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Full Length Research Paper

The effects of *Momordica charantia* on the liver in streptozotocin-induced diabetes in neonatal rats

M. Abdollahi[1], A. B. Z. Zuki[1]*, Y. M. Goh[1], Rezaeizadeh A.[2] and M. M. Noordin[3]

[1]Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, University Putra Malaysia, 43400 UPM, Serdang, Selangor.
[2]Institute of Bioscience, University Putra Malaysia, 43400 UPM, Serdang, Selangor.
[3]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University Putra Malaysia, 43400 UPM, Serdang, Selangor.

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The aim of this study is to determine the effects of *Momordica charantia* (MC) fruit aqueous extract on the liver histopathological changes in neonatal rats streptozotocin (STZ)-induced diabetes mellitus type II. Diabetes mellitus was induced in one day old neonatal Sprague-Dawley rats with STZ (85 mg/kg) and monitored for 12 weeks thereafter. The diabetic rats were separated into three groups as follows: the diabetic control group (nSTZ), the MC treated diabetic group (nSTZ/M), and the glibenclamide treated diabetic group (nSTZ/G). At the end of the treatment, blood glucose, serum insulin, alanine amino transferases (ALT) and aspartate amino transferases (AST) level was measured. Malondialdehyde (MDA) concentration was measured in the plasma and liver. The liver samples were processed for light microscopy examination. The results showed a reduction of blood glucose, ALT and AST, and increment of insulin level in the nSTZ/M and nSTZ/G rats. Administration of MC reduced the MDA concentration in plasma and liver of the nSTZ/M rats. Glucose tolerance and insulin sensitivity was improved in the nSTZ/M and nSTZ/G groups. The degenerative changes in liver were alleviated in the nSTZ/M and nSTZ/G groups. These results suggested that MC fruit aqueous extract may have a significant role in alleviating liver damage in the nSTZ-diabetic rats.

Key words: Diabetes, *Momordica charantia*, liver, neonatal rat.

INTRODUCTION

Diabetes mellitus is a systemic metabolic disorder characterized by hyperglycemia due to deficiency of insulin secretion or insulin action. Diabetes mellitus is categorized into two major types, namely; non insulin dependent diabetes mellitus (NIDDM) and insulin dependent diabetes mellitus (IDDM) (Bastaki, 2005). NIDDM or type II diabetes is the most common form of the disease which is caused by impaired insulin secretion paralleled by a progressive decline in β-cell function and chronic insulin resistance (Lupi and Del Patro, 2008). Insulin resistance is a main reason in pathogenesis of type II diabetes and occurs when the cellular mechanisms fail to respond to insulin effects (Shulman, 2000). The neonatal rats treated with streptozotocin (STZ) on the first day of birth showed hyperglycemia and reduction in pancreatic insulin amount during neonatal period and which could be maintained up to adulthood. This diabetic rat's model resembles the human NIDDM (Portha et al., 2007). Chronic hyperglycemia in diabetes mellitus leads to disorder of carbohydrate, fat and protein metabolism and induced complications in organs (Bastaki, 2005). Diabetes is also associated with oxidative stress that plays an

*Corresponding author. E-mail: zuki@vet.upm.edu.my. Fax: 603-89468333.

Abbreviations: MC, *Momordica charantia*; **STZ,** streptozotocin; **nSTZ,** diabetic control group; **nSTZ/M,** *Momordica charantia* treated diabetic group; **nSTZ/G,** glibenclamide treated diabetic group; **ALT,** alanine amino transferases; **AST,** aspartate amino transferases; **MDA,** malondialdehyde; **NIDDM,** non-insulin dependent diabetes mellitus; **IDDM,** insulin dependent diabetes mellitus; **GLUT4,** glucose transporter 4; **ELISA,** enzyme-linked immunosorbent assay; **IPGTT,** intraperitoneal glucose tolerance test; **IPITT,** intraperitoneal insulin tolerance test.

important role in the development of diabetes complications. An advanced production of free radicals due to diabetes causes membrane damage and further promotes lipid peroxidation (Baynes and Thorpe, 1999).

