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Full Length Research Paper

Effects of *Momordica charantia* aqueous extract on renal histopathological changes associated with streptozotocin- induced diabetes mellitus type II in neonatal rats

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This study investigated the effects of *Momordica charantia* (MC) extract on the renal functional and morphological changes in neonatal diabetic rats, a model of non-insulin-dependent diabetes mellitus. Diabetes mellitus was induced in 21 one day old Sprague-Dawley neonatal rats using a single intrapretoneal injection of STZ (85 mg/kg) and monitored for 12 weeks thereafter. The diabetic rats were randomly divided into three groups as follows: the diabetic control group, the MC treated diabetic group, and the glibenclamide treated diabetic group. The blood samples were collected to measure blood glucose, serum urea and creatinine. Urine creatinine, urine total protein and glomerular filtration rate was determined. Determination of malondialdehyde in plasma and kidney was also carried out. The kidney samples were taken for light and electron microscopic examinations. The level of serum creatinine and urea was significantly low in treated diabetic rats. Glomerular filtration rate was improved in the MC and glibenclamide treated rats. The renal tissue and plasma malondialdehyde were markedly lower in the MC treated diabetic rats. The degenerative changes in kidney tissue were alleviated in the treatment diabetic groups. These results suggested that MC fruit aqueous extract might have a significant role in alleviating kidney damage in the nSTZ-diabetic rats.

Key words: *Momordica charantia*, diabetes, kidney, neonatal rat.

INTRODUCTION

Diabetes mellitus is a systemic metabolic disorder characterized by elevated blood glucose due to absolute or relative deficiency of insulin secretion from pancreatic cells (Leonardi 2003; kidambi and patel, 2008). Non-insulin dependent diabetes mellitus (NIDDM) or type II diabetes has been increasing alarmingly worldwide. The worldwide prevalence of diabetes mellitus is expected to increase by 42% from 51 to 72 million in the developed countries and by 170% from 84 to 228 million in the developing countries by the year 2025 (Nakano and Ito, 2007). Hyperglycemia is an important causal factor that leads to kidney and glomerular hypertrophy (Koya et al., 2003). Oxidative stress induced by diabetes has a critical role in the development and progression of diabetic vascular complications including nephropathy. The

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Abbreviations: MC, *Momordica charantia*; **STZ,** streptozotocin; **nSTZ/16,** diabetic control group; **nSTZ/M,** *Momordica charantia* treated diabetic group; **nSTZ/G,** glibenclamide treated diabetic group; **NC/16,** normal group; **MDA,** malondialdehyde; **GFR,** glomerular filtration rate; **NIDDM,** non-insulin dependent diabetes mellitus; **IDDM,** insulin dependent diabetes mellitus; **ELISA,** enzyme-linked immunosorbent assay.

oxidative stress may cause tissue to be more susceptible to oxidative damage and progression of disease in renal glomerolus (Brownlee, 2001; Yao et al., 2009). Histopathological evaluations on the diabetic kidney show expansion of mesangial matrix and uniform thickening of basement membranes in glomerulus and tubules (Ziyadeh and Wolf, 2008). Since ancient times, plants have been a worthy source of medicine, which not only control hyperglycemia at low dosages but can also be taken for longer periods in contrast to synthetic hyperglycemic drugs (Grover et al., 2002). One of these plants is *Momordica charantia* (MC), also known as karalla, or bitter melon, which belongs to the *cucurbitacea* family, grows in tropical areas, including parts of the Amazon, east Africa, Asia, and the Caribbean, and is cultivated throughout South America as a food and medicine (Grover and Yadav, 2004).

The MC contains anti-hyperglycemic chemicals include glycosides, saponins, alkaloids, fixed oils, triterpenes, proteins and steroids (Murakami et al., 2001; Erden et al., 2010). These chemicals are concentrated in fruits of the MC, therefore fruit of the MC has shown more pronounced anti-hyperglycemic activity (Grover and Yadav, 2004). Presence of antioxidants in the fruits and vegetables such as vitamin C, E, carotenoids, lycopenes and flavonoids are also important in prevent free radical injury (Semiz and Sen, 2007). Total flavonoid and phenol contents of MC extract were analyzed and revealed that MC extract possess potent diphenylpicrylhydrazyl (DPPH) radical scavenging activity (Wu and Ng, 2008). Several studies have reported the anti-diabetic effects of MC on renal functional and histological changes in IDDM rats induced by streptozotocin, but only limited data is available on the anti-diabetic effects of MC on renal functional and histological changes in NIDDM neonatal rats.

