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Full Length Research Paper

Antioxidant and antihyperglycaemic effects of an aqueous extract from *Momordica charantia* fruit in a type II diabetic rat model

Alireza Rezaeizadeh[1], Zuki Bin Abu Bakar Zakaria[2]*, Maryam Abdollahi[2], Goh Yong Meng[2], Noordin Mohamed Mustapha[3], Muhajir Bin Hamid[4] and Tengku Azmi Bin Tengku Ibrahim[1,2]

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

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The aim of this study was to determine the antioxidant and antihyperglycaemic activities of an aqueous extract from *Momordica charantia* (MC) fruit in a type II diabetic rat model. In this study, diabetes mellitus was induced in one-day-old neonatal rats by a single injection of streptozotocin (STZ) (85 mg/kg). The animals were separated into four groups as follows: the normal control group (NC), the diabetic control group (DC), the MC-treated diabetic group (DMO), and the glibenclamide-treated diabetic group (DPG). At the end of four weeks of treatment, the malondialdehyde (MDA) concentration was measured in the serum and pancreas. Ferric thiocyanate (FTC), thiobarbituric acid (TBA) and 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) levels, and the total phenol and flavonoid content of the MC extracts were evaluated. The results showed a reduction of blood glucose in the DMO and DPG groups as compared to the DC group. Administration of MC reduced the MDA concentration in plasma and in the pancreas of the DMO group. Antioxidant activity in the MC extract was indicated by a high IC_{50} value for free radical scavenging. Based on these results, it can be concluded that the MC fruit aqueous extract would alleviate oxidative stress induced by diabetes through antioxidant activity and free radical scavenging.

Key words: Diabetes type II, *Momordica charantia*, antioxidant activity, ferric thiocyanate, thiobarbituric acid, 1, 1-Diphenyl-2-picryl-hydrazyl, malondialdehyde.

INTRODUCTION

Diabetes mellitus is one of the most pressing global health problems. It is estimated that the prevalence of diabetes mellitus will be more than 300 million in 2025 (Ojewole et al., 2006). Diabetes mellitus is a disturbance of carbohydrate, fat and protein metabolism accompanied by an insufficiency of insulin secretion and/or action. The complications of diabetes mellitus affect the eyes, kidneys, nerves, arteries and skin (Virdi et al., 2003; Chandra et al., 2007). The development of these complications can be postponed by reducing blood glucose to normal levels (Virdi et al., 2003). Type II diabetes is the most common form of the disease and usually involves insulin resistance and β-cell dysfunction (Lupi and Del Prato, 2008). The Streptozotocin (STZ) treated neonatal rats, a model of type II diabetes, exhibit increased blood glucose and decreased insulin levels on Postnatal day one. Therefore, type II diabetes can be studied *in vivo* using this animal model (Portha et al., 2007).

Several studies have indicated that diabetes mellitus is accompanied by an increase in free radicals and a

*Corresponding author. E-mail: zuki@vet.upm.edu.my. Tel: +603-89463504. Fax: +603-89471971.

reduction in antioxidant activity. Thus, the balance between free radical formation and the defence system against them is impaired. This imbalance causes damage to cell components including proteins, lipids and nucleic acids (Rahimi et al., 2005) and eventually results in cell death (Wright et al., 2006). Evans et al. (2002) reported that an increase in reactive oxygen species (ROS) in diabetes mellitus leads to oxidative stress and cellular dysfunction. A previous study has shown that increased thiobarbituric acid reactive substances (TBARS) in rats with STZ-induced diabetes are an indirect measure of the production of free radicals (Semiz and Sen, 2007).

Based on ethnobotanical information, there are approximately 800 herbal medicinal plants for controlling diabetes mellitus (Chandra et al., 2007). There is some evidence that an increased consumption of fruits and vegetables that contain phytochemicals reduces the risk of chronic diseases, such as diabetes (Myojin et al., 2008), cancer and cardiovascular diseases (Semiz and Sen, 2007; Myojin et al., 2008). Previous studies have reported that plants with hypoglycaemi effect might work via a different mechanism such as inhibition of glucose absorption, enhanced use of glucose by the liver and increased insulin secretion (Sezik et al., 2005).

One of these plants is *Momordica charantia* (MC) or bitter melon. MC is a vegetable belonging to the Cucurbitacea family, which usually grows in Asia, South America and Africa (Virdi et al., 2003; Semiz and Sen, 2007). The unripe fruit, leaves, seeds and roots of MC have been used as anti-diabetic, anti-inflammatory, anti-cancer, anti-HIV, anti-ulcer and anti-tumour medications (Virdi et al., 2003; Raj et al., 2005; Ojewole et al., 2006; Myojin et al., 2008). Some studies have investigated hypoglycaemic chemicals in MC that include a mixture of steroidal saponins known as charantins, insulin-like peptides, oleanolic acid glycosides and alkaloids such as momordicine (Miura et al., 2001). The unripe fruit of MC is a good source of phosphorus, iron and vitamins C and A, (Raj et al., 2005); MC also contains active phytochemicals, including triterpenes, proteins and steroids (Raza et al., 2000). MC is an important source of phenolic compounds. The main phenolic acids in MC are gallic acid, gentisic acid, catechin, epicatechin, and chlorogenic acid (Horax, 2005, 2010; Burdrat and Shotipruk, 2009). Gallic acid is the most predominant phenolic compounds in MC (Kubola, 2008). It has been reported that the seeds of MC are effective on tissue lipid peroxides and enzymatic antioxidants in STZ-treated diabetic rats (Sathishsekar and Subramanian, 2005). Phenolic compounds as an antioxidant agent with redox properties that absorbs and neutralizes free radicals could prevent lipid peroxidation, cross-linking and DNA mutation, and tissue damage (Javanmardi et al., 2003; Horax et al., 2005).

Until now, there has not been a report on the aqueous extract of MC using methods that measure ferric thiocyanate (FTC) for the initial stage of lipid peroxidation and thiobarbituric acid (TBA) for the secondary stage of lipid peroxidation. Thus, the current study was carried out to characterise the antioxidant activity of this extract by various methods. The total phenol and flavonoid contents of the extract were also determined. In addition, this study was conducted to evaluate the effects of the aqueous extract of MC fruit on non-insulin-dependent diabetes mellitus (NIDDM) in diabetic neonatal rats.