Medications such as sulfonylurea are generally utilized in the management of type II diabetes, however, they are accompanied with side effects. Recently, some researchers had manifested an increasing interest towards traditional medicinal plants. Many traditional plants have been identified to have hypoglycemic activities (Kim et al., 2006). One of these plants is *Momordica charantia* (MC), also known as karalla or bitter melon that belongs to the *cucurbitacea* family. MC is consumed in South Asia, Africa, South America and oriental countries as a food item and medicinal plant for treating various diseases such as diabetes mellitus (Grover and Yadav, 2004). The hypoglycemic activity of MC fruit extract (Miura et al., 2001; Virdi et al., 2003), MC seed (Sathishsekar and Subramanian, 2005) and whole plant extract (Krawinkel and Keding, 2006) has been confirmed in experimental animals. MC is competent in lowering fasting serum glucose in patients with type II diabetes (Ahmad et al., 1999) and improving the glucose tolerance (Welihinda et al., 1986). Previous studies had reported that MC enhances insulin secretion (Sathishsekar and Subramanian, 2005; Fernandes et al., 2007) and increases the number of pancreatic B-cells in the islets of Langerhans (Ahmed et al., 1998). There are several possible mechanisms of hypoglycemic activity of MC. Some previous studies had also revealed that MC increases the glucose uptake in liver via promoting glucose-6-phosphate dehydrogenase and declining glucose-6- phosphatase activities (McCarty, 2004). In addition, it could also increase the mRNA expression of glucose transporter 4 (GLUT4) proteins in skeletal muscles (Shih et al., 2009). Mahomoodally et al. (2007) suggested that MC fruit extract can reduce glucose transport via the brush border of small intestine in albino rats. Wu and Ng (2008) had reported free radical scavenging activities of MC aqueous and ethanol extracts. The antioxidant compounds of MC include phenolic phytochemicals and vitamins such as C and A which were isolated from this plant (Grover and Yadav, 2004). Recently, cucurbitane-type triterpenoids were isolated from the stems of MC and demonstrated their antioxidant activity (Liu et al., 2010).

Majority of studies on antidiabetic activity of MC had focused on IDDM diabetic animals. Hence, this study was carried out to evaluate the effects of MC fruit aqueous extract on the liver histopathology of NIDDM diabetic neonatal rats.

MATERIALS AND METHODS

Animals

The protocol for animal experiment for this study was approved by the Animal Care and Use Committee of Faculty of Veterinary Medicine, University Putra Malaysia. Normal females Sprague-Dawley rats (200 - 250 g) were caged overnight with the normal males. Natural birth occurred 22 days after mating. The one-day-old neonatal rats were given a single intraperitoneal injection of STZ (85 mg/kg) (Sigma, S0130-USA) freshly dissolved in 0.9% saline solution (Li et al., 2004). Meanwhile, the normal control neonatal rats received equivalent volume of 0.9% saline solution only. The neonatal rats were placed with their own mothers for one month and kept in suitable temperature (22 ± 2°C), humidity and 12 h of day-night cycle in polyethylene cages. The animals were considered as diabetic only if their blood glucose concentration was more than11 mmol/l on the second post injection day (Li et al., 2004). Twelve weeks after STZ injection, the diabetic animals were divided into three groups with seven animals in each group. The treated groups were as follows: the nSTZ 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). Meanwhile, the non-diabetic rats were considered as a normal control group.

Preparation of *M. charantia* fruit aqueous extract

Fresh green whole fruits of MC were purchased from the local shops within 5 km radius from the preparation venue. Small pieces of fruits were soaked in water at a ratio of 10:25 w/v for one hour at room temperature (25±2°C). It was then filtered and evaporated by rotary evaporator to dryness under reduced pressure to get the yield (Virdi et al., 2003).

Mode of feeding

Treatments were given twice daily for a period of four weeks. The extract powders were orally fed to nSTZ/M at a dosage of 20 mg/kg body weight, while glibenclamide was orally administered in nSTZ/G at a dosage of 0.1 mg/kg body weight (Virdi et al., 2003).

Measurement of blood glucose concentration

To detect the 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 (Roche Diagnostics Corp.).

Measurement of serum insulin level

Insulin was measured at the end of four weeks treatment in the experimental animals using the enzyme-linked immunosorbent assay (ELISA) Rat/Mouse insulin kit (Millipore, USA).

Measurement of alanin aminotransferase (ALT) and aspartate aminotransferase (AST) serum activities

The levels of ALT and AST serums were determined after four weeks treatment in all the experimental rats by using the Automatic analyzer (Hitachi 902).

Measurement of insulin sensitivity

For the measurement of insulin sensitivity, the intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT) were performed at the end of four weeks treatment on the experimental groups.

Table 1. Non-fasting blood glucose concentration changed during four weeks treatment in the experimental animals.