MATERIALS AND METHODS

Animals

The protocol for animal experiment for this study had been approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, University Putra Malaysia. One day old neonatal rats received a single intraperitoneal injection of 85 mg/kg body weight of streptozotocin (Sigma, S0130-USA) freshly dissolved in 0.9% saline solution. Following the streptozotocin injection, mortality was observed in roughly 10% nenonatal rats. All these neonatal rats were assessed one day after the STZ treatment for blood glucose concentration using the Accu-Chek Instant Plus blood glucose monitor (Roche Diagnostics Corp.). The animals were considered as diabetes only if their non-fasting blood glucose concentration was more than 11 mmol/l on second post injection day (Abdollahi et al., 2010). All the experimental rats were kept under a suitable temperature (22 ± 2°C), humidity, and 12 h day-night cycle. Twelve weeks after STZ injection 21 male diabetic neonatal rats and seven normal neonatal rats were selected for experiment. Diabetic animals were randomly divided into three groups with seven animals in each group and were kept until the end of experiment at 16 week post-natal. The treated groups were as follows: the nSTZ/16 control group (STZ-injected neonatal rats), the nSTZ/M group (STZ-injected neonatal rats treated with MC fruit aqueous extract), and the nSTZ/G group (STZ-injected neonatal rats treated with glibenclamide). The non-diabetic rats were considered as a normal control group (NC/16). At the end of four-week treatment period, all these experimental rats were sacrificed and biochemical and histopathological evaluation was performed in these groups.

Preparation of *M. charantia* fruit aqueous extract

Fresh green whole fruits of MC were purchased from the local shops within five-kilometre radius from the preparation venue. After washing, small pieces of fruit were soaked in tap water at a ratio of 10:25 w/v for one hour at room temperature. It was then filtered with filter paper and evaporated by rotary evaporator (BUCHI Rotavapor R-220) to dry under reduced pressure to produce the dried yield. The MC powder was kept at -20°C until use (Abdollahi et al., 2010).

Mode of feeding

The experimental diabetic rats were administered the materials twice daily for a period of four weeks from the 13th week until the 16th week. The extract powders were dissolved in distilled water and orally fed (gavage) to the nSTZ/M group at a dosage of 20 mg/kg body weight, while glibenclamide was orally administered (gavage) in the nSTZ/G group at a dosage of 0.1 mg/kg body weight (Abdollahi et al., 2010).

Measurement of blood glucose concentration

To measure blood glucose level, the blood samples were collected from the saphenous vein. The blood glucose was measured once a week during four weeks treatment using the Accu-Chek Instant Plus blood glucose monitor.

Measurements of body weight and general characteristics

All experimental animals were weighed at the end of four weeks treatment by a digital weighing machine (Mettler-Toledo, 200). For measurement of food and water intake and urine output, the 16 week old experimental rats were placed in metabolic cages for 24 h and were provided with 40 g standard rodent chow and 250 ml water. After 24 h, the animals were removed and the consumed water and food were calculated.

The urine volume was also measured and then centrifuged to separate debris. The urine samples were kept at -80°C until further analysis.

Determination of glomerular filtration rate

Glomerular filtration rate (GFR) was calculated using the following formula (Shetty et al., 2005).

GFR (ml/min) = Urinary Creatinine (mg/dl) x Urine volume (ml) x 1000 (g) / Plasma creatinine (mg/dl) x Body weight (g) x 1440 (min)

Determination of serum creatinine and urea

Renal function was analyzed by determining serum urea and creatinine level by Automatic analyzer (Hitachi 902) using Roche kit.

Determination of urine creatinine and total protein

Renal function was estimated by determining urine creatinine concentration and total protein in all the experimental animals by automatic analyzer (Hitachi 902) using Roche kit.

Determination of malondialdehyde concentration in plasma

Lipid peroxidation was evaluated by measuring the malondialdehyde (MDA) concentration in plasma after four weeks treatment following the method described by Ohkawa et al. (1979) with slight modifications. 0.3 ml plasma sample was mixed with 2.4 ml sulfuric acid (H_2SO_4) and 0.3 ml 10% sodium tungstate dehydrate (Na_2WO_4). The mixture was left for 10 min at room temperature after vortexing. Then, the mixture was centrifuged at 5000 rpm for 10 min. The supernatant was discarded and the tubes placed upside down on absorbent papers. The reactive mixture was obtained by adding 450 µl distilled water, 50 µl 7 mM BHT (2, 6-di-tert-butyl-4-methylphenol), 3.0 ml 0.05 M hydrochloride acid (HCl), and 1.0 ml 1% thiobarbituric acid (TBA) and then heated (90°C, 10 min). After cooling to room temperature, 4 ml of n-butanol were added and vortexed for one min. The obtained mixture was centrifuged at 5000 rpm for 10 min. The butanol layer was separated. Its absorbance was measured at 532 nm using a spectrophotometer (Secomam, Domont, France).