MATERIALS AND METHODS

Animals

The procedures for the animal trials in this study have been authorised and approved by the Animal Care and Use Committee of the Faculty of Veterinary Medicine, University Putra Malaysia. Diabetes was induced in one-day-old Sprague-Dawley rats with a single intraperitoneal injection of STZ (85 mg/kg), freshly dissolved in 0.9% saline solution (Abdollahi et al., 2010). Meanwhile, the normal control neonatal rats (NC) received an equivalent volume of 0.9% saline solution only. After 12 weeks, diabetic animals were randomly divided into three groups consisting of seven animals per group. The three groups were as follows: the diabetic control group (DC), the diabetic group treated with the aqueous extract from MC fruit at a dosage of 20 mg/kg body weight twice daily for a period of four weeks (DMO), and the diabetic group was treated with glibenclamide at a dosage of 0.1 mg/kg body weight twice daily for a period of four weeks (DPG). The NC group was comprised of the saline-treated, non-diabetic rats. The rats were kept in cages at a suitable temperature (22 ± 2°C) and 12 h day-night cycle. The rats were given a standard pellet diet throughout the study period and water was available at all times. Diabetes was confirmed by determination of blood glucose concentration using a glucometer (Accu-Chek Instant Plus blood glucose monitor, Roche Diagnostics Corp, Germany) on the second post-injection day. The animals were considered diabetic if their blood glucose concentration was more than 11 mmol/l at that time (Abdollahi et al., 2010). Following confirmation of diabetes, five of these diabetic neonatal rats were randomly selected for pancreatic β-cell pathological study.

Blood collection and biochemical analysis

Blood glucose was measured by glucometer once a week during the period of treatment. At the end of the treatment period, blood samples were collected through the saphenous vein, and the plasma was separated and kept at -80°C for biochemical assays.

Preparation of the aqueous extract from *M. charantia* fruit

Fresh, unripe, whole fruit of the MC plant were collected from local markets within 5 km radius from the preparation venue in Malaysia and prepared within 24 h post harvest, and extraction was performed by evaporative procedures. The aqueous extract of MC was prepared according to the method of Virdi et al. (2003). Small pieces of the whole fruit were drenched in water (10:25 w/v) for one hour at room temperature. The mixture was then filtered and evaporated by a rotary evaporator (BUCHI Rotavapor R-220) to dry it under reduced pressure to yield powdered extract. The extract was kept at -80°C until use. Each day, the MC extract was weighed and dissolved in distilled water. The yield was 17 g powder per kg fresh whole fruit (1.7%).

Antioxidant activity determination

Antioxidant assays were performed using the following three

methods:

Ferric thiocyanate (FTC) method

Ferric thiocyanate assays were conducted following the method of Saha et al. (2004), with slight modifications. The amount of 4 mg of MC extract and standards butylated hydroxytoluene (BHT), vitamin C and vitamin E were dissolved in 4 ml of absolute ethanol and then 4.1 ml of 2.52% linoleic acid in absolute ethanol, 8.0 ml of 0.02 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water. The mixtures were then placed in a 40°C oven. Next, 0.1 ml of each mixture was mixed with 9.7 ml of 75% (v/v) ethanol and 0.1 ml of 30% ammonium thiocyanate and then precisely 3 min after adding 0.1 ml of 0.02 M ferrous chloride in 3.5% hydrochloric acid, absorbance was measured at 500 nm in a spectrophotometer (Secomam, Domont, France) every day until the absorbance of the control samples reached a maximum.

A mixture without added sample was used as a negative control. The percent of inhibition of lipid peroxidation was estimated by the following formula:

$$\% \text{ Inhibition} = 100 - [(A_1 - A_0) \times 100]$$

Where A_0 is the absorbance of the negative control and A_1 is the absorbance of the extract samples (Elmastas et al., 2007).

Thiobarbituric acid (TBA) method

The TBA assay was performed following the method of Saha et al. (2004). On the final 8th day of the FTC assay, 2 ml of the sample solution from the FTC assay were added to 1 ml of 20% aqueous trichloroacetic acid and 2 ml of 0.67% aqueous thiobarbituric acid. That mixture was placed in a boiling water bath for 10 min. After cooling, the tubes were centrifuged at 3,000 rpm for 30 min. Absorbance of the supernatant was evaluated at 532 nm in a spectrophotometer. The percentage of inhibition of the secondary stage of lipid peroxidation was calculated by the following formula:

$$\% \text{ Inhibition} = 100 - [(A_1 - A_0) \times 100],$$

Where A_0 is the absorbance of the negative control and A_1 is the absorbance of the extract samples (Elmastas et al., 2007).

Free radical scavenging activity

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method determines the free radical inhibitory ability of different antioxidants. DPPH is a purple-coloured stable free radical that receives an electron or hydrogen atom to become a stable diamagnetic molecule with a yellow colour (Myojin et al., 2008). The free radical scavenging activity of MC aqueous extracts was evaluated by determining the conversion of DPPH free radicals to diamagnetic molecules, as described by Jain et al. (2008).

Briefly, 3 ml of six different concentrations of the MC extract (15.62, 31.25, 62.5, 125, 250 and 500 µg/ml) or vitamin C as a standard (1.56, 3.12, 6.25, 12.5, 25 and 50 µg/ml) was aliquoted to different test tubes. Then, 1 ml of a 0.1 mM ethanolic solution of DPPH was added to each tube. After incubating the tubes in the dark for 30 min, the absorbance at 517 nm was measured against pure ethanol by a spectrophotometer. Radical scavenging activity was expressed as per centinhibition and was calculated by the following formula:

$$\text{Radical scavenging (\%)} = [(A_0 - A_1 / A_0) \times 100],$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the extract samples (Elmastaset al., 2007). Lower absorbance values of the mixtures indicate higher free radical scavenging activity. The IC_{50} values denote the effective concentration of sample that is required to scavenge 50% of the DPPH free radicals (Jain et al., 2008).

Total phenol content assay

The total phenolic content of the MC extract was analysed according to the Folin-Ciocalteau method (Kim et al., 2003). First, 0.5 ml (1000 µg/ml) of extract solution and 0.5 ml of each gallic acid standard solution (50, 100, 150, 250 and 500 mg/l) was mixed with 4.5 ml of deionised distilled water (ddH_2O), and subsequently 0.5 ml of Folin and Ciocalteau's reagent was added. The mixtures were left for 5 min at room temperature. Then, 5 ml of 7% sodium carbonate (Na_2Co_3) and 2 ml of ddH_2O were added to the mixture. The mixtures were incubated at 23°C for 90 min. The absorbance was then measured at 750 nm. Total phenolic content was indicated in milligrams of gallic acid (GAE) equivalents per gram of extract (mg GAE/g extract).

Total flavonoid content assay

The flavonoid content of the MC extract was estimated based on the colorimetric method (Ebrahimzadeh et al., 2008). Briefly, 0.5 ml of extract solution (5 g/l)and 0.5 ml of quercetin (as a standard; 12.5, 25, 50, 80 and 100 mg/l) each was mixed with 1.5 ml of methanol, followed by 0.1 ml of 10% aluminium chloride hexahydrate ($AlCl_3$) and subsequently mixed with 0.1 ml of potassium acetate and 2.8 ml of distilled water. After proper mixing, the solutions were incubated at room temperature for 30 min. The absorbance at 415 nm was measured by a spectrophotometer,and a standard curve was prepared using the absorbances of the quercetin solutions. Total flavonoid content was estimated in milligrams of quercetin equivalents per gram of extract (mg QE/g extract).

