Clinical Studies on Bitter Melon (MOMORDICA CHARANTIA)

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Effects of *Momordica charantia* fruit juice on islet morphology in the pancreas of the streptozotocin-diabetic rat

I. Ahmed [a], E. Adeghate [a,*], A.K. Sharma [a], D.J. Pallot [a], J. Singh [b]

[a] Department of Human Anatomy, Faculty of Medicine and Health Sciences, United Arad Emirates University, P.O. Box 17666, Al Ain, United Arab Emirates

[b] Department of Applied Biology, University of Central Lancashire, Preston PR1 2HE, UK

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Abstract

An investigation was made of the effect of *Momordica charantia* fruit juice on the distribution and number of α, β and δ cells in the pancreas of streptozotocin (STZ)-induced diabetic rats using immunohistochemical methods. The results indicated that there was a significant (Student's *t*-test, $P<0.004$) increase in the number of β cells in *M. charantia*-treated animals when compared with untreated diabetics, however, their number was still significantly less than that obtained for normal rats. There was also a significant ($P<0.006$) increase in the number of δ cells in STZ-diabetic rats compared to non-diabetic rats. This increase in the number of δ cells was not affected by *M. charantia* treatment. The number of α cells did not change significantly in *M. charantia*-treated rats when compared with untreated diabetic rats. Our results suggest that oral feeding of *M. charantia* fruit juice may have a role in the renewal of β cells in STZ-diabetic rats or alternately may permit the recovery of partially destroyed β cells.

1. Introduction

The bitter melon or Karela (*Momordica charantica*) of the family Cacurbitaceae is a plant widely cultivated in many tropical and subtropical regions of the world and is frequently used in South Asia and the Orient as a food stuff and medicinal plant. Extracts from various components of this plant have been reported to possess hypoglycaemic activity [1], protein synthesis inhibitory activity [2,3], anti-tumor [4,5], and abortifacient properties [6]. *M. charantia* fruit juice has also been shown to increase both glucose uptake by tissues in vitro and liver glycogen storage [7]. The freeze-dried juice of *M. charantia* can also stimulate insulin secretion by isolated β cells of the islets of Langerhans [8]. The hypoglycaemic activity of *M. charantia* fruit juice is demonstrated in animals with

experimental diabetes [9,10] and also in humans in both type 1 and type 2 diabetes mellitus [7,11,12].

In a previous study [13] we have shown that there is a significant increase in plasma insulin level in *M. charantia*-treated STZ-diabetic rats when compared to untreated STZ-diabetic rats. The morphological basis of such an increase is still unknown. Thus, this study was designated to ascertain this information.

2. Materials and methods

2.1. Experimental animals

All studies were performed in male Wistar rats (200-250 g body weight). Diabetes was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg body weight in 0.05 M sodium citrate, pH 4.5). The blood glucose estimations were made by One Touch II ®Glucometer (LifeScan, Johnson and Johnson, USA) for each individual animal. The animals were considered diabetic if blood glucose levels were more than 300mg/dl. The animals were kept in plastic cages and maintained on standard laboratory animal diet with water ad libitum.

2.2. Experimental setup

The experimental animals were divided into three groups with four to five animals in each group. These comprised of untreated diabetics, diabetics treated with fruit juice of *M. charantia* and age-matched non-diabetic controls. The *M. charantia* juice was prepared as described by Sharma et al. [13]. Briefly, fresh Karela fruit (1 kg) was obtained from local market. The seeds were removed and the juice was obtained by using a commercial juice extractor (Moulinex, France). The fresh juice was centrifuged at 5000 rpm (Beckman, USA) for 30 min at 4°C. The supernatant was aliquoted and stored at -20°C until used. The fruit juice was administered 1 week after induction of diabetes, daily at the dosage of 10 ml/kg body weight. At the end of the 10th week the animals were sacrificed and the pancreata were removed, cut into small fragments and fixed overnight in freshly prepared Zamboni's fixative [14]. Representative fragments were always taken from the tail of the pancreas. They were dehydrated in ethanol series,

leared in xylene embedded in paraffin. Sections of 7 µm thicknesses were cut on a microtome and transferred onto microscopic slides which were dried at 55°C for 30 min to enhance section attachment.

2.3. Immunohistochemical localization

The slides were deparaffinised with xylene, hydrated in decreasing concentration of ethanol and brought to Tris-buffered saline (TBS). The sections were then incubated for 30 min in a solution of hydrogen peroxide (0.3%, v/v in methanol) to block endogenous peroxide activity. The immunohistochemical staining was performed by the Labeled Streptavidin Biotin method using a Dako (LSAB)® peroxidase kit. The staining procedure was started by incubating the sections with the blocking reagent. After 15 min the blocking reagent was drained and appropriate dilutions of primary antibodies and negative control reagents were applied. The sections were incubated in primary antibodies for 1 hr at room temperature. The slides were then washed (3 times) with TBS and incubated for 30 min with prediluted biotinylated anti-rabbit or anti-mouse IgG for 30 min, washed in TBS (3 times) and incubated in streptavidin peroxidise conjugate for 45 min. After a final wash in TBS (3 times), the peroxidase activity was revealed by incubating for 5 min in 3,3-diaminobenzidine tetrahydrochloride containing 0.03% H_2O_2. The slides were then counterstained with haematoxylin, dehydrated in ethanol, cleared in xylene and mounted in Cytoseal 60 (Stephens Scientific, USA).

2.4. Antisera

The antiserum to insulin was monoclonal and purchased from Sigma. It was used at 1:500 dilutions. The antisera to somatostatin and glucagon were purchased from Dako (Copenhagen, Denmark). The antisomatostatin was used at 1:100 dilution while antiglucagon was supplied prediluted by the vendor. No specific immunostaining was observed in pancreatic tissue when primary antisera were omitted.

2.5. Statistical analysis

All data were expressed as mean ± SD. Student's *t*-test was performed for each experimental group. Data were also compared by analysis of variance (ANOVA) and only values with $P<0.05$ were considered as significant. Percentage distribution of α, β and δ cells were also calculated.

3. Results

3.1. Beta cells

Table 1 shows the distribution of insulin-positive cells in the experimental groups. The results reveal that insulin-positive cells were found in all three groups. The percentage of insulin-positive cells was about 60.01 and 27.04 in normal and STZ-diabetic animals, respectively. In contrast, when the diabetic animals were treated with *M. charantia* fruit juice for 9 weeks the percentage of insulin-positive cells increased significantly to 50.22 compared to untreated diabetic animals.