Determination of malondialdehyde concentration in renal tissue

Kidney samples were washed with saline solution and immediately frozen at -80°C. The tissues were separately homogenized in potassium chloride (1.15% / g tissue) as described by Ohkawa et al. (1979). Three hundred milliliter distilled water, 35 µl BHT, 165 µl sodium dodecyl sulphate (SDS), and 2 ml TBA, were added to mixture, and heated for 60 min at 90°C. After cooling under running water, 3 ml n-butanol were added to the mixture and then centrifuged at 5000 rpm for 10 min. The butanol layer was separated and its absorbance was read at 532 nm using a spectrophotometer.

Tissue preparation for light microscopy

The 16 week old experimental rats were sacrificed under intraperitoneal injection of ketamine (80 mg/kg) and xylazine (8 mg/kg) anesthesia. The kidney samples were taken and fixed in 4% formaldehyde (Yagil et al., 2005). The fixed tissues after dehydration using a series of alcohol were cleared in xylene and embedded in paraffin using paraffin embedding (SLEE MPS/P1). Serial sections of three µm thickness were cut from each paraffin block using a microtome (Microm / HM 315R)

Tissue preparation for electron microscopy

For electron microscopic examinations, 1 mm^3 small pieces of the kidney were immersed with 4% glutaraldehyde (Agar, R1010) for 24 hours at 4°C. Samples were then washed with 0.1 M sodium cacodylate buffer (PH 7.4) for 3 changes of 10 min each washing, and post-fixed in 1% Osmium tetroxide (Agar, R1017) for 2 hours at 4°C. After washing with 0.1 M sodium cacodylate buffer, the specimens were dehydrated in an acetone series. Tissues were infiltrated overnight with acetone and resine mixture at the room temperature and then embedded in 100% epoxy resin (Agar, R1043). The embedded capsules were polymerized in oven at 60°C for 24 h. The prepared ultrathin sections were stained with uranyl acetate (10 min), lead stain (10 min), and washed with double distilled water. The stained sections were cut (1 µm thickness) and examined using an H-7100 scanning electron microscope (Hitachi, Japan). All electron micrographs were analyzed using image analyzer software.

Lesion scoring of kidney

Lesion scoring of the kidney was performed with minor modification to the method described by Yoshida et al. (2006). To evaluate histopathological changes in kidney, hematoxylin and eosin (H&E) stained sections were examined under the light microscope at x200 magnifications (Olympus BX51, Japan). Histopathological findings include degeneration and necrosis of epithelium cells of distal and proximal tubules and degenerated glomeruli cells were observed in 10 tubules or glomerular per section and separately assessed as follows: I /. (0) if the extent of the injury was < 5% or there was no injury. II /. (1) If there was 5-25% injury, (2) if there was 25-50% injury and, (3) if there was more than 50% injury. In two randomly selected animals per group, the glomerular basement membrane thickness was measured from the electron micrographs at x2000 magnification. The glomerular basement membrane width was recorded at 20 different randomly selected sites (Sagen et al., 2003). The average score for each group was calculated for the histopathological evaluation.

Statistical analyses

Analyses were carried out using one-way analysis of variance, ANOVA (SPSS 15.0) followed by Tukey HSD test for multiple comparisons. Values are expressed as mean ± standard deviation (SD) and all statistical tests are conducted at 95% confidence level ($P<0.05$).

RESULT

Body weight and general characteristics

The animal's body weight is shown in Table 1. Body weights did not differ ($P>0.05$) across the groups throughout the experimental period. The general characteristics of the 16 week old experimental rats are shown in Table 1. Water intake was over increased 1.9-fold in the nSTZ/16 group of rats compared to the NC/16 group. The water in the nSTZ/M group was significantly lower ($P<0.05$) over 1.9-fold than that of the nSTZ/16 group. The nSTZ/G group showed a significantly decreased ($P<0.05$) over 1.5-fold in water intake compared to the nSTZ/16 group. Food intake was significantly enhanced ($P<0.05$) over 1.42-fold in the nSTZ/16 group compared to the NC/16 group. The treated diabetic group with MC showed insignificant decrease ($P>0.05$) by 1.08 fold in food intake compared to the nSTZ/16 group. The nSTZ/G exhibited a 1.1-fold significant ($P<0.05$) decrease in food intake compared to the nSTZ/16 group. The urine output was significantly enhanced ($P<0.05$) by more than 1.8-fold in the nSTZ/16 group in comparison with the NC/16 group. The nSTZ/M rats exhibited an approximately 2.04-fold decrease ($P<0.05$) in urine output compared to the nSTZ/16 group. The nSTZ/G group showed a 2.2-fold decrease ($P<0.05$)