Determination of MDA concentration in plasma

The lipid peroxidation in terms of malondialdehyde (MDA) was estimated in the plasma of the experimental animals after treatment following the method described by Ohkawa et al. (1979), with slight modifications. Briefly, 0.3 ml plasma, 2.4 ml of sulphuric acid (H_2SO_4) and 0.3 ml of 10% sodium tungstate dehydrate (Na_2WO_4) were mixed and left for 10 min at room temperature. The samples were centrifuged at 5,000 rpm for 10 min. The pellets were resuspended in 450 µl of distilled water (DW), 50 µl 2 BHT (7 mM), 3 ml chloridric acid (HCl, 0.05 M) and 1 ml thiobarbituric acid (TBA, 1%) and were incubated at 95°C for 1 h. After cooling in running water, 4 ml of n-butanol was added. The reaction mixture was then centrifuged at 5,000 rpm for 10 min. A clear butanol fraction obtained after centrifugation was used to measuring the absorbance at 532 nm by spectrophotometer. The standard curve was prepared with absorbance values for different concentrations of 1,1,3,3-tetraethoxypropane (TEP; 0.1-5 µM/l).

Determination of MDA concentration in tissue

The MDA was measured in the pancreatic tissue of the animals after treatment following the method described by Ohkawa et al. (1979), with slight modifications. The pancreatic tissue samples were separated and rinsed with normal saline and then immediately frozen at -80°C. One gram of tissue was homogenized in 4 ml of potassium chloride (1.15%). Then, a 200 µl homogenized sample

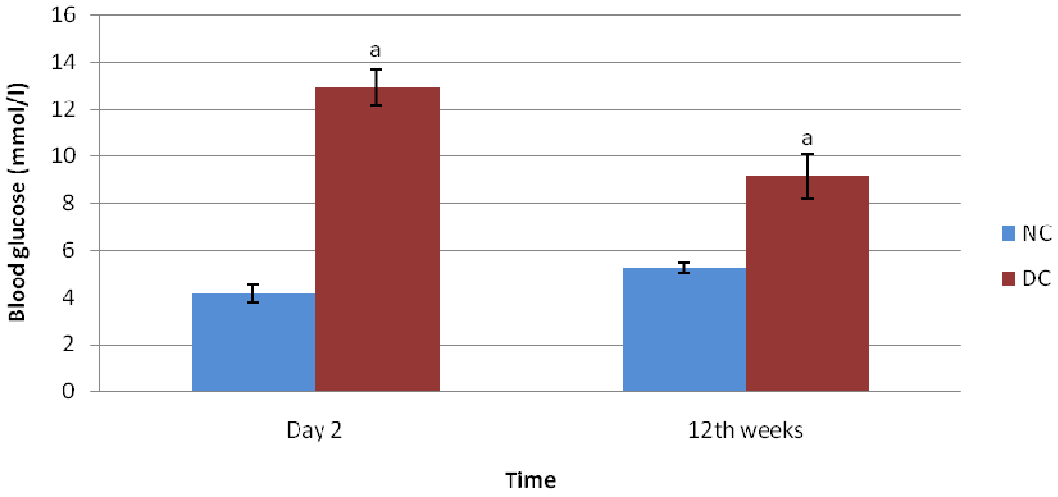

Figure 1. Non-fasting blood glucose concentrations changed after STZ injection at the second post-injection day and at 12 weeks of age. The lowercase letters indicate a significant difference between the NC and DC groups. (P ≤ 0.05, mean±SD, n=7).

was mixed with 300 µl DW, 35 µl BHT, 165 µl sodium dodecyl sulphate (SDS) and 2 ml TBA. Next, the mixture was heated for 60 min at 90°C.The solution was immediately cooled in running water. After adding 3 ml of n-butanol, the solution was centrifuged at 5,000 rpm for 10 min. The butanol fraction obtained was used to estimate the absorbance at 532 nm by spectrophotometer. The standard curve was prepared with different concentrations of TEP (2.5-50 µM/l).

Immunohistochemical localization

To identify and quantify the pancreatic insulin-positive cells, immunohistochemical staining was performed on pancreatic sections from the experimental animals. The pancreatic tissue was fixed in 4% paraformaldehyde in PBS overnight at 4°C and then was dehydrated by submersion in increasing concentrations of alcohol, cleared in xylene and embedded in paraffin. Tissue sections (3 µm) were deparaffinised with xylene (3 × 5 min) and rehydrated with a series of ethanol washes (100, 90 and 70%). After boiling tissue for 20 min in target retrieval solution (tris EDTA, pH 9.0) in a microwave, the slides were cooled down for 20 min and placed in TBS with 0.05% Tween20. The slides were incubated with peroxidase block solution (3% H_2O_2) for 10 min and then rinsed with distilled water for 5 min. The sections were immersed in TBS with 0.05% Tween20. The slides were then incubated with mouse monoclonal anti-insulin primary antibody (abcam, ab6995 England) for 30 min at room temperature and washed four times in TBS with 0.05% Tween20 and incubated with secondary antibody (biotinylated anti-mouse, BA-2001, Vector Laboratories) for 40 min, washed four times in TBS with 0.05% Tween20 and then incubated with ABC alkaline phosphatase kit solution (PK-6100, Elite kit, Vector Laboratories) for 1 h. Then, DAB substrate (Dako; dilution 1:50) was added for 2 to 5 min. The slides were counterstained with Mayer's haematoxylin, mounted with coverslips and examined by light microscopy (× 200) (Olympus BX51, Japan).

Statistical analysis

The results were described as mean ± standard deviation (SD). The data were subjected to one-way analyses of variance (ANOVA) and student's t-tests using the Statistical Analysis System (SPSS 15.0) program. The correlation between IC_{50} values and the total phenolic and flavonoid contents was established using a regression analysis at a 95% significance level. For all analyses, P values ≤ 0.05 were considered significant.

RESULTS

Blood glucose concentration

The results of the blood glucose assay on the second post-injection day and at the beginning of the treatment (12th week after STZ injection) showed a significant increase in the DC (4.15±0.389 and 5.25±0.195, respectively) as compared to the NC group (12.91±0.763 and 9.15±0.944, respectively) (P≤0.05) (Figure 1). The administration of MC fruit extract in the DMO group reduced the levels of blood glucose in the second, third and fourth weeks post-treatment as compared to the DC group (P≤0.05). Similarly, the DPG group had reduced blood glucose levels in the second, third and fourth weeks post-treatment as compared to the DC group (P≤0.05). However, during the treatment period, our results showed that blood glucose levels in the DC group were significantly higher than in the NC group (P≤0.05) (Table 1). The results also showed that the blood glucose levels in both treatment groups (DMO and DPG) and the control group (NC) were not significantly different (P>0.05) after the first week of treatment.