Groups	1st week*	2nd week*	3rd week*	4th week*
Normal	5.70 ± 0.12 [b]	5.87 ± 0.23 [b]	5.74 ± 0.23 [b]	5.77 ± 0.15 [b]
nSTZ	8.91 ± 0.97 [a]	9.13 ± 0.86 [a]	9.54 ± 099 [a]	9.33 ± 0.93 [a]
nSTZ/M	8.19 ± 0.68 [a]	6.34 ± 0.19 [b]	6.39 ± 0.23 [b]	6.06 ± 0.21 [b]
nSTZ/G	7.43 ± 097 [a]	5.93 ± 0.39 [b]	5.84 ± 0.4 [b]	5.47 ± 0.34 [b]

*Valus are means ± SD (n=7). Different alphabet notation within column differ significantly at P<0.05.

IPGTT test

IPGTT was done at the end of four weeks treatment with minor modifications to the method described by Goren et al., (2004). Briefly, after an over-night fasting period, 2 g/kg glucose was intra-peritoneally injected into all the groups at the end of four weeks treatment. The blood glucose was measured at 15, 45 and 90 min after the glucose injection, and the serum was analyzed for the measurement of insulin level.

IPITT test

IPITT test was performed with minor modifications following the method described by Wendel et al. (2008). After an over-night fasting period, 1.5 IU/kg insulin was intraperitoneally injected into all the groups at the end of four weeks treatment. The blood glucose was measured at 15, 45 and 90 min after insulin injection (Wendel et al., 2008).

Determination of malondialdehyde (MDA) concentration in plasma

MDA concentration was assessed in the plasma of the experimental animals after four weeks treatment following the method described by Ohkawa et al. (1979) with slight modifications. Briefly, a mixture of 0.3 ml plasma, 2.4 ml sulfuric acid (H_2SO_4) and 0.3 ml sodium tungstate dehydrate (Na_2WO_4) was centrifuged at 5000 rpm for 10 min. The reactive mixture was obtained by adding 450 µl distilled water, 50 µl of 2,6-di-tert-butyl-4-methylphenol (BHT), 3 ml hydrochloric acid (HCl, 0.05 M) and 1 ml thiobarbituric acid (TBA). It was then heated at a temperature of 95°C for 60 min. After cooling under running water, 4 ml n-butanol was added and centrifuged at 5000 rpm for 10 min. Its absorbance was measured at 532 nm using the spectrophotometer (secomam, Domont, France). The standard curve was prepared by 1,1,3,3-tetraethoxy-propane (TEP) in different concentration (0.1 - 5 µM/lit).

Determination of MDA concentration in liver tissue

At the end of four weeks treatment, the liver samples were taken from the animals and washed with saline solution frozen immediately at -80°C. The tissue was homogenized in 4 ml of potassium chloride 1.15%/g as described by Ohkawa et al. (1979). The freshly obtained mixture (200 µl) was then added with 300 µl water, 35 µl BHT, 165 µl sodium dodecyl sulphate (SDS) and TBA (2 ml), and heated for 60 min at 90°C. The solution was immediately cooled in running water. After adding 3 ml n-butanol, the solution was centrifuged at 5000 rpm for 10 min and its absorbance was measured at 532 nm using spectrophotometer. The standard curved was prepared by TEP in different concentration (2.5 - 50 µM/lit).

Tissue preparation for light microscopy

The male rats were sacrificed by being given ketamine (80 mg/kg) and xylazine (8 mg/kg) anesthesia at the end of the treatment. The liver tissue was examined under the light microscope. The samples were fixed in Bouin's solution and embedded in paraffin. The sections were stained with hematoxylin and eosin (H and E) using routine protocol and examined under the light microscope (Olympus BX51, Japan). Lesions relating to histopathological changes, hydropic swollen, cell degeneration, microvesicular vacuoles, macrovesicular vacuoles and cell necrosis in the liver were scored in 10 fields of each H and E-stained slide and examined under the light microscope (×200). The histopathological findings were scored as none (0), mild (1), moderate (2) and severe damage (3) (Guven et al., 2006), and examined in a blinded manner.

Statistical analyses

One-way analysis of variance (ANOVA) (SPSS 15.0) and the corresponding post hoc test were used for data analysis. All data were expressed as mean ± SD and P<0.05 were identified as significantly different.

RESULTS

The effect of MC fruit extract on blood glucose concentration

The diabetic rats showed a significant increase in the blood glucose level when compared to the normal rats (P<0.05). The administration of MC fruit extract in the diabetic treated rats reduced the elevated levels of blood glucose in the 2nd, 3rd and 4th weeks post-treatment compared to the untreated diabetic control (P<0.05). Similarly, the treated diabetic rats with glibenclamide exhibited reduced blood glucose level in the 2nd, 3rd and 4th weeks post-treatment compared to the diabetic control (P<0.05) (Table 1).