Table 1. The body weight and general characteristics at four weeks post-treatment in the 16 week old experimental rats.

Parameters \ Groups	NC/16	nSTZ/16	nSTZ/M	nSTZ/G
Body weight (g)	373.43 [a]	361.14 [a]	373.43 [a]	372.42 [a]
Water intake (ml/24h)*	19.71±4.2[a]	37.14±3.9[b]	19 ± 3.5[a]	24 ± 1.6[a]
Food intake (g/24h)*	14.56±1.5[a]	20.66±0.7[b]	19.01±0.7[b]	18.1± 1.1 [c]
Urine output (ml/24h)*	11.57±2.9[a]	21.29±3.9[b]	10.43±1.4[a]	9.57±1.6[a]

Normal group (NC/16), diabetic group (nSTZ/16), treated diabetic groups with MC (nSTZ/M) and with glibenclamide (nSTZ/G). *Values are means ±1SD (n=7). Different superscripts within rows indicate significant difference at P<0.05.

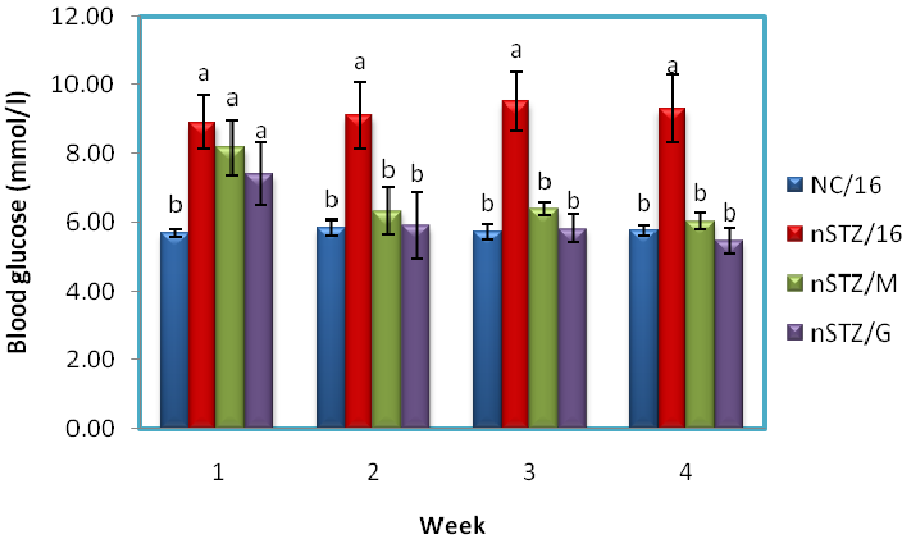

Figure 1. Non-fasting blood glucose concentration changed during four weeks treatment (1st, 2nd, 3rd, 4th week) in the normal group (NC/16), diabetic group (nSTZ/16), treated groups with MC (nSTZ/M) and glibenclamide (nSTZ/G). Error bar = ±1 SD (n=7)[a,b] Bars with different alphabet notation differ significantly at P<0.05.

in urine output compared to the nSTZ/16 group.

Blood glucose concentration

The blood glucose concentration was shown at the 16 week old experimental rats (Figure 1). As it is apparent from the current results, the nSTZ/16 group manifested mild hyperglycemia during of study and have exhibited significant (P<0.05) elevation in the blood glucose level compare to those of normal group. The results obtained from effect of MC fruit extract on blood glucose showed a significant (P<0.05) blood glucose lowering activity in the 2nd, 3rd and 4th weeks post-treatment in the nSTZ/M group (6.34 ± 0.19, 6.39 ± 0.23, 6.06 ± 0.21 mmol/l, respectively) as well as glibenclamide in the nSTZ/G group (5.93 ± 0.39, 5.84 ± 0.4, 5.47 ± o.34, respectively) compare to the nSTZ/16 group (9.13 ± 0.86, 9.54 ± 0.99, 9.33 ± 0.93 mmol/l, respectively). However, at the end of treatment the extract of MC fruit showed a blood glucose lowering activity, which was roughly a 35.01% reduction as compared to the nSTZ/16 rats. This reduction was approximately 41.37% in diabetic rats treated with glibenclamide in comparison with the nSTZ/16 rats.