Immunohistochemical study of pancreatic β-cells

Quantitative study of immunohistochemical staining in pancreatic β-cells of neonatal rats demonstrated that the numbers of insulin-positive cells were reduced in the

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

Groups	Parameter			
	1st week*	2nd week*	3rd week*	4th week*
NC	5.49 ± 0.242 [b]	5.82 ± 0.339 [b]	5.55 ± 0.232 [b]	5.53 ± 0.545 [b]
DC	8.65 ± 0.828 [a]	8.43 ± 0.929 [a]	9.83 ± 0.947 [a]	8.90 ± 0.663 [a]
DMO	8.31 ± 1.081 [a]	6.05 ± 0.713 [b]	5.80 ± 0.592 [b]	6.01 ± 0.207 [b]
DPG	8.090 ± 0.987 [a]	5.60 ± 0.249 [b]	5.64 ± 0.217 [b]	5.40 ± 0.388 [b]

* Data represents mean±SD (n=7). Different lowercase letters in the same column indicate significant difference at P ≤ 0.05.

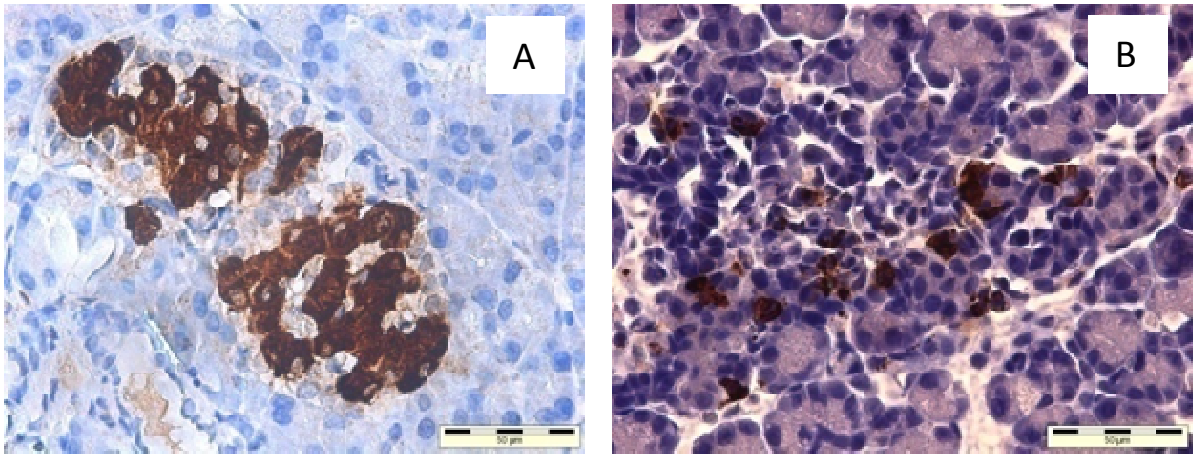

Figure 2. Immunohistochemical staining for insulin-positive cells in pancreatic islets of the (A) normal and (B) diabetic neonatal rats on the second day after STZ injection.

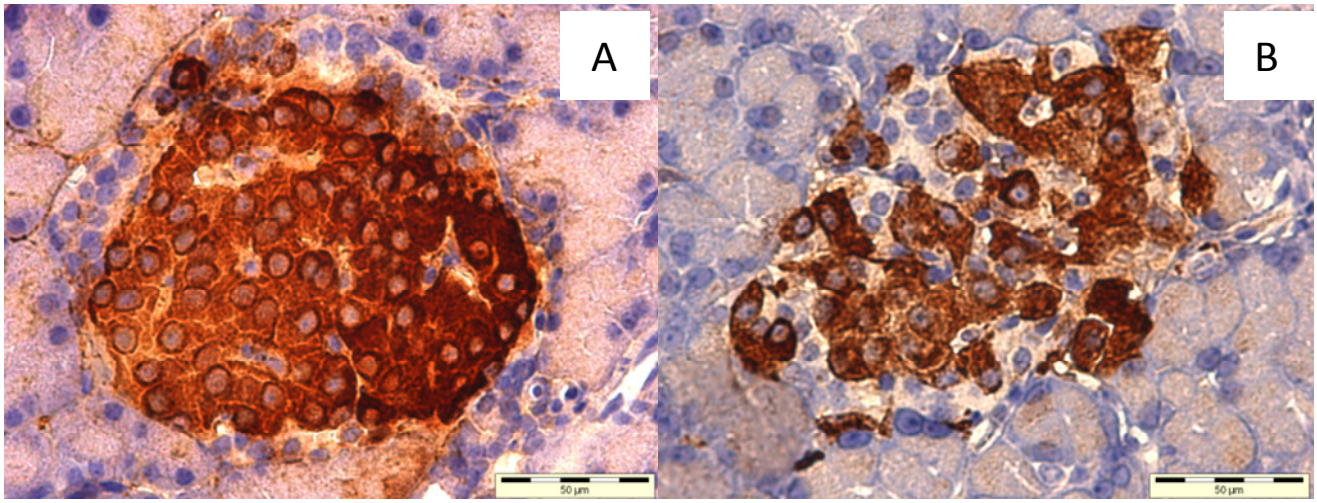

Figure 3. Immunohistochemical staining for insulin-positive cells in pancreatic islets of the (A) normal and (B) diabetic neonatal rats in the 12th week after STZ injection.

diabetic neonatal rats compared to the normal control neonatal rats on the second day (Figure 2) and in the 12th week after STZ injection (Figure 3).