Table 1 : Number of insulin-positive cells per islet (expressed as mean ± SD) in the pancreas of (a) control rats, (b) STZ-treated rats and (c) STZ-treated animals fed with *M. charantia* fruit juice (n=5 animals for each group)

Experimental group	Islets counted	Number of insulin-positive cells per islet (mean ± SD)
(a) Control	45	22.97 ± 20.17
(b) Diabetic	45	10.79 ± 8.29
(c) Treated diabetic	45	16.61 ± 10.27

Student's *t*-test: a-b, $P<0.0003$ (very highly significant); b-c, $P<0.004$ (highly significant) a-c, $P<0.06$ (not significant); ANOVA, $P<0.002$.

The number of insulin-positive cells per islet, expressed as mean ± SD, was 22.97 ± 20.17, 10.79 ± 8.29 and 16.61 ± 10.27 in normal (control), untreated diabetic and treated diabetic rats respectively. These results indicate that *M. charantia* can markedly (Student's *t*-test, $P<0.004$) improve the viability of β cells. Fig. 1 shows representation of insulin staining in the pancreas of (a) normal, (b) diabetic and (c) *M. charantia* treated diabetic rat.

(Please login at http://www.diabetesresearchclinicalpractice.com/article/S0168-8227(98)00022-9/abstract to view **fig. 1**)

3.2. Alpha cells

Glucagon-positive cells were located in the periphery of the islets in normal animals. However, in STZ-diabetic animals many glucagon-positive cells were seen scattered within the central portion of the islets as well. A representation of glucagon

immunostaining is shown in Fig. 2. The data on the number and distribution of glucagons positive cells are shown in Table 2.

(Please login at http://www.diabetesresearchclinicalpractice.com/article/S0168-8227(98)00022-9/abstract to view **fig. 2**).

Table 2 : Number of glucagon-positive cells per islet (expressed as mean ± SD) in (a) control rats, (b) STZ-treated rats and (c) STZ-treated animals fed with *M. charantia* fruit juice (n=5 animals for each group)

Experimental group	Islets counted	Number of glucagon-positive cells per islet (mean ± SD)
(a) Control	40	21.9 ± 20.03
(b) Diabetic	40	14.2 ± 11.13
(c) Treated diabetic	40	17.4 ± 13.69

Student's *t*-test: a-b, *P*>0.06 (not significant); b-c, *P*>0.33 (not significant) a-c, *P*>0.24 (not significant); ANOVA, *P*>0.18.

The number of glucagons-positive cells per islet expressed as mean ± SD was 21.9 ± 20.03, 14.2 ± 11.13 and 17.4 ± 13.69 in normal (control), STZ-diabetic and *M-charantia* fruit juice-treated diabetic rats, respectively. There was no significant increase in the number of glucagons-positive cells in STZ-diabetic animals compared to controls. In addition to this there was no change in the number of glucagons-positive cells between *M. charantia*-treated and untreated diabetic animals.

3.3 Delta cells

Somatostatin-positive cells were found mainly in the outer part of the islets. The statistical analysis of the number of δ cells in all three groups is presented in Table 3. The number of somatostatin-immunopositive cells per islet expressed as mean ± SD was 4.27 ± 3.26, 7.82 ± 4.97 and 6.73 ± 4.65 in normal controls, untreated diabetic and *M. charantia*-treated diabetic rats. There was a significant increase in the number of somatostatin-immunopositive cells in untreated diabetic rats compared to normal rats (Student's *t*-test, *P*<0.0002). There seemed to be a decrease in the number of pancreatic δ cells of *M. charantia*-treated diabetic rats compared to untreated diabetic rats. The percentage of somatostatin-positive cells was 6.4 in control animals and it increased significantly to 26% in STZ-diabetic animals. The percentage of somatostatin-positive cells was only marginally

reduced (20.78%) with *M. charantia* treatment. The immunostaining of somatostatin in all three groups of animals is shown in Fig. 3.

(Please login at http://www.diabetesresearchclinicalpractice.com/article/S0168-8227(98)00022-9/abstract to view **fig. 3**).

4. Discussion

The results of this study have demonstrated marked changes in the pattern of distribution of insulin-, glucagons- and somatostatin-positive cell in pancreatic tissue of diabetic animals compared to that of normal controls. The relative distribution of pancreatic islet in the control animals is similar to the results of previous studies in the rat [15]. There were, however, significant changes in the pattern of distribution of insulin-, lucagons- and somatostatin-positive cells in the islet of Langerhans of *M. charantia*-treated and untreated diabetic rats. The arrangement of these endocrine cells in the islets of diabetic rats appeared differently as compared to controls. The number of insulin-positive cells decreased markedly in both *M. charantia*-treated and untreated diabetic rats compared with control animals but the decrease was much greater in the untreated rats.

Table 3 : Number of somatostatin-positive cells per islet (expressed as mean ± SD) in the pancreas of (a) control rats, (b) STZ-treated rats and (c) STZ-treated animals fed with *M. charantia* fruit juice (n=5 animals for each group)

Experimental group	Islets counted	Number of insulin-positive cells per islet (mean ± SD)
(a) Control	45	4.27 ± 3.26
(b) Diabetic	45	7.82 ± 4.97
I Treated diabetic	45	6.73 ± 4.65

Student's *t*-test: a-b, $P<0.0002$ (very highly significant); b-c, $P>0.2$ (not significant) a-c, $P<0.006$ (highly significant); ANOVA, $P<0.002$.

The number of insulin-positive cells per islet was significantly (Student's *t*-test, $P<0.004$) higher in the *M. charantia*-treated animals compared to untreated diabetic rats. This interesting observation indicates that *M. charantia* may play an important role in increasing the number of insulin-positive cells in the pancreas. There are two possible

explanations for this finding: First, *M. charantia* may exert its effect by preventing in the death of β cells and/or second, it may permit the recovery of partially destroyed β cells. Another interesting observation is the significant increase in the number of somatostatin-positive cells in the diabetic group. In diabetes, it appears that lucagons- and somatostatin-producing cells increase in number to compensate for the relative decrease in insulin-secreting cells. An increase in the number of lucagons-producing α and somatostatin-producing δ cells has also been reported previously in the STZ-treated rats [17]. There was a slight, but not significant ($P>0.6$) decrease in the number of glucagons-positive cells in the diabetic group. This slight decrease may be indicative of α cell damage by STZ.