The effect of MC fruit extract on serum insulin level

In the nSTZ group, the serum insulin level after four weeks treatment was lower than that of the normal group (P<0.05). The serum insulin level in the nSTZ/M and nSTZ/G groups was higher than that of the nSTZ group (P<0.05) (Figure 1).

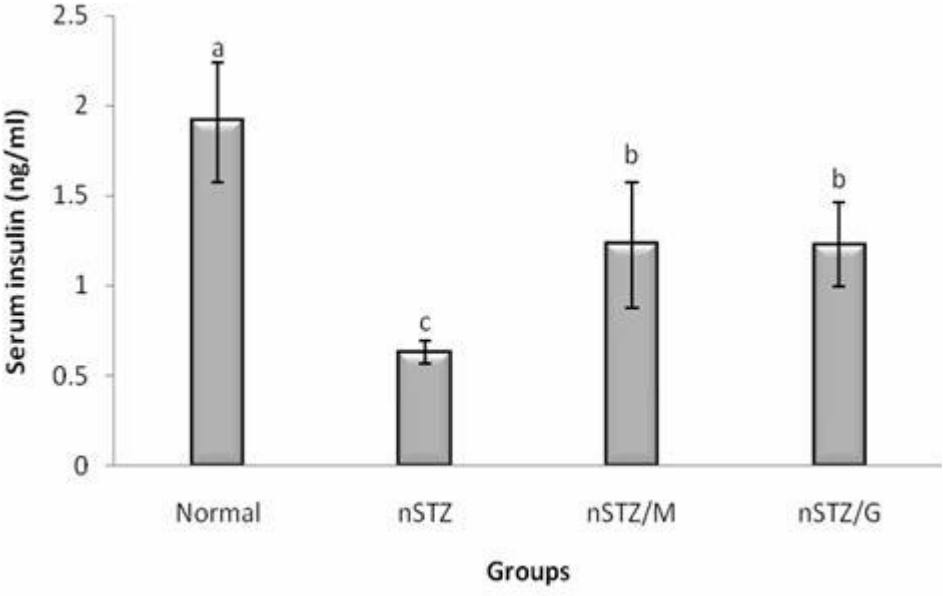

Figure 1. Serum insulin level in non-fasting experimental animals after four weeks treatment. Error bar = ± SD (n=7). [a,b,c] Bars with different alphabet notation differ significantly at P<0.05.

Table 2. Serum levels of ALT and AST after four weeks treatment in the experimental animals.

Groups	ALT (U/l)*	AST (U/l)*
Normal	53.13 ± 4.52[b]	121.61 ± 21.17[b]
nSTZ	70.53 ± 6.56[a]	158.47 ± 10.55[a]
nSTZ/M	53.43 ± 6.83[b]	131.14 ± 13.50[b]
nSTZ/G	45.67 ± 5.53[b]	113.23 ± 7.22[b]

*Values are means ± SD (n=7). Different alphabet notation within column differ significantly at P<0.05.

The effect of MC fruit extract on the activity of ALT and AST serum

As shown in Table 2, the levels of ALT and AST serums were significantly enhanced in the nSTZ group compared to the control (P<0.05). The results obtained from the nSTZ/M and nSTZ/G groups showed significant decrease in ALT and AST levels compared to the untreated nSTZ group (P<0.05).

The effect of MC fruit extract on glucose tolerance

Impaired glucose tolerance was seen in the nSTZ group. The blood glucose increased in the nSTZ group (24.5 ± 1.4) peaking at 45 min after glucose injection and was higher during 90 min after glucose was administered, compared to the control (10 ± 0.5) (P<0.05). Significantly, lower blood glucose level was observed in the nSTZ/M and nSTZ/G groups during 90 min after glucose injection when compared to the nSTZ rats (P<0.05) (Figure 2a). The fasting serum insulin level was significantly lower in the nSTZ group compared to the control (P<0.05). However, the serum insulin level was significantly higher in nSTZ/M (P<0.05) and nSTZ/G (P<0.05) than that of the nSTZ group (Figure 2b).

The effects of MC fruit extract on insulin sensitivity

The blood glucose started to diminish after insulin was administered. The results of IPITT showed increased insulin sensitivity in the treated diabetic groups after four weeks of treatment (Figure 2c). The blood glucose level slowly decreased within 90 min after insulin injection in the nSTZ group compared to the control (P<0.05). However, the glucose concentrations rapidly diminished after insulin injection in the nSTZ/M and nSTZ/G groups compared to the untreated nSTZ group (P<0.05) and were closed to the normal control.