Ferric thiocyanate (FTC) method

As Figures 4 and 5 show the percentage of inhibition

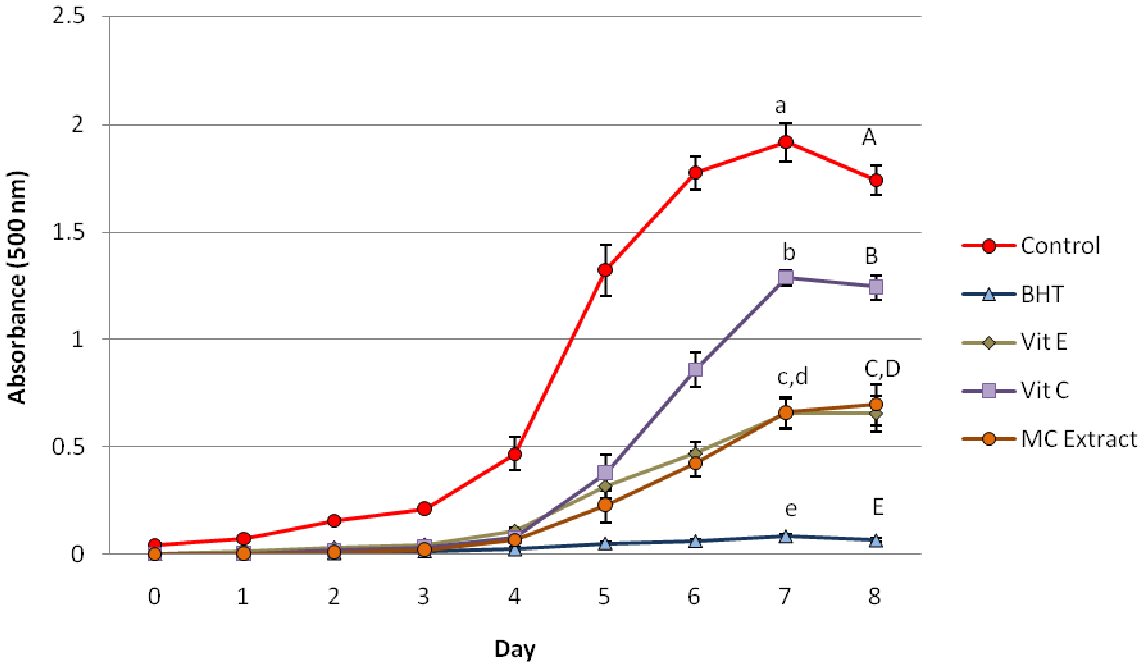

Figure 4. Antioxidant activity of the MC extract measured by the FTC method (mean±SD). Each experiment was executed in triplicate and repeated three times. The different lowercase letters on day 7 and the uppercase letter on day 8 indicate significant differences P≤0.05).

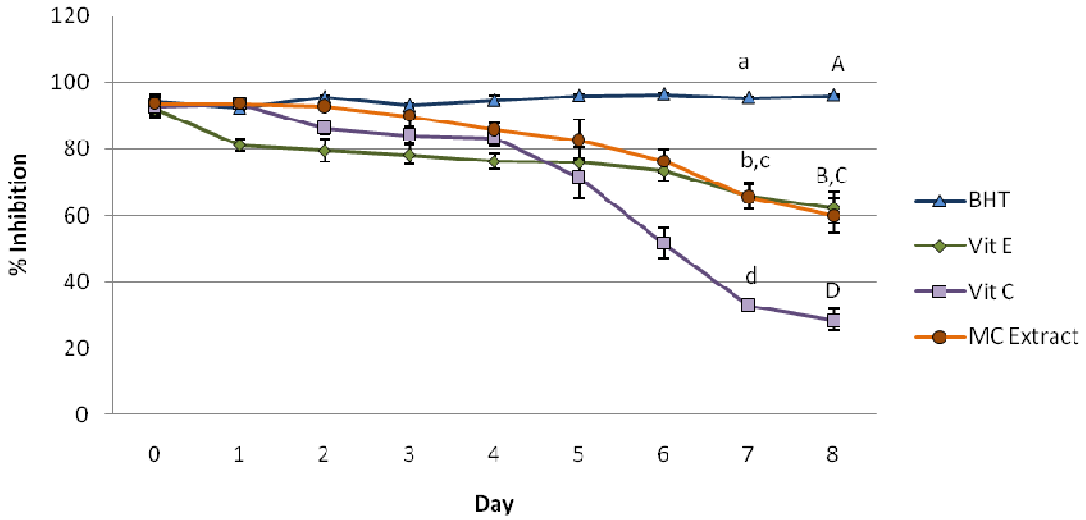

Figure 5. Percentage of inhibition of linoleic acid peroxidation as measured by the FTC method (mean±SD). Each experiment was executed in triplicate and repeated three times. The different lowercase letters on day 7 and the uppercase letter on day 8 indicate significante differences (P≤ 0.05).

(as determined through absorbance measurements) of the MC extract (65.60±3.599%, 60±5.338%) was significantly (P≤0.05) higher than of vitamin C (32.91±1.773%, 28.54±3.266%) on the seventh and eighth days, respectively. However, there was no difference between the MC extract and vitamin E (65.76±3.803%, 62.30±4.739%) on the seventh and eighth days, respectively. Meanwhile, the percentage of inhibition of the MC extract was significantly (P≤0.05) lower than BHT (95.48±0.312%, 96.14±0.376%) on the seventh and eighth days, respectively.

In this study, lipid peroxidation was elevated

Table 2. Total antioxidant activity of samples by TBA method.

Samples	Absorbance (532 nm)	Percentage of inhibition
Control	1.23±0.067[a]	0[a]
BHT	0.04±0.005[b]	96.22±0.435[b]
Vitamin E	0.45±0.080[c]	63.41±6.509[c]
Vitamin C	1.02±0.056[d]	16.75±4.577[d]
MC Extract	0.49±0.092[e]	59.99±7.501[e]

Data represents mean±SD. Each experiment was executed in triplicate and repeated three times. Different lowercase letters in the same column indicate significant difference at P≤0.05.

Table 3. Comparison evaluation of IC_{50} values.

Sample	IC_{50} value (µg/ml)
Vitamin C	25.55±0.404[a]
MC aqueous extract	248.14±11[b]

Data represents mean±SD of duplicate analysis. Different lowercase letters within column indicate significant difference at P≤0.05.

at each 24 h time point over a period of eight days (the optical density of the control reached a maximum on the seventh day). The control showed an increase in the absorbance value from day zero onwards and reached the maximum level on day 7 of the experiment, and the absorbance value dropped on day 8.

Thiobarbituric acid (TBA) method

Table 2 shows the absorbance and percentage of inhibition of the samples. The absorbance values of the MC extract were significantly (P≤0.05) higher than those of BHT and lower than those of vitamin C. However, the results showed no difference between the MC extract and vitamin E. The percentage of inhibition for the MC extract was significantly (P≤0.05) lower than for BHT and higher than for vitamin C. Meanwhile, the percentage of inhibition for the MC extract was similar to that for vitamin E. The results of the TBA assay correlate well with those of the FTC assay.

Free radical scavenging activity

The IC_{50} for DPPH radical scavenging activity in the aqueous extract from MC was significantly higher than that for vitamin C (Table 3).

The data represented in Table 4 show that free radical inhibition correlated with the concentration of the aqueous extract for six different concentration of aqueous extract.

Total phenolic and flavonoid content assays

The total amounts of phenol compounds, as determined by the Folin-Ciocalteu method, are reported as Gallic acid equivalents by reference to the standard curve (y=0.003x + 0.077, r^2=0.997). The total flavonoid contentis reported quercetin equivalents, by reference to the standard curve (y=0.002x + 0.014, r^2=0.992). Figure 6 shows the total phenol (16.88±1.283 mg of gallic acid/g of extract) and flavonoid (12.06±1.146 mg of quercetin/g of extract) contents of the aqueous extract from MC.

Correlation between the phenol assay and the DPPH assay

Figure 7 shows the strong negative correlation between the total phenolic content and the total antioxidant capacity (IC_{50}) of the MC fruit extract. Regarding the antioxidant capacity of the MC extract as measured by the DPPH method, the correlation coefficients resulting from the linear regression analysis were high for total phenols (y = -8.579x + 393, r^2 = 0.99 and, P = 0.024). These results also showed that a decrease in radical scavenging ability could be related to an increase in phenolic compounds.

