Key words: glomerular capsule, glomerulus, hyperglycemia, Momordica dioica, renal tubules

SUMMARY

The aim of the present study was to investigate the antidiabetic and renal protective effect of Momordica dioica extract (MDMtE) in streptozotocin-diabetic rats. MDMtE treatment markedly reduced serum glucose, increased serum insulin and urea levels. Study results indicated that the antioxidant enzyme activity of kidney was increased, while thiobarbituric acid reactive substances (TBARS) were reduced in MDMtE treated diabetic rats. Furthermore, histologic observation of kidney of diabetic rats showed degenerative changes in glomerulus and renal tubules and extract treatment rejuvenated kidney histoarchitecture. In conclusion, the present results suggest that MDMtE protects kidney in severe diabetes and thus may provide a promising antidiabetic drug for managing diabetic kidney disorders.

INTRODUCTION

Severe diabetic nephropathy results in asymptomatic kidney failure and may cause narrow and clogged glomerulus; waste products cannot be excreted and remain in the blood and may cause cytotoxicity (1). Diabetes causes reduced nerve sensitivity including response to a filled bladder and the ensuing pressure can damage kidneys. Urinary tract may also cause bacterial infection due to high sugar concentration in urine. Kimmelstiel-Wilson and kidney hypertrophy-hyperfunction syndromes are also well established in diabetic nephropathy (2).

Currently there are over 240 million diabetics worldwide and about 40% of them develop severe diabetic nephropathy (3). Despite much research work, the diabetic kidney epidemic is increasing rapidly. Patients with diabetes kidney failure undergo either painful dialysis or kidney transplantation (4), which is both costly and harmful. Presently, researches to develop drugs that slow the progression of diabetic kidney damage with fewer side effects are being conducted, however, showing no significant outcome (5). This has led to increasing exploration of complementary and alternative medicine from natural sources having potent antidiabetic as well as nephroprotective activity with fewer side effects.
Momordica (M.) dioica (family Cucurbitaceae) is a climbing creeper and reported as an important medicinal herb since ancient times for headache, urinary calculi, jaundice, asthma, bronchitis, leprosy, fever, tumors, urinary discharges, excessive salivation and heart troubles (6).

Phytochemical investigations of M. dioica have shown the presence of lectins, β-sitosterol, saponin glycosides, triterpenes of ursolic acid, hederagenin, oleanolic acid, α-spiranosterol, stearic acid, gypsogenin and momodicaursenol (7-9).

In the present study, M. dioica fruits were used to evaluate the antidiabetic and renoprotective activity using diabetes-oxidative damage in kidney.

MATERIALS AND METHODS

Preparation of extract

M. dioica fruits were freshly collected from University of Rajasthan and authenticated by deposition of specimen with herbarium (Department of Botany, University of Rajasthan, RUBL 20394). The fruits (3 kg) were cleaned, shade dried and powdered for methanol extraction at room temperature. The filtrate was vacuum dried and concentrated to obtain final extract (yield 25.7 g).

Experimental animals

The present study was approved by the ethics committee (Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur, India). INSA, New Delhi guidelines were followed throughout the experiment for the use of animals. Colony bred male albino rats of Wistar strain weighing 170-190 g were randomized into ten groups of seven rats each and provided free food access and water ad libitum.

Experimental design

Streptozotocin (STZ; Sigma-Aldrich, USA) was dissolved in 0.1M sodium citrate buffer just prior to use and injected intraperitoneally to rats. Control rats (group I, n=9) received an equivalent volume of citrate buffer. After 72 h of STZ administration, the rats with serum glucose levels >250 mg/dL were selected for the study. The MDMtE was dissolved in distilled water and orally given to the rats for 21 consecutive days.

Group I: vehicle treated control group.
Group II: diabetic group.
Group III: diabetic rats treated with MDMtE (300 mg/kg b.w./day) dissolved in vehicle.
Group IV: diabetic rats treated with glibenclamide (0.3 mg/kg b.w./day) dissolved in vehicle.

Estimation of serum glucose

Serum glucose was measured in overnight fasted rats using a commercially available kit based on glucose oxidase method (10).