Glomerular filtration rate

Diabetic rats in the nSTZ/16 group showed a significant (P<0.05) reduction in GFR compared to the NC/16 group. Treatment with both MC fruit extract and glibenclamide partly improved GFR in diabetic treated groups (Table 3).

Serum creatinine and urea concentrations

Diabetic rats in the nSTZ/16 group (Table 2) exhibited a significantly (P<0.05) higher serum creatinine as well as

Table 2. Serum creatinine and urea, level at four weeks post-treatment in the 16 week old experimental rats.

Parameters \ Groups	NC/16	nSTZ/16	nSTZ/M	nSTZ/G
Serum creatinine (µmol/L)*	48±4.76 [a]	64.14±2.91 [b]	52.28±4.27 [a]	50.86±3.71 [a]
Serum urea (mmol/L)*	6.96±0.99 [a]	10.73±1.41 [b]	6.45± 0.92 [a]	6.5± 0.8 [a]

Normal group (NC/16), diabetic group (nSTZ/16), treated diabetic groups with MC (nSTZ/M) and with glibenclamide (nSTZ/G). *Values are means ±1SD (n=7). Different superscripts within rows indicate significant difference at P<0.05.

Table 3. The urine creatinine and total protein at four weeks post-treatment in the 16 week old experimental rats.

Parameters \ Groups	NC/16	nSTZ/16	nSTZ/M	nSTZ/G
Urine creatinine(µmol/L)*	5705±560.7 [a]	2658± 457.4 [b]	5008.4±527.7 [a]	5215.4±500.3 [a]
Urine Total protein (g/L)*	<0.001 [a]	0.42 ± 0.12 [b]	0.08± 0.04 [a]	0.13± 0.06 [a]
GFR (ml/min)*	2.56±0.78 [a]	1.71±0.48 [b]	2.08±0.41 [ab]	2.01±0.42 [ab]

Normal group (NC/16), diabetic group (nSTZ/16), treated diabetic groups with MC (nSTZ/M) and with glibenclamide (nSTZ/G). *Values are means ±1SD (n=7). Different superscripts within rows indicate significant difference at P<0.05.

Table 4. Malondialdehyde (MDA) concentration of plasma and kidney tissue at four weeks post-treatment in the 16 week old experimental rats.

Parameters \ Groups	NC/16	nSTZ/16	nSTZ/M	nSTZ/G
MDA(plasma)* (µmol/l)	1.60 ± 0.76 [a]	3.23 ± 0.93 [b]	1.73 ± 0.74 [a]	3.27 ± 1.17 [b]
MDA(kidney)* (µmol/g)	20.34±4.11 [a]	32.11± 5.09 [b]	22.43±3.94 [a]	30.02± 3.42 [b]

Normal group (NC/16), diabetic group (nSTZ/16), treated diabetic groups with MC (nSTZ/M), and glibenclamide (nSTZ/G). *Values are means ±1SD (n=7). Different superscripts within rows indicate significant difference at P<0.05.

serum urea compared to normal rats in the NC/16 group. Treatment with MC and glibenclamide were effective among diabetic rats. As results showed, serum creatinine and urea level was significantly (P<0.05) decreased following the administration of MC fruit extract to the nSTZ/M rats compared to the nSTZ/16 group. Diabetic rats treated with glibenclamide in the nSTZ/G also showed a significant (P<0.05) reduction in serum creatinine and urea level compared to the nSTZ/16.

Urine creatinine and total protein concentrations

Urine creatinine and total protein was measured in the 16 week old experimental rats (Table 3). A significant (P<0.05) reduction in the urine creatinine level was observed in the nSTZ/16 group compared to the NC/16 group. Urine creatinine level in the treated diabetic rats with MC in the nSTZ/M group was significantly (P<0.05) higher than those of the nSTZ/16 group. Diabetic rats treated with glibenclamide also exhibited a significantly (P<0.05) increased urine creatinine in the nSTZ/G group compared to the nSTZ/16 group. The mean values of urine total protein in the nSTZ/16 group was significantly (P<0.05) higher than those of NC/16 group. However, it was significantly (P<0.05) lower in the nSTZ/M and nSTZ/G groups when compared to the nSTZ/16 group.