In this study, a correlation between the IC_{50} value and the flavonoid compound content in the extract was not found (R = 0.331 and P = 0.785).

Determination of MDA concentration in plasma and tissue

Figure 8 shows the MDA activity in plasma. In the DC group (3.06±0.59 µmol/l), the activity of MDA in plasma was significantly higher (P≤0.05) than that of the NC (1.62±0.28 µmol/l) and the DMO (1.60±0.33 µmol/l) groups. Interestingly, the MDA level in the DMO group approached the level in the NC group. Conversely, glibenclamide did not reduce the increased MDA level in the DPG (3.24±0.71 µmol/l) group as compared to the DC group (P≤0.05). Figure 9 shows the MDA activity in the pancreatic tissue. In the DC (13.75±1.32 µmol/g) group, the activity of MDA in the pancreatic tissue was significantly higher than in the NC group (5.69±1.47 µmol/g) (P≤0.05). The treated diabetic rats in the DMO group (6.95±0.96 µmol/g) showed decreased MDA activity as compared to the DC group (P≤0.05). There

Table 4. Percentage of free radical scavenging activity (% inhibition) of aqeueous extract of MC.

Concentration (µg/ml)	500	250	125	62.5	31.25	15.62
Inhibition (%)	97.84±0.169	57.46±0.716	26.53±0.447	9.3±0.309	5.09±0.091	2.44±0.304

Each experiment was executed in triplicate and repeated three times (mean±SD).

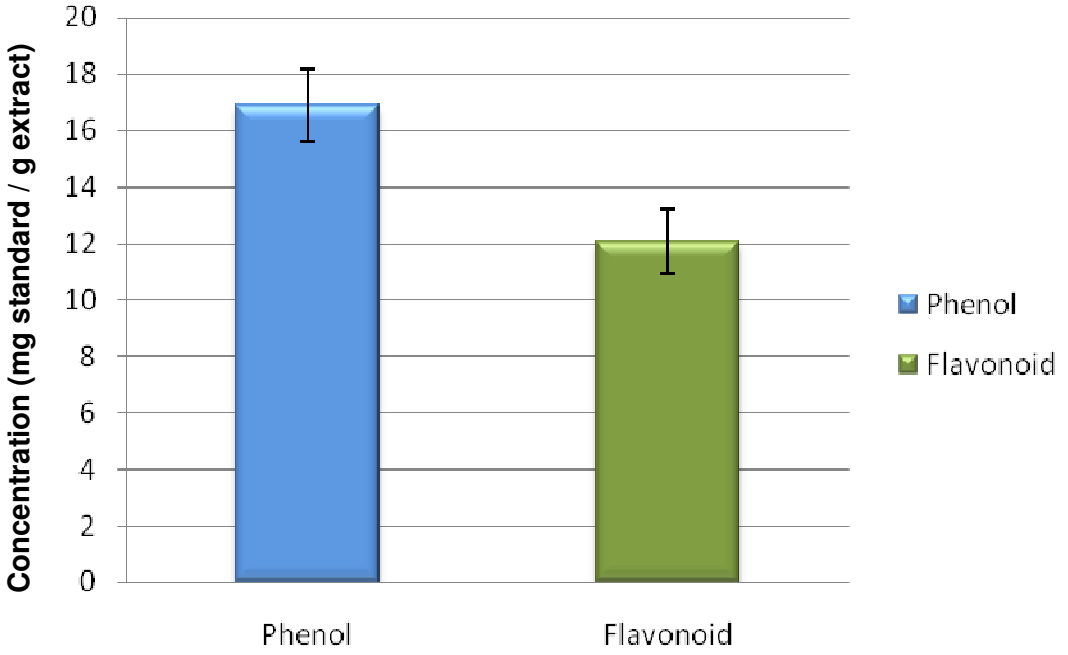

Figure 6. Total phenolic and flavonoid contents of the aqueous extract of MC (mean±SD). Each experiment was executed in triplicate and repeated three times.

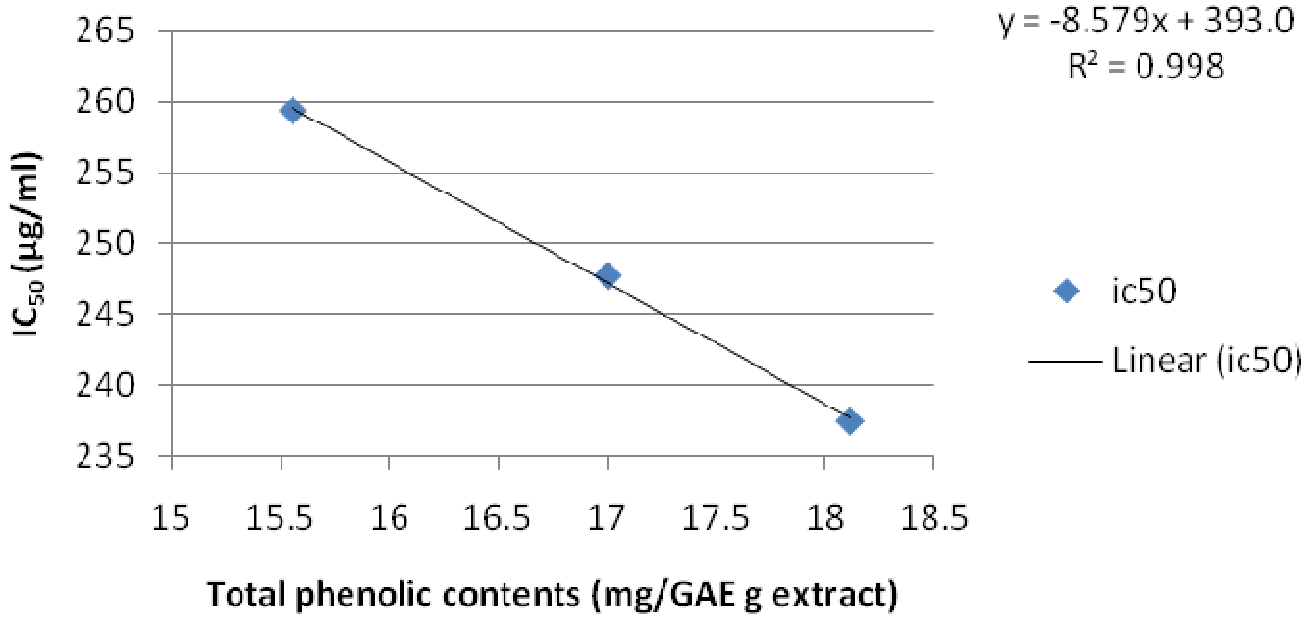

Figure 7. Relationship between total phenolic contents and DPPH (IC_{50}) in the extract from MC. Data represent mean±SD.