There is much controversy regarding the changes in the number of α cells in streptozotocin diabetes. It has been demonstrated that the number of pancreatic α cells do not change significantly in STZ-treated rats [16]. Pons and Aoki [17], however, have reported a remarkable increase in the number of α cells in diabetic rats.

Physiological experiments have shown that *M. charantia* can stimulate unsulin secretion [8] and induce glucose uptake in liver [7]. It seems therefore that the induction of an increase in the number of insulin-producing cells may be one of the several pathways of action of this vegetable. It is possible that *M. charantia* may have initiated cell proliferation, since it has been reported that pancreatic endocrine cells have the potential to proliferate after induction of diabetes with STZ [18]. It is also very interesting that the absolute values of insulin-positive cells in the *M. charantia*-treated rats are still lower than that of the controls. The reason for this discrepancy may be attributed to the fact that some β cells may have been completely destroyed with no possibility of recovery, whereas the others were partially damaged. In this respect, *M. charantia* may act to prevent the destruction of the insulin-positive cells by hilherto, unknown mechanism.

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Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats

Ajit Kar [a,*], B.K. Choudhary [b], N.G. Bandyopadhyay [c]

[a] Satsang Herbal Research and Analytical laboratories, PO Satsang-814 116 Deoghar, India

[b] Vashistha Institute of Bio-molecular Studiesand Natural Medicines, Deoghar, India

[c] Government Ayurveda College, Patna, India

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Abstract

In our experiments 30 hypoglycaemic medicinal plants (known and less known) have been selected for thorough studies from indigenous folk medicines, Ayurvedic, Unani and Siddha systems of medicines, In all the experiments with different herbal samples (vacuum dried 95% ethanolic extracts), definite blood glucose lowering effect within 2 weeks have been confirmed in alloxan diabetic albino rats. Blood glucose values are brought down close to normal fasting level using herbal samples at a dose of 250 mg/kg once, twice or thrice daily, as needed. While evaluating comparative hypoglycaemic activity of the experimental herbal samples, significant blood glucose lowering activities are observed in decreasing order in the following 24 samples-*Coccinia indica, Tragia involucrate, G. sylvestre, Pterocarpus marsupium, T. foenum-graecum, Moringa oleifera, Eugenia jambolana, Tinospora cordifolia, Swertia chirayita, Momordica charantia, Ficus glomerata, Ficus benghalensis, Vinca rosea, Premna integrifolia, Mucuna prurita, Terminalia bellirica, Sesbenia agyptiaca, Azadirachta indica, Dendrocalamus hamiltonii, Zingiber officinale, Aegle marmelos, Cinnamomum tamala, Trichosanthes cucumerina* and *Ocimum sanctum*. Present studies besides conforming hypoglycaemic activities of the experimental herbal samples; help identify more potent indigenous hypoglycaemic herbs (in crude ethanolic extract) from the comparative study of the reported experimental results.

1. Introduction

More than 100 medicinal plants are mentioned in the Indian system of medicines including folk medicines for the management of diabetes, which are effective either singly or in combinations. While going through the previous works of different investigators, some variations in their experimental results have been observed. Incompete extraction procedure, inadequate pharmacognosy and insensitive animal model may be the reasons for showing variable and even negative results. We have guarded against all these factors in our experiments and selected 30 hypoglycaemic medicinal plants for our studies. Among these plants, 20 are mostly known for their hypoglycaemic activities, (presented in Tabel 1A) and were investigated by different workers including us (Nadkarni, 1954; Nagarajan et al., 1982; Kar et al., 1999). The remaining ten plants (presented in Table 1B) are less known for their hypoglycaemic activity and have been thoroughly investigated by the present workers in the form of crude ethanolic extracts.

We have already studied and reported the effect of inorganic parts (in the form of carefully prepared ash containing mainly mineral elements) of these 30 hypoglycaemic plants on oral glucose tolerance test (Kar et al., 1999). However, though improvement in glucose tolerance factor (especially significant in the case of latent or early mild diabetes) was noted, no significant blood glucose lowering effect of such herbal ash samples was found by us while working with moderately high or high diabetic models of animals. A simple reason may be that minerals are not hypoglycaemic agents as such and most essential trace mineral elements act primarily as catalysts or co-factors in enzyme systems (Underwood and Mertz, 1986). In the present study, comparative hypoglycaemic activities of the organic parts (in the form of 95% ethanolic extract) of above referred herbal samples have been thoroughly evaluated using alloxan-diabetic albino rats.

2. Materials and methods

2.1 Plant materials

The specific parts of the 30 full grown matured plant samples were collected (tho-

roughly cleaned and dried) and identified by Professor N.G. Bandyopadhyay (Govt. Ayurveda College, Patna, India). The voucher samples of the plant materials were on deposit in our herbarium of Satsang Herbal Research and Analytical Laboratories (Satsang-814116, India). For every reference, full particulars of the experimental plants are presented in Table 1 (A and B) including the w/w yield % of the prepared extracts.

Each of the dried plant samples (75 g) was soxhlet extracted with 95% ethanol (450 ml) at controlled temperature. The collected extract was concentrated under reduced pressure below 45ºC using vacuum pump and rotary evaporator ensuring complete removal of the solvent. Pure and dried ethanolic extracts of the samples thus prepared were stored at 4-5 ºC until used.

2.2. Experimental animals

Charles Foster strain male albino rats (wt. 150-200 g) were used throughout the studies. Animals had adlibitum access to standard laboratory diet water, expect during the day of the blood sampling when animals were used after an overnight fast. Diabetes was induced by inter-peritonial injection of alloxan monohydrate (Sigma Chemicals, USA) to overnight fasted animals at a dose of 100 mg/kg body weight by partially destroying pancreatic beta cells. Steady diabetes was confirmed noting urine sugar regularly and then measuring blood glucose values before starting an experiment. Groups of five animals were selected for the oral feeding of each of the herbal samples. The dose selected was 250 mg/kg of the experimental herbal samples, prepared and stored as mentioned above (emulsified in 10% ethanol-water vehicle solution) and fed orally. The control group having five animals was fed equal amount of the vehicle solution only.