Measurement of kidney weight

Relative kidney weight was expressed by using the following equation:

\[
\text{Relative kidney weight} = \frac{\text{Kidney weight (g)}}{\text{(mg/100 g b.w.)} \times 100} \times \text{Total body weight (g)}
\]

Tissue and serum biochemical estimation

After autopsy under mild ether anesthesia, kidneys were quickly removed from the sacrificed rat, placed on cotton presoaked in ice cold saline and trimmed of adipose tissue. Kidneys were finely minced, homogenized and used for protein (11), lipid peroxidation (LPO) (12), ascorbic acid (13), antioxidant defense system assays (superoxide dismutase (SOD) (14), catalase (CAT) (15), reduced glutathione (GSH) (16), glutathione peroxidase (GPx) (17) and glutathione-s-transferase (GST) (18) estimations.

Kidney sample (200 mg) was homogenized with 5 mL of phosphate buffer with Na2EDTA and refrigerated, centrifuged at 10000 rpm for 20 min at 4°C.
°C. The clear supernatant was taken as the enzyme extract. Then 2.0 mL of phosphate buffer and 0.8 mL of H2O2 was added, and finally 0.2 mL enzyme extracts, and absorbance was immediately determined at 240 nm.

Kidneys were rinsed with phosphate buffer saline (PBS) solution, pH 7.4 to remove blood cells and clot. Tissue was homogenized in 50mM Tris-HCl, 5mM EDTA and 1mM DTT per gram tissue and centrifuged at 10000 xg for 15 min at 4 ºC. The supernatant was removed and absorbance measured at 240 nm.

GSH was spectrophotometrically measured using 2-nitrobenzoic acid per one mole of glutathione. The amount of GSH was expressed as nmol/mg protein. The lipid peroxides expressed as nmol of TBARS production per mg protein was measured spectrophotometrically using 1,1,3,3-tetraethoxypropane.

**Histopathology**

For histologic examination, kidneys were fixed in 10% formalin, embedded in paraffin wax, sectioned at 5 μm and stained with Harris hematoxylin and eosin (19).

### Statistical analysis

Values are given as mean ± SEM (standard error of the mean) and were compared using one way ANOVA to judge difference between various groups. Values of P<0.05 were considered statistically significant.

### RESULTS

The protective activity of *MDMtE* in diabetes-induced kidney damage was investigated. Glycemia and glycosuria levels as well as body-kidney weight ratio of control rats were within the normal range (Figs. 2 and 3, Table 1). Serum glucose level and body-kidney weight ratio were found to be elevated significantly (P<0.001) in diabetic rats. *MDMtE* administration to normal rats did not cause any alteration, whereas in diabetic rats *MDMtE* treatment caused significant decrease (P<0.001) in serum glucose level and body-kidney weight ratio.

A significant decrease (P<0.001) was observed in serum protein content of diabetic rats (group II). *MDMtE* as well as glibenclamide treatment of normoglycemic and hyperglycemic rats showed marked increase in serum protein level (Fig. 4).
Serum contents of renal markers, i.e. urine protein, uric acid, creatinine and urea nitrogen were significantly increased ($P<0.001$) in group II diabetic rats as compared to group I control rats (Table 1). Diabetic rats treated with MDMtE showed markedly decreased renal markers and urinary protein contents closer to normal rats. However, nonsignificant decreases were observed in MDMtE treated normal rats.

The concentration of GSH and LPO in kidney of control and diabetic rats is presented in Table 2. GSH content of diabetic rats was found to be significantly decreased, whereas LPO content markedly increased. MDMtE as well as glibenclamide treatment to diabetic
rats significantly restored the GSH and LPO contents to near normal. The effects were more pronounced in the groups of rats orally treated with 300 mg/kg dose of MDMtE.

The activities of antioxidant enzymes, i.e. SOD, CAT, GPx, GST and ascorbic acid in kidney of control and experimental diabetic rats are presented in Table 2. The activities of SOD, CAT, GPx, GST and ascorbic acid in kidney of group II diabetic rats were significantly decreased \((P<0.001)\). MDMtE treatment to diabetic rats significantly increased the enzyme activities in a dose-dependent fashion as compared to group II diabetic rats.