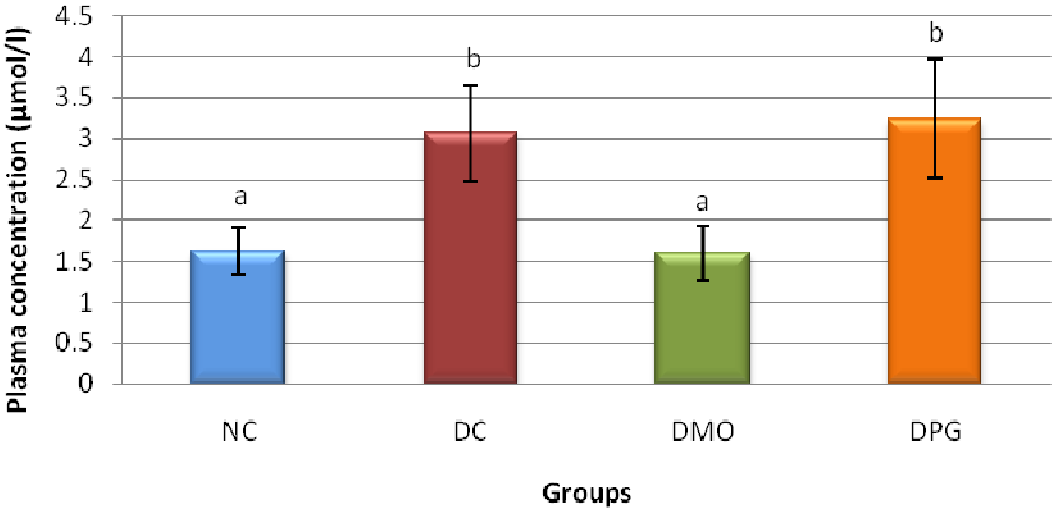

Figure 8. Plasma MDA levels in experimental animals after treatment (mean±SD, n=10). Different lowercase letters indicate significant differences (P≤0.05).

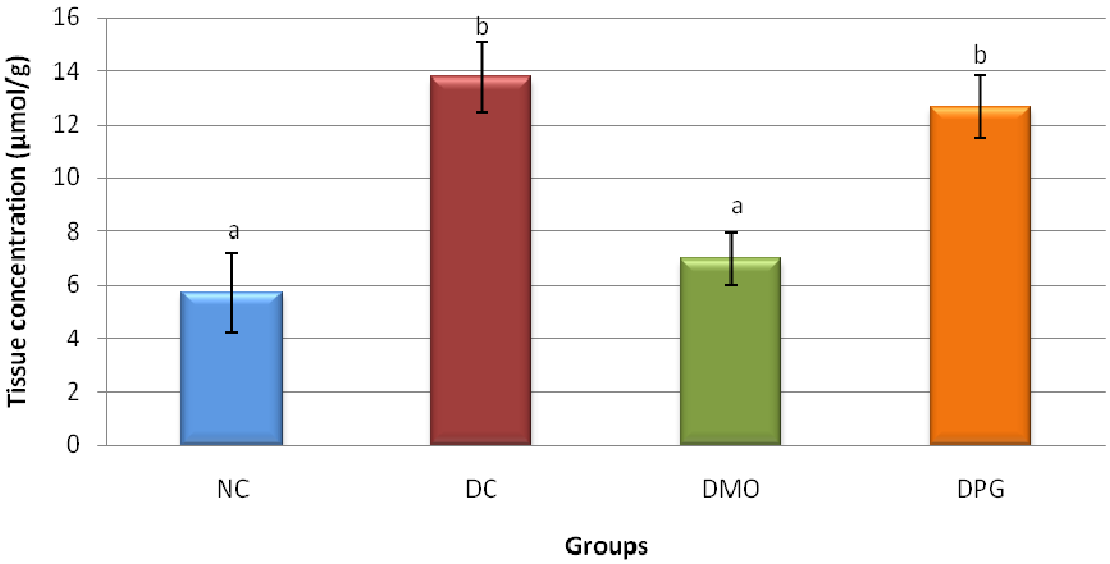

Figure 9. Pancreatic tissue MDA levels in experimental animals after treatment (mean±SD, n=10). Different lowercase letters indicate significant differences (P ≤ 0.05).

was no significant reduction in the MDA concentration of pancreatic tissue in the DPG (12.67±1.16 µmol/g) rats when compared to the DC rats (P>0.05).

DISCUSSION

The neonatal rat STZ-induced diabetes model induces a deficiency in insulin secretion and action, much like NIDDM in humans (Portha et al., 2007). This research was designed to investigate the antioxidant and antihyperglycaemic effect of an MC fruit aqueous extract in this animal model. The results of this study showed that blood glucose levels fell significantly in both MC- and glibenclamide-treated diabetic rats. These findings are in agreement with previous studies (Virdi et al., 2003; Chandra et al., 2007). Glibenclamide is a sulphonylurea that decreases blood glucose levels in diabetic subjects by increasing insulin secretion from pancreatic β-cells, decreasing blood glucagon concentrations and improving

insulin's action on target tissues. Traditional antidiabetic plants could affect blood glucose through mechanisms similar to those of the sulphonylureas (Ojewole et al., 2006).

Immunohistochemical quantitative study of β-cells in pancreatic islets revealed a decrease in the number of β-cells in diabetic neonatal rats as compared to normal rats on the second day and twelfth weeks after STZ injection. Chronic hyperglycaemia causes pancreatic islet damage, leading to pancreatic dysfunction and development of type II diabetes (Nivitabishekam et al., 2009). Diabetic conditions produce oxidative stress and cause destruction of pancreatic β-cells (Cemek et al., 2008). In support of this association between oxidative stress and β-cell destruction, Sathishsekar and Subramanian (2005) observed β-cell death and a reduction in the number of islets in the pancreas of diabetic rats. The MC fruit extract increases the number of insulin-positive cells (Ahmed et al., 2004).

Our prior research had shown that the MC fruit extract increase the serum insulin levels in type II diabetic rats (Abdollahi et al., 2010). Some studies have also reported the importance of the hypoglycaemic components of MC, which consist of a mixture of saponins such as charantin, insulin-like peptides and alkaloids that are concentrated in the fruit. Polypeptide insulin-like compounds, such as glucoside, are also reported to be useful for both insulin dependent diabetes mellitus (IDDM) and NIDDM (Ojewole et al., 2006). Therefore, the aqueous extract of MC fruit might improve increase blood insulin levels and improve hyperglycaemia through its insulin-like component peptides.

Diabetes-induced oxidative stress is due to an increase in free radicals and a reduction in antioxidant protection. Therefore, compounds with hypoglycaemic and antioxidative properties would be useful as antidiabetic agents (Sathishsekar and Subramanian, 2005). In this research, the results of FTC and TBA assays indicated that the aqueous extract of MC has stronger antioxidant properties than vitamin C. In addition, in comparing the FTC and TBA levels, which represent the first and second stages of lipid peroxidation, respectively, the results showed that the amount of FTC was 16% higher than the amount of TBA. Abas et al. (2006) have shown that the percent inhibition of FTC is higher than that of TBA in methanolic and aqueous extracts from several plants. Higher antioxidant activity found in the FTC assay in this study indicates that the amount of peroxide in the initial stage of lipid peroxidation is greater than the amount of peroxide in the secondary stage. Therefore, the results of this study suggest that the aqueous extract of MC has beneficial effects on the initial stage of lipid peroxidation.