The effects of MC fruit extract on plasma MDA activity

Table 3 shows the MDA activity in the plasma of all groups. In the nSTZ group, the activity of MDA in plasma was significantly higher than that of the normal group (P<0.05). Interestingly, the nSTZ/M group showed a significant decrease in the MDA level compared to the diabetic group (p<0.05) and was close to the normal group. On the contrary, glibenclamide did not reduce the increased MDA level in the nSTZ/G group compared to the nSTZ group (P<0.05).

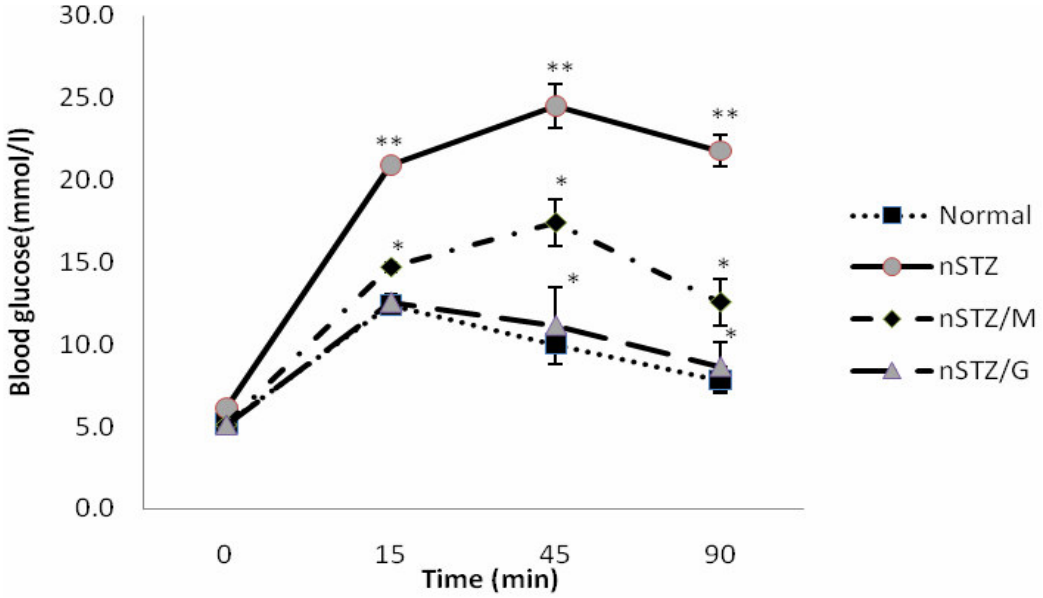

Figure 2a. Blood glucose level changed during IPGTT after four weeks treatment. Error bar = ± SD (n=7). *P<0.05 compared to the nSTZ group. **P<0.05 compared to the normal group.