Kidney histopathology

Histologic study of kidney of diabetic rats showed severe degeneration, whereas treatment with MDMtE as well as with glibenclamide to diabetic rats caused marked regeneration in kidney histoarchitecture. Kidney of MDMtE treated control rats showed well rejuvenated renal corpuscles (glomerulus and Bowman’s capsule) with normal proximal and distal convoluted tubules. In addition to proximal and distal convoluted tubules, STZ also caused degeneration in glomerulus with increased capsular space. Oral MDMtE administration rejuvenated proximal and distal convoluted tubules with well regenerated glomerulus and Bowman’s capsule.
DISCUSSION AND CONCLUSION

The present study assessed the antidiabetic and renoprotective effect of *M. dioica* on STZ-induced diabetes. STZ administration resulted in destruction of β-cells (20), which generate the superoxide, hydrogen peroxide and hydroxyl radicals. Furthermore, STZ releases nitric oxide that reduces aconitase activity and damages DNA at the O6 position of guanine (21). Oral *MDMtE* administration to diabetic rats resulted in marked diminution of serum glucose level, but no significant changes in fasting serum glucose level in *MDMtE* treated normal rats, thus also proving its antihyperglycemic action. Hypothetically, active principles act through a variety of mechanisms; however, in the present study it was not possible to pinpoint the exact mechanism of action of the extract. At the present level, only hypothetical suggestions can be made.

The body-kidney weight ratio of diabetic rats was found to be elevated in comparison to control rats. This may be due to fatty infiltration, enlargement of tubular cells lining, large hemorrhagic area and lymphocyte infiltration in hyperglycemic rats (22). In the present research, extract administration decreased the body-kidney weight ratio to near normal value, thus proving the kidney ameliorative activity of *MDMtE* in diabetic rats by maintaining or regenerating the renal cell histoarchitecture.

STZ administration elevated renal markers, i.e. serum urea nitrogen, uric acid and creatinine, which are found responsible for proper maintenance, functioning of kidney and change in the glomerular filtration rate (23). In the present study, an increase in renal markers of diabetic rats was observed, while *M. dioica* decreased the level of serum urea nitrogen, uric acid and creatinine. These results are in agreement with other previous studies (24,25).

A decrease in serum protein contents with concomitant increase in urinary protein level of diabetic rats were observed in the present study. Advanced oxidative protein products (AOPP), reactive oxygen species (ROS) and free radicals produce protein carbonyl products (PCO) and are considered as markers of oxygen-mediated protein damage, also indicating changes in glomerular filtration barrier that result in the increased permeability of the membrane (26,27). Extract administration increased the serum protein levels and decreased the urinary protein level, showing improvement in renal function.

Elevated lipid peroxidation (LPO) in the liver, kidney and pancreas is a characteristic feature of chronic diabetes (28). LPO damages cell membrane and leads to disturbed membrane integrity (29). Inhibition of free radical generation and oxidative damage could be considered as an important mechanism in the management of diabetes. In the present study, an increase in kidney LPO content was observed in STZ-diabetic rats. Previous studies have also reported increase in renal lipid peroxidation of diabetic rats (25,30). *MDMtE* administration to diabetic rats is likely to reverse the peroxide levels to near normal value. This indicates that *MDMtE* moderates oxidative stress-mediated complication.

The kidney of diabetic animals showed decrease in free radical and reactive oxygen scavenging activity of the key antioxidant enzymes SOD, CAT, GPx, GST and GSH. Reduced antioxidant activity results in over accumulation of O$_2^-$ and H$_2$O$_2$, which further generate OH$^-$ diabetic kidney damage (25). *MDMtE* administration increased the activities of antioxidants in the kidney of diabetic rats. This may be due to the excess production of antioxidants due to *MDMtE* administration and further protection from toxic effects of free radical intermediates, so it is concluded that the extract is very effective in diabetes and that the effects could be mediated through the pancreatic antioxidant without side effects.

In conclusion, the abnormal changes in antioxidant enzymes in the kidney of diabetic rats may reflect susceptibility to increased oxidant stress. It can be concluded that the possible mechanism by which *M. dioica* exerts its renoprotective activity against STZ-induced diabetes could be due to regeneration of kidney cells through an improved synthesis of proteins and/or its accelerated detoxification, as well as to the potential to minimize the deleterious effects of free radicals including peroxynitrate and its antioxidant
activity in combination with the inhibition of LPO and NO. Thereby *M. dioica* can be referred to as a natural antioxidant.

**Acknowledgments** The authors are thankful to the Head and Coordinator, CAS, Department of Zoology, University of Rajasthan, Jaipur, India, for providing necessary facilities.

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