are means ± S.D for six rats.
Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

as compared with normal rats. Diabetic rats treated with 3-HMX showed the reversal of these parameters towards normalcy. Urine output and the levels of urea, uric acid, creatinine and albumin in the urine of diabetic rats were shown in Table 2. Diabetic rats had decreased levels of urea, uric acid, creatinine and increased albumin and urine output as compared with normal rats, and treatment with 3-HMX reversed these parameters towards normalcy.

DISCUSSION

The increase in the activities of AST, ALT, ALP and GGT in plasma may be mainly due to the leakage of these enzymes from the liver cells into the blood stream (Navarro et al., 1993), indicated on the hepatotoxic effect of streptozotocin. Administration of 3-HMX, the compound isolated from *C. esculenta* root lowered the serum AST, ALT, ALP and GGT activities in diabetic rats. Our findings were in agreement with (Prakasam et al., 2004); the *C. esculenta* root extract has been beneficial effect on protein metabolism and liver marker enzymes in streptozotocin-induced diabetic rats. On treatment with 3-HMX to normal rats, did not produced any hepatotoxicity after 45 days. In this context, reports are available on a related compound, xylitol, were administered to normal rats did not observe any hepatotoxicity after 2 weeks (Truhaut et al., 1977).

The levels of plasma total proteins were found to be decreased in this study. This could be due to increased lipid peroxidation in the diabetic rats. Decreased protein content of serum in diabetic patients were reported by Mahboob et al., (2005) indicating elevated lipid peroxidation process and decreased antioxidant defensive system. Insulin generally has an anabolic effect on protein metabolism in that it stimulates protein synthesis and retards protein degradation (Murray et al., 2000). Protein synthe-

sis is decreased in all tissues due to decreased production of ATP in absolute or relative deficiency of insulin (Chatterjee et al., 1994). This may be responsible for the decreased level of plasma proteins in diabetic rats. In our study, the elevated level of plasma total proteins, albumin and globulins in diabetic rats treated with 3-HMX and glibenclamide may be related to the increased level of plasma insulin. It is well known that, our control drug of glibenclamide is insulin secretory action involves intracellular mechanisms because 90% of its binding sites are located inside the β-cell. Glibenclamide stimulate insulin release by inhibiting carnitine palmitoyltransferase 1 activity, which switches fatty acid metabolism from β-oxidation to protein kinase C–dependent insulin exocytosis (Akira et al., 2007). Plasma A/G ratio was lower in diabetic animals (Chatterjee et al., 1994). Increased protein catabolism in diabetes might have induced a direct adverse effect on the synthesis and secretion of albumin. Diabetic rats treated with 3-HMX and glibenclamide, brought A/G ratio also to near normal level.

Kidneys maintain optimum chemical composition of body fluids by acidification of urine and removal of metabolite wastes such as urea, uric acid and creatinine. In renal disease, the concentrations of these metabolites increase in blood. Our data showed that plasma uric acid, urea, and creatinine levels were increased in diabetic rats. This may be due to metabolic disturbance in diabetes reflected in high activities of xanthine oxidase, lipid peroxidation, and increased triacylglycerol and cholesterol levels (Madinov et al., 2000). Moreover, protein glycation in diabetes may lead to muscle wasting and increased release of purine, the main source of uric acid, as well as increased activity of xanthine oxidase (Madino et al., 2000). Our data showed that 3-HMX decreased the plasma uric acid, urea, and creatinine levels in diabetic rats. Elevation of the plasma urea and creatinine, as significant renal function markers, are related to renal dysfunction in diabetic hyperglycemia (Almadal and Vilstrup, 1988).

Albuminuria is a major prognostic factor for progressive diabetic renal disease, cardiovascular disease (Dinneen and Gerstein, 1997; Parving et al., 2004) and diabetic retinopathy (Rossing et al., 1998) in diabetic patients. Gomes et al. (1997) observed that untreated diabetic animals, developed albuminuria that may be due to impaired tubular reabsorption or leakage of albumin due to damaged glomerular membrane. Treatment with 3-HMX reduced urine albumin towards normal level, which reflects the reduced kidney damage from the hyperglycemia, induced oxidative insult, by improved glycemic control and defense mechanisms. 3-hydroxymethyl xylitol is having beneficial effect on hepatic and renal functional markers and protein levels and its activity is similar to glibenclamide.

Conclusion

In conclusion, our study suggests that liver and kidney functions are highly altered in diabetic state. Treatment with 3-hydroxymethyl xylitol and glibenclamide reversed these changes in diabetic rats, which indicates that 3-hydroxymethyl xylitol and glibenclamide protect the hepatic and renal function in the diabetic condition. Further detailed investigation is necessary to find out its mechanism of action and to establish its therapeutic potential in the treatment of diabetes and diabetic complications.

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