2.3 Experimental procedures

Blood glucose was measured by Tringer's glucose oxidase method (Varley et al., 1976). Initial glucose values were measured on the first day of the experiment without sample load. Urine sugar was monitored every day but blood glucose values were determined at 1 week interval. If the desired lowering of blood glucose values was not found with the single dose of ethanolic extract of the sample for the 2 weeks, the double doses of 250 mg/kg were given twice a day (total 500 mg/day) and blood glucose values were noted at 1 week interval as before, when double dose failed to show any significant lowering

effect, triple doses of 250 mg/kg were given thrice a day (750 mg/day) up to 2 weeks.

3. Results and Discussion

To confirm definite hypoglycaemic effect, final blood glucose values of each sample were brought down closer to normal fasting level using different doses of the concerned ethanolic extract (Table 2). The present result indicates that most of the experimental samples show definite blood glucose lowering effects within 1-2 weeks in single, double or triple doses.

The following experimental samples show confirmed blood glucose lowering effect within 1 week using only single dose of the ethanolic extract of the sample (250 mg/kg). In terms of hypoglycaemic activity in decreasing order, these are *Coccinia indica, Tragia involucrate, Gymnema sylvestre, Pterocarpus marsupium, Trigonella foenum-graecum, Moringa oleifera, Eugenia jambolana* and *Tinospora cordifolia*. The next higher hypoglycaemic activity was confirmed in similar manner in 2 weeks using single dose of the experimental sample in the cases of *Swertia chirayita* and *Momordica charantia*.

Plants showing comparatively lower hypoglycaemic activity (in decreasing order) using double doses of the sample for 1 week only are *Ficus glomerata, Ficus benghalensis, Vinca rosea, Premna integrifolia, Mucuna prurita, Terminalia bellirica, Sesbenia aegyptiaca* and *Azadirachta indica*. The next range of hypoglycaemic activity was confirmed in the following samples in 2 weeks instead of 1 week, using double doses (250 mg/kg twice daily) of the samples. In terms of hypoglycaemic activity in decreasing order, these are *Dendrocalamus hamiltonii, Zingiber officinale, Aegle marmelos, Piper longum,* and *Cinnamomum tamala*.

Trichosanthes cucumerina confirmed blood glucose lowering effect within 1 week of oral feeding of the sample in comparatively higher dose (triple doses), in 250 mg/kg- thrice daily. Lastly, *Ocimum sanctum* and *Asparagus racemosus* show comparatively lesser activity in 2 weeks with triple doses of the sample (250 mg/kg- thrice daily).

As pointed out by WHO, prevention of diabetes and its complications is not only a major challenge for the future, but essential if health for all is to be an attainable target. This, WHO study groups emphasise strongly in this regard the optimal, rational uses of

traditional and natural indigenous medicines, the hypoglycaemic plants have been in use generally in their natural forms (fresh juice, paste or dry powder). These include both the inorganic and organic constituents of the concerned herbs. Further it is important to note that the inorganic part of a medicinal plant containing mainly mineral elements, sometimes plays a contributory role in enhancing medicinal properties (including hypoglycaemic activity) of that plant (Kar and Choudhary, 1994; Kar et al., 1999). Besides, a number of essential minerals (Ca, Zn, K, Mn and Cr) are known to be associated with the mechanisms of insulin release and its activity or glucose tolerance factor in different laboratory animals and in human beings (Mertz, 1981; Niewoehner et al., 1986; Chen et al., 1995; Schroeder, 1996; Castro, 1998).

Further, from our studies, more potent hypoglycaemic herbs of Indian origin can be selected for their use in indigenous medicinal preparations in crude forms either singly or in combinations. For a final co-ordinated result, the effects of both the organic and inorganic constituents of the concerned medicinal plant may be taken into consideration.

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Bitter Melon (*Momordica Charantia*) Reduces Serum Sialic Acid in Type2 Diabetics: Evidence to Delay the Process of Atherosclerosis

Inayat Ur Rahman[1*], Mohammad Bashir[2], Mohammad Salman[2], Mohammad Idrees[3], Mohammad Iqbal Khan[4]

[1]Gandhara College of Pharmacy, Gandhara University, Peshawar, Pakistan
[2]Institute of Medical Science, Kohat University of Science & Technology, Kohat, Pakistan

[3]Department of Pathology, Khyber Medical College, Peshawar, Pakistan
[4]Department of Chemistry, Kohat University of Science & Technology, Kohat, Pakistan

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Abstract

More than 1000 herbal products have been used by diverse cultures of the world to treat hyperglycemia and among them bitter melon (*Momordica charantia*) is one of the most popular herbal resource. The beneficial effects of bitter melon is not limited to hypoglycaemia only, but it also ameliorates diet induced obesity, insulin resistance and exhibit cardio protective effects. The present study attempts to investigate the effect of bitter melon fruit juice on a newly investigated risk factor, sialic acid in type2 diabetics. A total of 40 type2 diabetic patients, divided into group A (n = 20) and group B (n = 20) were investigated during the present study. The patients of group A were following bitter melon fruit juice treatment along with diet control, whereas the patients of group B were on diet control only. Serum sialic acid (SSA) decreased in group A from 66.20 ± 2.30 mg/dl to 63.50 ± 2.10 mg/dl (<0.11) but, increased in group B from 66.50 ± 1.70 mg/dl to 68.20 ± 2.50 mg/dl (<0.12), compared to baseline. Post-treatment between group comparison revealed a significant difference (<0.05). The beneficial effects on fasting plasma glucose (FPG) and glycohemoglobin (HbA1-c) were also greater in group A compared to group B as was the case with blood lipids, weight and blood pressure. The study provides another mechanism for the cardio protective effect of bitter melon and further strengthens its value in the management of type2 diabetes.