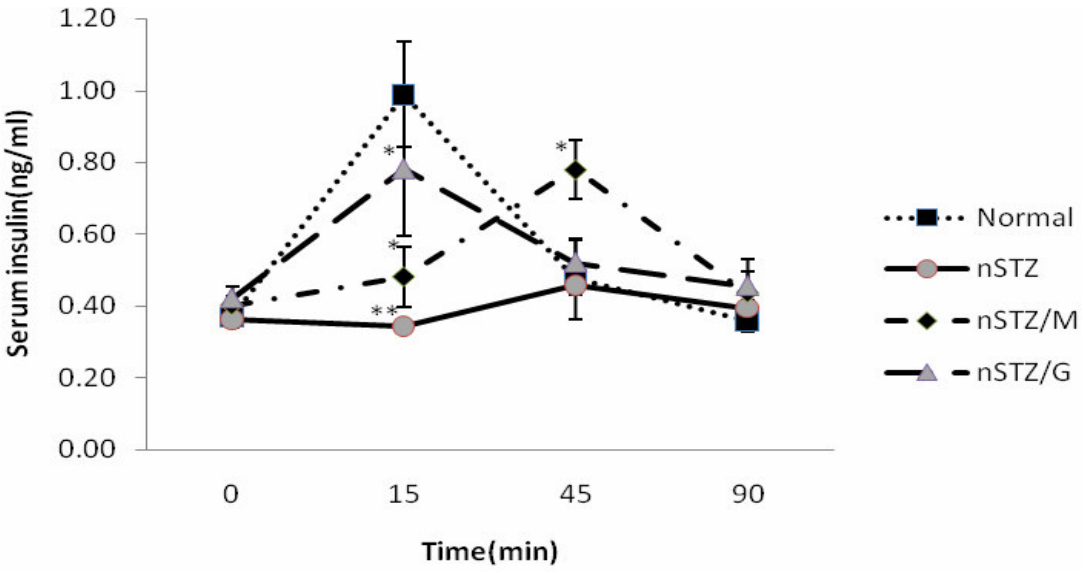

Figure 2b. Serum insulin level changed during IPGTT after four weeks treatment. Error bar = ± SD (n=7). *P<0.05 compared to the nSTZ group. **P<0.05 compared to the normal group.

The effects of MC fruit extract on the liver tissue MDA activity

In the nSTZ group, the activity of MDA in the liver tissue was significantly higher than that of the normal control (P<0.05). The treated diabetic rats in nSTZ/M showed decreased MDA activity compared to the nSTZ group (P<0.05). There was no significant reduction in the MDA concen-tration of liver tissue in the nSTZ/G rats when compared to the nSTZ rats (P>0.05) (Table 3).

Histopathological findings

Figure 4 shows the structural features of hepatocytes in the experimental animals. In the diabetic control rats, degeneration and scattered necrotic cells, swollen cyto-plasmic hydropic and microvesicular vacuoles were observed. Interestingly, in nSTZ/M and nSTZ/G, the severity extent of abnormal histological signs of hepato-cytes was less than those of the nSTZ group (P<0.05) (Table 4).

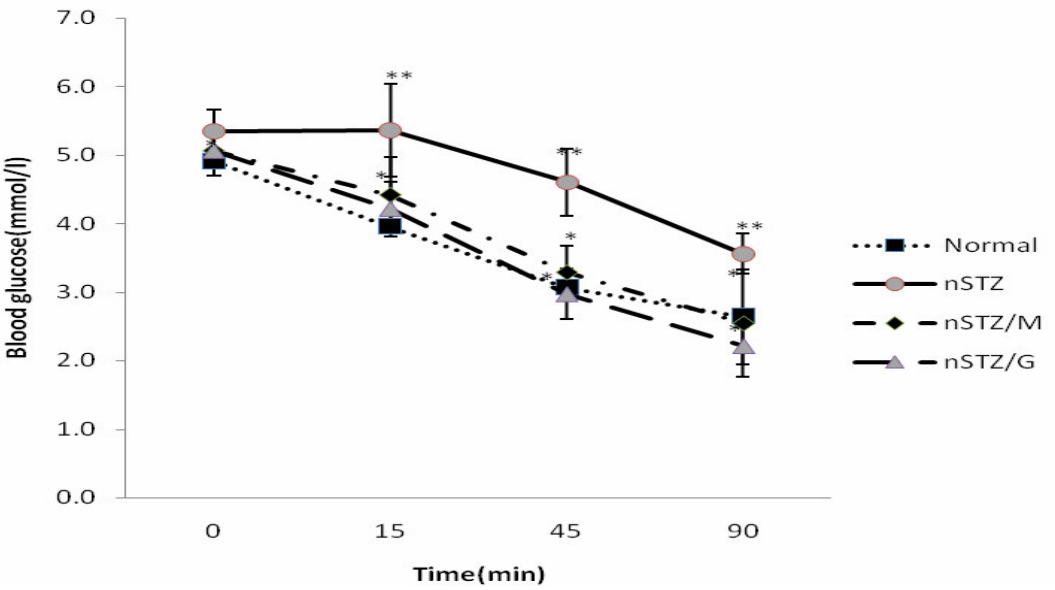

Figure 2c. Blood glucose level changed during IPITT after four weeks treatment. Error bar = ± SD (n=7). *P<0.05 when compared to the nSTZ group. **P<0.05 compared to the normal group.

Table 3. Malondialdehyde (MDA) values for all groups in plasma and liver tissues after four weeks treatment.

Groups	MDA(plasma)*µmol/l	MDA(tissue) *µmol/g
Normal	1.60 ± 0.76[b]	27.2 ± 7.68[b]
nSTZ	3.23 ± 0.93[a]	45.8 ± 4.69 [a]
nSTZ/M	1.73 ± 0.74[b]	29.38 ± 5.51[b]
nSTZ/G	3.27 ± 1.17[a]	39.2 ± 7.12[a]

*Values are means ± SD (n=7). Different alphabet notation within column differ significantly at P<0.05.