Malondialdehyde concentration in plasma

Table 4 shows plasma Malondialdehyde (MDA) activity in all of the 16 week old experimental rats. In the nSTZ/16 group of rats, the activity of MDA in plasma was significantly (P<0.05) higher than those of the NC/16 rats. Interestingly, diabetic rats treated with the MC fruit aqueous extract showed a significant (P<0.05) reduction in the MDA level compared to the nSTZ/16 group. This was close to those of the NC/16 group. The glibenclamide did not decrease MDA level in the nSTZ/G rats compared to the nSTZ/16 group.

Malondialdehyde concentrations in kidney tissue

In the STZ/16 group (Table 4), the activity of MDA was markedly (P<0.05) higher than those of the NC/16 group.

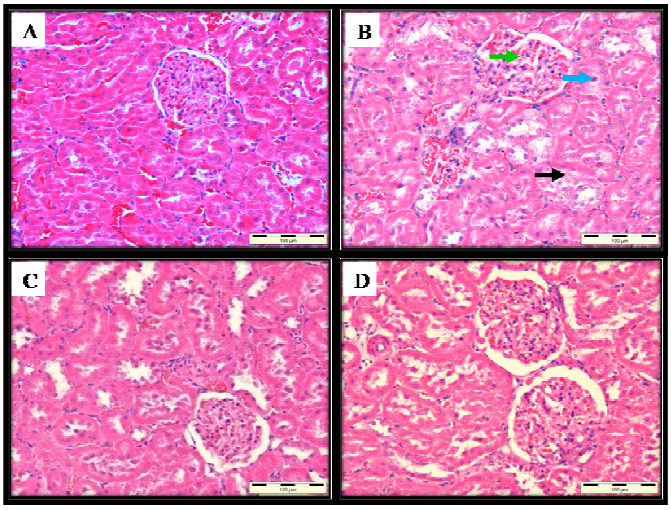

Figure 2. Photomicrographs of the kidney in the diabetic rats at 4 weeks post-treatment (A) Normal structure of kidney in the normal (NC/16) group. (B) Degeneration (black arrow) and necrosis (blue arrow) in epithelium cells and degeneration (green arrow) in the glomerular cells clearly existed in the nSTZ/16 group H and E. Scale bar 100 µm. x200.

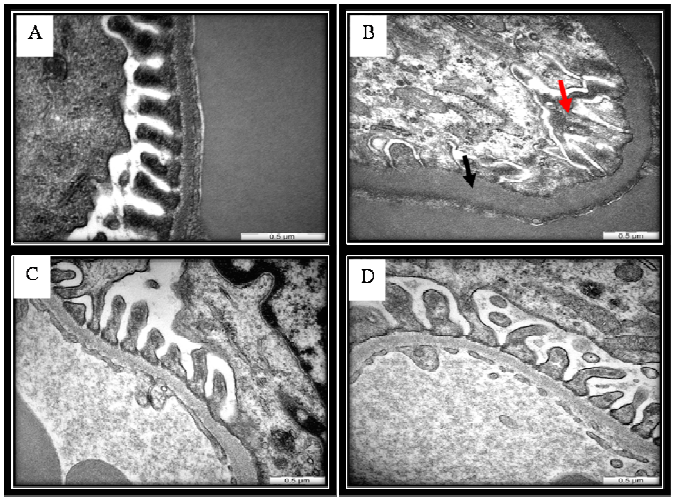

Figure 3. (A) Electronmicrographs showing a normal structure of glomerular basement membrane and foot processes in the NC/16. (B) Thickness glomerular basement membrane (black arrow) and foot processes fusion (red arrow) was observed in the nSTZ/16. A reduction in severity of thickening glomerular basement membrane and foot processes fusion in treated diabetic animals (C) with MC (nSTZ/M) and (D) glibenclamide (nSTZ/G). Scale bar 0.5 µm x40000.

Treatment with the MC fruit extract was effective in restoring the MDA concentration in the experimental tissue and exhibited significant ($P<0.05$) decrease in MDA concentration in the kidney (~30.15%) compared to the nSTZ/16 group. There was no significant ($P>0.05$) reduction in MDA concentration of renal tissue associated with the glibenclamide treatment in the nSTZ/G group compared to the STZ/16 group (Table 4).