1. Introduction

Plant medicines have a long history of use in various pathological conditions. Bitter melon (*Momordica charantia*) is traditionally used for treating diabetes in developing world including India and Pakistan, which have a long history of the use of herbal remedies in diabetes [1]. Bitter melon is a traditional plant used by ayurvedic doctors of medicines to benefit various conditions including diabetes [2]. The effect of bitter melon on various diabetes associated cardiovascular risk factors like plasma lipids, obesity and insulin resistance is known [3,4] and the present study is designed to determine the effect of bitter melon on a recently investigated cardiovascular (CV) risk marker/factor, sialic acid. Sialic acidis used to be a group for acetylated derivatives of neuraminic acid and the serum level of it is increased in type 2 diabetes mellitus [5-8]. The mechanistic aspect of raised levels of serum sialic acid (SSA) is not very clear, but several possibilities have been suggested for elevated levels in diabetic patients. There may be generalized endothelial cell dysfunction or macrovascular disease, either through loss of sialic acid containing glycoproteins from vascular cells into blood stream or through an acute phase response. Circulating sialic acid is mostly covalently attached to glycoproteins, particularly the acute phase group. Type 2 diabetes mellitus may be considered an acute phase disease, since in type 2 diabetes (not type 1 diabetes), even without tissue complications, the serum levels of acute phase proteins, C-reactive protein and haptoglobin are elevated [9-13].

2. Materials and Methods

2.1. Study Design

A total of 40 recently diagnosed type2 diabetic patients at-tending Afghan Dawakhana, Lakki Marwat, was investigated during the present study. They were divided into two groups, A and B. The patients in group A (20) were on diet control only, whereas, the patients in group B (20) were also taking freeze-dried bitter melon juice in a dose of 1 g/d along with diet control. The volunteer patients of type2 diabetes were explained the research protocol and their written informed consents obtained for the study. Participants were informed of their right to withdraw from the study at any time. The study was conducted for a period of 12 weeks. Therapeutic goal was to achieve FPG level

<140 mg/dl, while observing the changes in SSA.

2.2. Procedures and Measurements

Fasting blood samples were collected after 12 -14 h overnight fast without the use of a tourniquet. Metabolic variables were determined at baseline and at the end of the study period.

Sialic acid was determined by using a method pro- posed by Shamberger [18]. In brief, sialic acid in the sample reacts with Ehrlich's reagent and this result in the formation of a white precipitate. After incubation in water bath the color of the mixture turned blue from white. Sodium chloride was added to the mixture and centrifuged. The intensity of the color of supernatant is directly proportional to the concentration of sialic acid and is read in a spectrophotometer at 525 nm.

Fasting plasma glucose (FPG), glycohemoglobin (Hb- A1-c), total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and triglyceride (TG) levels were analyzed by enzymatic methods, using commercially available kits.

SSA, HbA1-c and blood lipids were determined at start and at the end of the study period whereas FPG was determined at start and then after every 2 weeks.

Standing height and weight were measured with the subjects in light clothing and without shoes. Height was recorded to the nearest centimeter and weight to the nearest 0.1 Kg. The weighing scales (Detecto-Medic, NY, USA) were standardized on each visit using standard weights of 20 and 70 Kg. Body mass index (BMI), defined as weight in Kg/height (in meters) squared was calculated, and used as an index for obesity. Obesity was defined according to the WHO standards as BMI <30 [19]. Blood pressure was measured according to a stan- dard protocol [20]. Hypertension was defined as systolic blood pressure 160 mmHg and/or diastolic blood pres- sure 95 mmHg and/or current history of antihypertensive medications, according to the WHO criteria [21].

2.3. Source and Preparation of Bitter Melon Fruit Juice

Momordica charantia (family Cucurbitaceae, commonly known as Ku gua, bitter melon, karela or bitter gourd) was purchased from a local market in Khyber Pukh- toonkhwa

Province, Pakistan and authenticated by a pharmacognosy expert before juice preparation. The juice was prepared by the method proposed by Chen *et al.* [22]. Unripe bitter melon fresh fruit was washed thoroughly with water, cut open and the seeds removed. The juice was extracted from the edible portion by crushing the fruit in electric juicer (NOVA-Osaka, Japan) and strain- ing through a muslin cloth. The yield was 390 ml/Kg. The juice was frozen and then completely lyophilized by continuous freeze drying operation for 72 h (Dura Bulk Tray Dryer, FTS System, Stone Ridge, NY). The yield was 7 g powder/Kg of fresh fruit. The powder was filled in hard gelatine capsules in a dose of 0.5 g and 1 g.

Results are expressed as means ± SD. Data were ana- lysed using the Statistical Package for Social Sciences, SPSS (SPSS Inc., Chicago, IL, USA) and a $P \leq 05$ was taken as the cut-off level for significance. Because the distribution of most variables was not symmetric, we used non-parametric statistical methods. The Mann-Wh- itney U-test was used for between group comparisons and Kruskal-Wallis one-way ANNOVA test was used for comparisons involving more than two groups.

3. Results

Table 1 shows baseline characteristics of the study groups. There were no significant differences of metabolic variables among the groups. Table 2 shows the post-treat- ment values of metabolic variables. Serum sialic acid (SSA) showed a trend towards decrease in group A from 66.20 ± 2.30 mg/dl to 63.50 ± 2.10 mg/dl (<0.11) whereas, increased in group B from 66.50 ± 1.70 mg/dl to 68.20 ± 2.50 mg/dl (<0.12). Post-treatment between group comparisons revealed a significantly high SSA in group B (Figure 1).

Fasting plasma glucose (FPG) decreased to <140 mg/dl within the first month of study in both group A and group B. After that, the level deteriorated (≥140 mg/dl) more rapidly in group B than group A (Figure 2). HbA1-c significantly decreased in both group A and gr- oup B, compared with baseline (Table 2).

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Table 1: Baseline characteristics of group A and group B.

Variable	Group A	Group B
N	20	20
Age (y)	52 ± 3.40	52.20 ± 4.70
Sex (M/F)	8/12	9/11
BMI (Kg/m2)	26 ± 2.20	26.70 ± 2.80
Weight (Kg)	77.60 ± 4.30	77 ± 4.40
SBP (mmHg)	139 ± 6	138 ± 5
DBP (mmHg)	94 ± 3	95 ± 3
Smoking (cig/d)	8	10
SSA (mg/dl)	66.20 ± 2.30	66.50 ± 1.70
FPG (mg/dl)	151 ± 6.20	148 ± 6.80
HbA1-c (%)	8.60 ± 0.40	8.70 ± 0.50
TC (mg/dl)	196.30 ± 6.10	194 ± 5.60
HDL-c (mg/dl)	49.20 ± 2.90	48.40 ± 1.50
LDL-c (mg/dl)	150.20 ± 4.20	151.30 ± 3.50
TG (mg/dl)	172.90 ± 5.10	172.40 ± 3.60

Data are mean ± SD; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pres-sure; SSA: Serum Sialic Acid; FPG: Fasting Plasma Glucose; HbA1-c: Glyco-hemoglobin; TC: Total Cholesterol; HDL-c: High Density Lipoprotein cholesterol; LDL-c: Low Density Lipoprotein cholesterol; TG: Triglyceride.