DISCUSSION

This study was designed to evaluate the effects of MC fruit aqueous extract on improving the damages of liver parenchyma in the n-STZ rats. The neonatal rats treated with streptozotocin on the first day of birth exhibited deficiency in insulin secretion and action similar to NIDDM in humans (Portha et al., 2007). The results of this study showed a significant effect of MC fruit extract in reducing the blood glucose concentration as well as glibenclamide. Glibenclamide is one of the most widely common used medications against hyperglycemia which stimulates insulin secretion from β-cells through inactivation of ATP-sensitive potassium channel (Sakamoto et al., 2006). It also increases the number of insulin receptors (Hribal et al., 2001). MC was found to oppose the hyperglycemic actions in the diabetic treated rats (Virdi et al., 2003). Previous studies had also noted the importance of hypoglycemic components of MC, which consist of a mixture of saponins such as charantin, insulin-like peptides and alkaloids that are concentrated in the fruit (Krawinkel and Keding, 2006). The results of this study showed increased serum insulin level in the nSTZ/M and nSTZ/G groups. Previous studies had reported increasing serum insulin level in diabetic animals treated with MC (Chen et al., 2003; Garau et al., 2003).It is also reported that MC fruit juice would enhance the number of B-cells of pancreatic islets in diabetic treated rats (Ahmed et al., 1998). The increase in insulin levels suggested that MC would enhance the secretion of insulin from B-cells of islets of Langerhans. ALT and AST are common intracellular enzymes that increase the liver damage induced by diabetes (Can et al., 2004). The results of this study clearly showed the high level of ALT and AST serums in the nSTZ group. In contrast, they were decreased in the nSTZ/M and nSTZ/G groups. The MC juice and alcoholic extract caused a significant decrease in the levels of ALT and AST serums and had a protective effect on the liver damage of diabetic treated rats (Abdelsattarelbatran et al., 2006).

It was likely that the reduced levels of ALT and AST serums by the MC fruit extract was an indication of alleviation of plasma membrane damage produced by diabetes. Cell resistant to insulin is one of the most important factors in the initiation and development of type II diabetes (Zhang et al., 2008). The diabetic rats showed significant hyperglycemia in IPGTT and IPITT compared to the control. However, it was evident that the MC treatment restored the plasma glucose clearance rate, in contrast to that of the normal animal (Figures 2a and 2c). The diabetic rats treated with glibenclamide had significantly better glucose tolerance and clearance ability followed by the MC treated animals. This clearly shows that MC has the ability to improve glucose tolerance and