Light microscopy findings

A normal structure of renal tubules and glomerular was observed in the NC/16 rats (Figure 2). When histopathological changes in the kidneys of experimental animals were examined, the necrotic and degenerated tubular epithelium cells were observed in the nSTZ/16 (Figure 2 and Table 5) group. In addition, degeneration in glomerulus was clearly observed in the nSTZ/16 group. It also shown that glomerular capillaries were irregular, widened, and attached to the Bowman's capsule. These histopathological findings were significantly ($P<0.05$) higher in diabetic rats than those of the normal rats. The results of these study showed that the severity of these lesions in the nSTZ/M and nSTZ/G groups was less than those of the nSTZ/16 group (Figure 2) (Table 5).

Electron microscopy findings

Renal tissue sections were observed under electron microscope. It was found that glomerular lesions in the nSTZ/16 group of rats were characterized by significant ($P<0.05$) thickening of the glomerular basement membrane compared to the NC/16 (Figure 3 and Table 5) rats. In addition, partial fusion of foot processes was observed in glomerular micrographs of the nSTZ/16 group. Treated diabetic rats in the nSTZ/M and nSTZ/G groups exhibited less severity of thickness glomerular basement membrane and foot processes fusion than those of the nSTZ/16 group (Figure 3 and Table 5).

DISCUSSION

This study was designed to evaluate the effects of MC fruit aqueous extract on improving the structural and functional damages of renal nephritic system in the streptozotocin-induced diabetes in neonatal rats. The data presented clearly exhibit the beneficial role of MC fruit extract during diabetes. The importance of hypoglycemic components of MC, which consist of a mixture of saponins such as charantin, insulin-like peptides (Polypeptide-P) and alkaloids those are concentrated in the fruit (Krawinkel and Keding, 2006). These components have reported to be effective in reducing blood glucose concentration. Glibenclamide, a synthetic drug, was used as a reference for comparison. Glibenclamide is often used as an insulin stimulant and blood glucose reducer in many studies. It is also one of the most widely used medications against hyperglycemia, which stimulates insulin secretion from β-cells through

Table 5. Renal lesion scores at four weeks post-treatment in the 16 week old experimental rats.

Parameters \ Group	NC/16	nSTZ/16	nSTZ/M	nSTZ/G
Extent of renal tubule epithelial cell necrosis	0[a]	2.8 ± 0.43[b]	1 ± 0.26[c]	1.17± 0.2[c]
Extent of renal tubule epithelial cell degeneration	0[a]	1.95 ± 0.14[b]	0.73 ± 0.18[c]	0.8 ± 0.27[c]
Extent of renal glomerular cell degeneration	0[a]	1.48 ± 0.5[b]	0.85 ± 0.34[c]	1.02 ± 0.35[c]
Thickening of glomerular basement membrane (nm)*	120.5±12.77[a]	271.42±26.42[b]	145.26±11.27[c]	175.61±33.21[c]

Normal group (NC/16), diabetic group (nSTZ/16), and treated diabetic groups with MC (nSTZ/M) and with glibenclamide (nSTZ/G). *Values are means ±1SD (n=7). Different superscripts within rows indicate significant difference at $P<0.05$.

inactivation of ATP-sensitive potassium channel (Sakamoto et al., 2006).

The untreated diabetic rats demonstrated typical characteristics of diabetes mellitus such as polyuria, polydipsia, and polyphagia. The MC fruit extract was found effective in controlling the polyuria, and polydipsia condition during diabetes. Similar results were observed in glibenclamide treated rats. Shetty et al. (2005) demonstrated that dry MC supplementation prevent polyuria and polydepsia in diabetic rats that could be attributed to the high fiber content of the MC. Fibers facilitate slow absorption of glucose along the passage through gastrointestinal tract (De Leeuw et al., 2004). Polyuria, polydipsia, and polyphagia are the typical triad of diabetes, which increase due to increased levels of blood glucose. Hyperglycemia leads to hyperosmolarity and reduction of intracellular water (Collins, 2007). It also causes a negative energy balance and enhanced hunger in diabetic patients. The enhanced blood glucose level spills over into the kidney as well as increasing an osmotic diuresis leading to polyuria (Magee and Bhatt, 2001). Administration of MC effectively reduced blood glucose levels in diabetic subjects (Garau et al., 2003). Therefore, the MC fruit aqueous extract may moderate polyuria, and polydipsia through hypoglycemic activity.