Figure 1 : Comparison of baseline and post-treatment SSA in group A and group B.

(Please login at http://www.SciRP.org/journal/cm to view **fig. 1**)

Figure 2 : Changes in FPG overtime.

(Please login at http://www.SciRP.org/journal/cm to view **fig. 2**)

Table 2 : Post-treatment biochemical profile of group A and group B.

	Group A			Group B
Variable	Post-treatment value	Change from baseline P-value	Post-treatment value	Change from baseline P-value
SSA (mg/dl)	63.50 ± 2.10	<0.11	68.20 ± 2.50	<0.12
FPG (mg/dl)	142 ± 3.10	<0.05	142.70 ± 5.60	≤0.05
HbA1-c (%)	7.50 ± 0.30	<0.01	7.50 ± 0.60	<0.02
TC (mg/dl)	187.30 ± 5.90	<0.05	198.30 ± 8.70	<0.07
HDL-c (mg/dl)	53 ± 5.30	<0.12	51.30 ± 4.50	<0.18
LDL-c (mg/dl)	144.10 ± 5.20	<0.05	154.70 ± 7.10	<0.12
TG (mg/dl)	170.50 ± 8.80	<0.24	171.40 ± 7.50	<0.20
Weight (Kg)	75.50 ± 4.50	<0.13	77.90 ± 4.30	<0.19
SBP (mmHg)	137 ± 5.10	<0.19	139 ± 6.70	<0.20
DBP (mmHg)	92 ± 6.70	<0.22	97 ± 4.10	<0.21

Changes occurred in blood lipids during the study pe- riod are given in Table 2. Total cholesterol (TC) changed by −9 mg/dl and +4.30 mg/dl in group A and group B respectively. High density lipoprotein cholesterol (HDL-c) changed by +3.80 mg/dl and +2.90 mg/dl. Low density lipoprotein cholesterol (LDL-c) changed by −6.10 mg/dl and +3.40 mg/dl. Triglyceride (TG) changed by −2.40 mg/dl and −1 mg/dl in group A and group B respectively. Weight (Kg) decreased by −2.10 in group A and in-creased by +0.90 in group B. Both systolic and diastolic blood pressure (SBP and DBP) decreased in group A by −2 mmHg each, whereas increased by +1 mmHg and +2 mmHg in group B respectively (Table 2).

4. Discussion

Diabetes is the metabolic syndrome associated with both microvascular and macrovascular complications and am- ong these cardiovascular complications is the main cause of death in such patients [22]. Therefore modulation of the cardiovascular risk factors will definitely prove help- ful to reduce the incidence of cardiovascular complica- tions in these patients. Results of the present study suggest that the use of bitter melon in type2 diabetes de- creases the SSA level thereby indicating the usefulness of bitter melon in protecting against CV complications in diabetes. Lindberg *et al.* [23] have shown that the pre- dicting power of sialic acid for coronary heart disease is more

or of the same magnitude to that of cholesterol. Recent studies also indicate sialic acid as an independent risk factor for cardiovascular disease [24] and thus any positive change (decrease) in SSA may lead to control and deceleration of atherosclerotic process. Indeed, a sig- nificant decrease in SSA levels in growth hormone defi- cient patients after receiving growth hormone therapy has been related to the prognostic effect of this hormone in groups at risk of cardiovascular disease [25].

There may be several reasons for beneficial effects of bitter melon on SSA including, the inhibitory effect on glycation of LDL-c [26], protecting the biological sys- tems from potentially harmful effects of free radicals due to antioxidant properties [27,28], reduces adiposity and exerts antihyperlipidemic effects [29,30]. A positive as-sociation between SSA, blood lipids and BMI has been reported [31]. The role of bitter melon in protecting ag- ainst cardiovascular complications of diabetes has been reported previously [32,33] and a decrease in SSA shown in this study is the novel finding because it is an indicative of a decrease in the development and progression of atherosclerosis.

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Medicinal Chemistry of the Anti-Diabetic Effects of Momordica Charantia: Active Constituents and Modes of Actions

Jaipaul Singh[1], Emmanuel Cumming[2], Gunasekar Manoharan[1], Huba Kalasz[3] and Ernest Adeghate*[4]

[1]School of Pharmacy and Pharmaceutical Sciences and School of Forensic and School Investigative Sciences, University of Central Lancashire, Preston, England, UK.

[2]School of Medicine, Faculty of Health Sciences, University of Guyana, Turkeyn, Georgetown, Guyana

[3]Department of Pharmacology & Therapeutics, Semmelweis University, Budapest, Hungary

[4]Department of Anatomy, Faculty of Medicine and Health Science, United Arab Emirates, University Al Ain, United Arab Emirates

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Abstract

Diabetes mellitus (DM) is one of the oldest known human disease currently affecting more than 200 million people worldwide. Diabetes mellitus is derived from two Greek words meaning siphon and sugar. In DM, patients have high blood level of glucose and this passes out with urine. This is because the endocrine pancreas does not produce either or not enough insulin or the insulin which is produced is not exerting its biochemical effect (or insulin resistance) effectively. Insulin is a major metabolic hormone which has numerous functions in the body and one main role is to stimulate glucose uptake into body's cells where it is utilized to provide energy. The disease is classified into type 1 and type 2 DM. Type 1 DM develops when the insulin producing β cells have been destroyed and are unable to produce insulin. This is very common in children and is treated with insulin. Type 2 DM (T2DM) develops when the body is unable to produce an adequate amount of insulin or the insulin which is provided does not work efficiently. This is due to life style habits including unhealthy diet, obesity, lack of exercise and hereditary and environmental factors. Some symptoms of DM include excess urination, constant thirst, lethargy, weight loss, itching, decreased digestive enzyme secretion; slow wound healing

and other related symptoms. If left untreated, DM can result in severe long-term complications such as kidney and heart failure, stroke, blindness, nerve damage, exocrine glands insufficiency and other forms of complications. T2DM can be treated and controlled by prescribed drugs, regular exercise, diet (including some plant-based food) and general change in life style habits. This review is concerned with the role of plant-based medicine to treat DM. One such plant is *Momordica charantia* which is grown in tropical countries worldwide and it has been used as a traditional herbal medicine for thousands of years although its origin in unknown. This review examines the medicinal chemistry and use(s) of *M. charantia* and its various extracts and compounds, their biochemical properties and how they act as anti-diabetic (hypoglycemic) drugs and the various mechanisms by which they exert their beneficial effects in controlling and treating DM.