The DPPH scavenging assay is commonly used as a rapid analysis of the free radical scavenging activity of various plants extracts (Abas et al., 2006). Antioxidant agents may react through their electron- and/or hydrogen-donating ability in the DPPH assay. A faster reaction indicates more potent free radical scavenging activity. DPPH is a stable free radical with a purple colour and receives an electron or hydrogen to become a stable diamagnetic molecule with a yellow colour (Gülçin et al., 2004; Elmastas et al., 2007). The current study found that the aqueous extract of MC is a free radical inhibitor or scavenger.

Our prior research had shown that the methanolic extract of MC is also a free radical inhibitor (unpublished results). However, its activity was lower than that of the aqueous extract of MC. We had found a dose-dependent relationship in the DPPH assay between free radical scavenging activity and the concentration of the aqueous extract. The findings of the current study are consistent with those of Jain et al. (2008) and Wu and Ng (2008), who found high free radical antioxidant activity of the aqueous extract of the leaves (*Momordica dioica* Roxb.) and fruit of MC (wild MC). Yilmaz et al. (2004) have reported that the level of lipid peroxidation and the activities of antioxidant enzymes were enhanced in rats with STZ-induced diabetes. One of the important roles of antioxidants is to inhibit the chain reaction of lipid peroxidation (Pitipanapong et al., 2007). This extract might react with free radicals, which are the main promoters of the auto-oxidation of fatty acid chain of fat, thereby terminating the chain reaction and limiting free radical cellular damage (Elmastas et al., 2007; Gülçin et al., 2004).

However, in this study, the aqueous extract of MC showed good antioxidant ability. Meanwhile, the difference in the level of antioxidant activity could be due to differences in the concentration and type of antioxidants present in these extracts. Based on the results of the antioxidant assay on the MC fruit extract, we suggest that the consumption of MC fruit protects against lipid peroxidation.

The present study estimated the phenolic and flavonoid content of the aqueous extract of MC. Our results showed that the MC extract contains suitable phenol and flavonoids, although the phenol level was higher than the flavonoid level. The findings of the current study are consistent with those of Horax et al. (2005), who found that the Indian variety and Chinese variety of MC contains a potent supply of phenolic compounds and is a source of powerful antioxidants. In addition, Wu and Ng (2008) indicated that both the aqueous and ethanolic extracts of wild MC contain phenol and flavonoid components. Previous studies have reported that flavonoids are a group of secondary plant phenols with antidiabetic and antioxidant effects (Heim et al., 2002; Coskun et al., 2005). Polyphenols are known to be important compounds that play a major role in antioxidant activity (Sathishsekar and Subramanian, 2005; Elmastas et al., 2007). They play a critical role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen moleculesand in peroxide decay (Zheng and

Wang, 2001).

The present study revealed that the aqueous extract of MC is a polar solvent with a high concentration of phenols and flavonoids, which have significant free radical scavenging activity. Thus, the current results strongly suggest that phenols and flavonoids are important components of this plant, and some of its antidiabetic and antioxidative effects could be attributed to the presence of these valuable constituents.

In this study, we have found a strong correlation between phenolic content and DPPH radicals. These results showed that the IC_{50} value of DPPH radical scavenging decreases with an increase in the phenolic content. Meanwhile, we could not find any correlation between flavonoids and antioxidant activity (data not shown). Our results are in accordance with others', which have indicated a strong correlation between antioxidant activity and the total amount of phenols (Holasova et al., 2002; Aljadi and Kamaruddin, 2004). This result confirms the efficacy of the phenolic contents in the antioxidant activity of MC. Although the flavonoids did not show an important role in the antioxidant activity of MC, there might be other polyphenolic components that contribute to that activity.

As has been previously reported, increased formation of toxic free radicals is associated with increased lipid peroxidation through attack on unsaturated lipids (Sathishsekar and Subramanian, 2005; Pitipanapong et al., 2007). Malondialdehyde is a general indicator of lipid peroxidation (Sathishsekar and Subramanian, 2005). It was obvious from our study that the MDA level in the serum and pancreatic tissue was significantly higher in the DC group than in the NC group. The current study demonstrated that in diabetic rats treated with the MC fruit extract (DMO), the MDA levels in pancreatic tissue and plasma were lower than in the DC and DPG groups and comparable to levels in the NC group. It is possible that free radical formation was increased in the diabetic animals (Sathishsekar and Subramanian, 2005), as previous studies have shown a high level of MDA in erythrocyte ghost membranes of diabetic patients (Ahmed et al., 2006) and in pancreatic (Yazdanparast et al., 2007) and liver tissue of diabetic animals (Abdollahi et al., 2010). In addition, Mahboob et al. (2005) have shown a significant increase in the serum MDA level of patients with type II diabetes mellitus. The presence of free radicals and the occurrence of oxidative stress in diabetes probably cause damage to the diabetic pancreas (Yazdanparast et al., 2007).

The MC fruit extract raises the level of antioxidant enzymes (Semiz and Sen, 2007), which could be effective on preventing oxidative stress damage through the scavenging of free radicals (Wu and Ng, 2008). It has been shown that the chronic administration of MC fruit juice reduced the MDA level in diabetic animals (Sitasawad et al., 2000). These data clearly indicate that MC, by decreasing lipid peroxidation, could be effective in preventing the oxidative damage that is thought to be involved in diabetic pancreatic damage.

Conclusion

The results of this study indicated that the aqueous extract of MC fruit at a dose of 20 mg/kg body weight can alleviate STZ-induced hyperglycaemia in a rat model of type II diabetes for a period of four weeks. MC has potent antioxidant and free radical scavenging activities that could be derived from compounds such as phenols. Thus, MC could be safely recommended to diabetic patients, which might postpone or prevent the onset of secondary complications. However, with this speculation on the mechanism of action of the MC extract, we do not eliminate the possibility of other, unidentified mechanisms.

Abbreviations: MC, *Momordica charantia*; **STZ,** streptozotocin; **NC,** the normal control group; **DC,** the diabetic control group; **DMO,** the MC-treated diabetic group; **DPG,** the glibenclamide-treated diabetic group; **MDA,** malondialdehyde; **FTC,** ferric thiocyanate; **TBA,** thiobarbituric acid; **DPPH,** 1,1-diphenyl-2-picryl-hydrazyl; **NIDDM,** non-insulin dependent diabetes mellitus; **TBARS,** thiobarbituric acid reactive substances; TBS, tris-buffered saline; **DAB,** 3,3'-diaminobenzidine; **PBS,** phosphate-buffered saline; **BHT,** butylated hydroxytoluene.

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