According to our result, GFR was restored in MC fruit extract and glibenclamide treated diabetic groups. These results support by previous reports, which demonstrated the MC improved renal function and induced a significant reduction in serum creatinine and urea concentration in the diabetic rats. It was reported that the MC, because of its high fiber content, improved the GFR in the early stage of diabetes in diabetic rats (Shetty et al., 2005). The end stage of diabetic renal disease is usually characterized by changes in both proteinuria and a subsequent decline in GFR. Development of lesions in the glomerular capillaries of the kidneys allows proteins to escape because of changes in the basement membrane (Rasch et al., 2002). In untreated individuals with diabetes, GFR has been estimated to decrease during this stage (Lorenzo et al., 2008). The best predictor of the rate of decrease in GFR in type II diabetes is the severity of the glomerular structural lesion leading to thickening of glomerular basement membrane (Rasch et al., 2002). Kumar et al. (2008) also showed MC could ameliorate the level of glomerular basement membrane architecture. Therefore, the MC fruit extract could improve GFR in treated diabetic rats by restoring the thickness of the glomerular basement membrane, as observed in the present study.

It was apparent from the present study that the MDA levels in the plasma, and renal tissue were significantly higher in the diabetic group than those of the normal ones. The high level of MDA in the diabetic rats was decreased after MC fruit extract administration as previously reported by Mahboob et al. (2005) in the serum of patients with Type II diabetes mellitus. High level of the MDA in the plasma (Ahmed et al., 2001), pancreas (Martinez et al., 2005), liver (Can et al., 2004; Ashokkumar and Pari, 2005), and kidney tissues (Koya et al., 2003) has reported in diabetic animals. Diabetic conditions produce oxidative stress and cause a variety of tissue injury in patients with diabetes (Lipinski, 2001). Hyperglycemia is a cause of the oxidative stress in diabetic patients (Robertson and Harmon, 2006). During diabetes, an enhanced oxidative stress in tissues promotes lipid peroxidation. Malondialdehyde as a secondary production of lipid peroxidation was measured in serum and tissue as a marker for enhanced lipid peroxidation in diabetic rats (Tan et al., 2008). Malondialdehyde is chemically reactive if not remove from the cell by antioxidant mechanisms, and may cause cellular damage such as enzyme inactivation, protein and DNA damage (Del Rio et al., 2005). The MC fruit aqueous extract increased the level of antioxidant enzymes in diabetic animals (Semiz and Sen, 2007) and could be effective through scavenging of free radicals (Wu and Ng, 2008). There is a correlation between the decrease in hyperglycemia, the reduction of oxidative stress, and the histopathological results (Martinez et al., 2005). Therefore, it was likely that the MC fruit aqueous extract alleviated the lipid peroxidation and tissue injuries through anti-hyperglycemia and antioxidant enzyme activity.

In the present study, necrosis and degeneration of the epithelial cells in the tubules, and degeneration of the glomerular cells were observed in the kidney of diabetic rats. It was observed that the MC fruit extract alleviated these abnormalities in the treated diabetic rats supporting previous reports of Kumar et al. (2008) in diabetic rats. MC also has shown promising effects in prevention as well as delay in progression of renal damage in diabetic animals (Shetty et al., 2005).

Hyperglycemia is an important causal factor in mediating the development and progression of diabetic kidney disease (Nandi et al., 2004). After the onset of hyperglycemia, glomerular hypertrophy (Koya et al., 2003) and thickening of the glomerular basement membrane develop in diabetic subjects (Goumenos et al., 2009). The changes in the expression of L-glutamine-fructose-6-phosphate amino transferase (GFAT) may be one of the reasons for the thickening of glomerular basement membrane (Nandi et al., 2004). However, the MC was found to be effective in controlling the changed activities of these enzymes during diabetes and this effect is chiefly attributed to hypoglycemic effect of MC during diabetes (Kumar et al., 2008). Hyperglycemia combined with insulin resistance induces vascular oxidative stress in diabetic animals (El Midaoui and De Champlain, 2005). Numerous reports have demonstrated that oxidative stress induced by diabetes plays an important role in the development and progression of diabetic vascular complications including nephropathy (Brownlee et al., 2001; Koya et al., 2003). They also showed that these alterations were normalized by the treatment with antioxidants. It was reported that the MC fruit extract possesses the anti-oxidant effects besides having protective activities in rats (Semiz and Sen, 2007). Hence, hypoglycemic and antioxidant components of MC fruit aqueous extract could improve glomerular injury in treated diabetic rats.

Conclusion

The MC fruit aqueous extract improved hyperglycemia, possibly alleviated basal membrane damage through the reduction of damaging oxidative activities. These resulted in improved renal lesion scores and renal function in treated diabetic rats.

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