1. Introduction

Diabetes mellitus (DM) is one of the oldest known human disease currently affecting about 200 million people worldwide [1]. It is estimated that by 2025, more than 300 people globally will have confirmed DM and other 50 million undiagnosed [2,3]. This disease is the most common metabolic disorder in human and it is characterized by hyperglycemia, due to relative or absolute lack of insulin, the insensitivity of insulin or both [4,5]. DM is classified into type 1 or insulin-dependent DM (IDDM) or type 2 or non-insulin dependent DM (NIDDM) or T2DM [5,6]. Type 1 DM represents about 5-10% of all cases of DM whereas T2DM accounts for 90-95% of diabetes. Type 1 DM is characterized mainly by auto-immune-mediated destruction of beta cells of the endocrine pancreas leading to reduced insulin secretion. This form of DM is prevalent in young children [5,7]. On the other hand, T2DM is characterized by insulin resistance and relative insulin deficiency and it is due to sedentary life style, genetic disposition, obesity, human behavior and environmental factors. Both forms of DM can lead to such long-term complications as neuropathy, retinopathy, cardiomyopathy, nephropathy, exocrine gland insufficiency and several other complications and eventually to death [5]. Type 1 DM is treated mainly with insulin whereas T2DM is controlled by hypoglycemic drugs, regular exercise, general change in life style habits and diet including some plant-based food. The plant kingdom is a good potential source for the discovery of novel medicines to treat numerous diseases including DM. Currently; about 400 plants incorporated more than 700 recipes

and compounds which have been evaluated extensively for the treatment of diabetes throughout the world [8-14]. In many parts of the world, especially in poor countries, this may be the only available form of therapy for the treatment of diabetic patients. One such plant is *Momordica charantia* (family name: Cucurbitaceae), nature's own cure for DM. *M. charantia* has been used extensively as an anti-viral, anti-bacterial agent and more so to treat a number of infections and diseases [13]. These include DM, indigestion, fever, skin disease, HIV, viral and bacterial infections, hypertension, reduced cholesterol and inflammation, detoxification of the body, expelling worms from the body, balance certain hormones in the body, enhances immunity, promotes milk flow, prevents different tumors and several other reported medicinal benefits. This review is concerned specifically with the medicinal chemistry of *M. charantia* and its extracts and active constituents to treat DM.

1.1 Plant-based Anti-diabetic medicine

Plant-based medicine has been used cost-effectively worldwide to treat DM. In fact, in many parts of the world, especially poor countries, this may be the only form of therapy available to treat diabetic patients. There are several reviews by different authors about anti-diabetic herbal plants [9-11, 13, 15-17]. One review has listed more than 300 plant species which possess hypoglycemic properties and classified them according to their biochemical names, country of origin, parts used and nature of the active agent(s) [11]. From the current literature, it is evident that *M. charantia* is the most widely used and popular anti-diabetic plant. Thus, this review will concentrate mainly on *M. charantia* and its anti diabetic properties [13].

1.2 Characteristics of M. charantia

Scientific name: *Momordica charantia*
Kingdom: Plantae
Division: Magnoliophyta
Family: Cucurbetaceae
Genus: Momordica
Species: Charantia
Duration: Annual

Some common names of *M.charantia* include bitter melon, papilla, bitter gourd, salsamino, corrila or karela, hanzal, assorossie, ampalaya, nigauri or goya, pare, kho gua, sora, balsam apple, pear or balsamina, and several other common names (see Taylor, 2002 [13] for extensive review and technical data). *M. charantia* is cultivated in many damp and wet tropical countries of the world including parts of South America and the Amazon basin including Brazil, Guyana and the Caribbean, East Africa and Asia including India, China, Philippines, Pakistan, Nepal and Sri Lanka. *M. charantia* is harvested both as food and as a medicine. It is a slender annual climbing vine with long leaves and at reproductive stage it produces warty or wrinkled gourd green fruits, resembling a squash or a cucumber. *M. charantia* is known for its very bitter taste and this is found in the leaves, the fruits, the stems and other parts of the plant [17]. The bitter taste is a distraction for eating the fruit but this is sometimes overshadowed by its beneficial effect. People normally boil the green leaves and stem and drink the bitter content as tea. Some people cook the fruit as a curry or with meat, while others eat it as a salad, fried it in oil or liquidize it into a herbal juice. In some cases people neutralize the bitter taste with the addition of a fruit (e.g. papaya) or a tropical juice such as mango or with a dash of salt. Commercially, the plant is used to make a powder which is sold commercially as tea or as in capsule form. Medicinally, the plant, its fruit and its powder extract possess a long history of use in the treatment of numerous diseases including diabetes [9-11,13,15,17].

1.3 Extracts and Active Ingredients of *M. charantia*

Generally, the public have used different parts of *M. charantia* including the leaves, the stem and mainly the green fruits or seeds to treat diabetes. Table 1 shows the chemical structures of momocharin (1) and momordicin (2) which is believed to possess insulin-like chemical structure and properties. People eat the fruit raw, boil or cook the different parts or drink the pulp of the fruit as a juice. Over the years several scientists have tried to isolate the various active ingredient of *M. charantia* for commercial purpose.

(Please visit http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3174519/ to view **Table 1**)

Fig. (1) shows a schematic diagram of the different stages of the isolation procedure for the active ingredient(s) of *M. charantia* fruit employing water and organic solvents. Initially, the fruits are chopped into small pieces and liquidized in deionized water. The

green supernatant is separated from the cellulose and subsequently, the water is extracted using a rota-evaporator. The residue is dried in an oven and the green powder extract is used for experimentation or for further extraction, purification and identification employing HPLC, affinity chromatography, SDS-PAGE and NMR mass spectroscopic methods. Two medicinal compounds extracted from *M. charantia* include, charantin, a steroidal saponin agent with insulin-like properties and momordicin (2), an alkaloid responsible for the bitterness of the fruit [18]. In laboratory and clinical in vitro and in vivo studies scientists and clinicians have employed different water, ethanol and ether extracts as well as isolated biologically active phytochemicals including glycosides (momordin and charantin), alkaloids (momordicin (2)), polypeptide-P, oils from the seeds (linoleic, stearic and oleic acids), glycoproteins (alpha–momorcharin (1), beta-momorcharin and lecitins) and others active compounds including protein MAP30 and vicine (pyrimidine nuclease) to study their hypoglycemic properties using both human and animal models [13]. Of these constituents, charantin, insulin-like peptides and alkaloids possess hypoglycemic properties. They are more effective when they are combined and they produce effects almost similar to the crude water soluble extract [13].