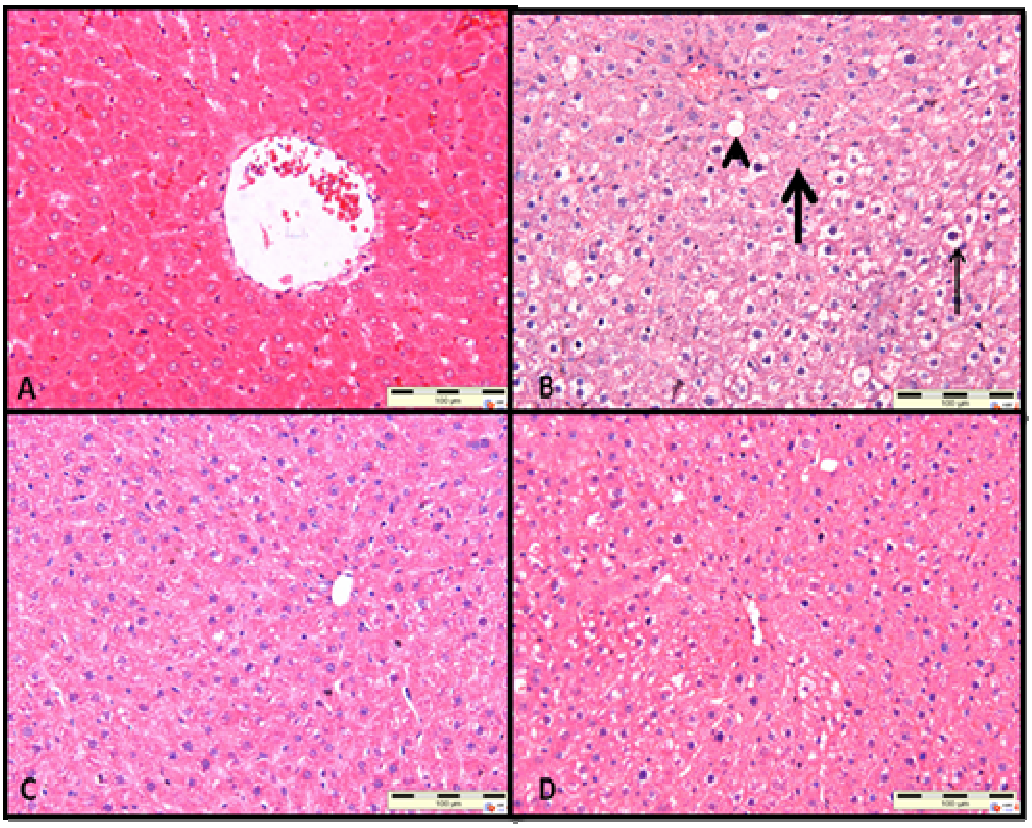

Figure 3. Light microscopic photographs of the livers of experimental animals showed (**A**) the liver of normal control group, (**B**) microvesicular fattening (arrow head), scattered necrotic cells (thick arrow) and degeneration (thin arrow) in the nSTZ group, **(C)** in nSTZ/M and **(D)** nSTZ/G, the severity of these changes was less than those in the nSTZ group. H&E. Scal bar 100 µm.

Table 4. Histopathological findings of liver after four weeks treatment in the experimental animals.

Groups	Hydropic swollen*	Necrotic cells*	Microvesicular vacuole*	Macrovesicular vacuole*
Normal	0^{b}	0^{b}	0^{b}	0
nSTZ	2.9 ± 0.12^{a}	1.9 ± 0.13^{a}	0.4 ± 0.05^{a}	0
nSTZ/M	0.9 ± 0.08^{c}	0.56 ± 0.08^{c}	0^{b}	0
nSTZ/G	1.2 ± 0.17^{d}	0.59 ± 0.09^{c}	0^{b}	0

*Values are means ± SD (n=7). Different alphabet notation within column differ significantly at $P<0.05$.

increase cellular insulin sensitivity as previously reported by Miura et al. (2001) and Chaturvedi et al. (2004). The administration of MC extract improved insulin tolerance test (ITT) and glucose tolerance test (GTT) in the high-fat-fed rats by the increment of skeletal muscle insulin-stimulated IRS-1 tyrosine phosphorylation (Sridhar et al., 2008).

The increased production of free radicals and oxidative damage is a feature of chronic diseases such as diabetes (Bayens and Thorpe, 1999). Hyperglycemia, due to diabetes, leads to the production of free radicals that are associated with the development of diabetic complications (Traverso et al., 2004). Free radicals are extremely toxic compounds that target biomolecules such as lipids with unsaturated double bonds and react with these lipids leading to lipid peroxidation. However, the increment of lipid peroxidation has being found to be involved in observed tissue damages in diabetes (Sathishsekar and Subramanian, 2005). Malondialdehyde is a secondary product and general indicator of lipid peroxidation. It was apparent from this study that the MDA levels in liver tissue and plasma were significantly higher in the nSTZ group than that of the normal group. In addition, there was possibility that the free radical generation was elevated

in the diabetic animals. Previous researches had also reported the high level of MDA in erythrocyte ghost membranes of diabetic patients (Ahmed et al., 2006) and liver tissue of diabetic animals (Can et al., 2004). Mahboob et al. (2005) had shown a significant elevation in the serum MDA level of patients with type II diabetes mellitus. According to the results of this study, the increment of liver tissue damage in the nSTZ group was found to be decreased after MC fruit extract feeding. This was in tandem with the reduction of MDA in the treated diabetic rats with MC fruit extract. The chronic administration of MC fruit juice decreased the MDA concentration in the diabetic animals (Sitasawad et al., 2000). The presence of degenerated hepatocytes and necrotic cells are possibly associated with the generation of free radicals in the liver of diabetic rats (Packer et al., 2000). There is increasing evidence that free radicals play an importance role in the initiation and progression of liver injury (Vitaglione et al., 2004; Sun et al., 2003) and causes apoptosis, necrosis, and regeneration in hepatocyte and endothelial cells (Vitaglione et al., 2004). The MC fruit extract increased the level of antioxidant enzymes (Semiz and Sen, 2007) and could be effective through scavenging these free radicals (Wu and Ng, 2008). Therefore, it was likely that the MC fruit extract alleviated lipid peroxidation and tissue liver injuries through antioxidant enzyme activity. In conclusion, the results of this investigation revealed that the aqueous extracts of MC fruit alleviated the histopathological changes caused by diabetes in the liver of NIDDM rats.

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