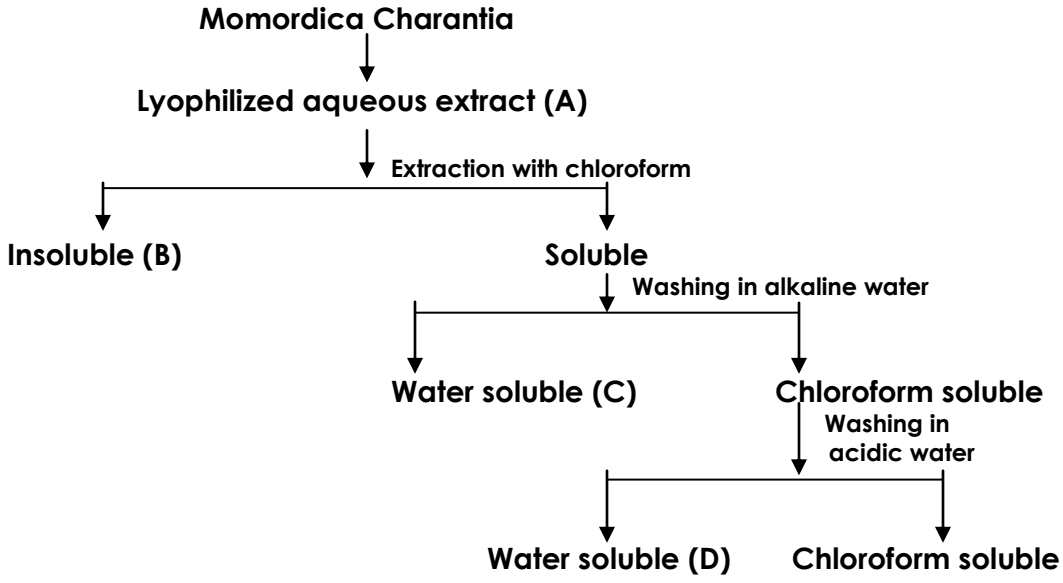

Fig. 1 : Schematic diagram showing the isolation procedures for the active ingredients(s) of M. charantia fruit juice (redrawn from Day *et al* [19]). Further purification and identification procedures employ HPLC, affinity chromatography, SDS-page and mass spectrometry.

1.4 Compounds Present in *M. charantia*

Today around 228 different medicinal compounds have been isolated from the stems, leaves, pericap, entire plant, aerial parts of the plant, endosperm, callus tissues, cotyledons and mainly the seeds and unripe fruit in different laboratories in India, Japan, USA, Thailand, Egypt, China, Taiwan, Australia, Nigeria, Pakistan, Brazil, Nepal, Philippines and Peru [19].

These different compounds have been classified into different chemical types. These includes proteids, triterpenes, lipids, inorganic compounds, phenylpropanoids, carotenoids, steroids, alkaloids, monoterpenes, alkene to C3, carbohydrates, benzanoids, alkanol C5 or more, other unknown structure (e.g. kakara I-B, II-A and III-B) sterol and sesquiterpene. Of the 228 different compounds, most of these fall under the groups of proteids and triterpenes [13].

The plant has many different chemical components, which help medicinally either alone or when combined. One of the hypoglycemic components is a steroid saponin called momocharin (charantin) (1) with insulin-like chemical effect. Charantin has a molecular weight of 9.7 kDa and it is the belief that charantin is the active hypoglycaemic agent of *M. charantia*.

Table 2 shows similarities in the chemical structures of momocharin (1), momordicin (2) and other commercially available hypoglycemic drugs (glibenclamide (3), gliclazide (4), Glipizide (5), Metformin hydrochloride (6), Pioglitazone hydrochloride (7) used in the treatment of T2DM. The hypoglycemic action of *M. charantia* and its isolated components may be due to its insulin-like structure. Recently, two other anti-diabetic constituents were isolated from *M. charantia* and both substances exerted hypoglycemic effects in mice. The cucurbitane triterpenoids were found to have the structures, 5β, 19-epoxycucurbita-6, 5β, 19-epoxy-19, 25-dimethoxycucurbita-6 23-(E)-dien-3β-ol and 3β-7β-25-trihydroxy-cucurbita-5, 23 (E)-dien-19-al. These two compounds have more or less the same parent structure as a-β momocharin (1), and momordicin (2) [20]. More recently, momordicin 1 (2) was isolated from M. charantia and its chemical structure was characterized as momordicin1 3, 7, 23,-Trihydroxycucurbitan-5,24-dien-19-al [20]. This compound is more or less similar to the one identifies by Harinantenaina et al. [21].

(Please visit http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3174519/ to view **Table 2**)

1.5 Traditional Remedy

The literature has suggested that one-half of one cup of a standard leaves or whole herb concoction, 1-2 times daily is adequate for a hypoglycemic effect. Alternatively, an amount of one to two grams of the water extracted powder from either the leaf or fruit is adequate as a daily dose. Many people liquidize two to three green fruits with water or with a tropical juice and drink 10 to 20 ml twice per day prior to meal. The question which people often ask is: can they take *M. charantia* with traditional commercial anti-diabetic drugs? The answer is to discuss the matter with their General Practitioner first. There may be an element of drug interaction between *M. charantia* and commercially available anti-diabetic glucose lowering drugs but further experiments are required to determine the kind of interaction which may occur between a commercially available hypoglycemic drug and *M. charantia* or its hypoglycaemic extract [22]. For people with T2DM there is no harm in using *M. charantia* alone, but combine this regimen with regular physical exercise and modification of daily diet. Previous studies have described the pharmacology, clinical efficacy, adverse effects, drug interactions and place in therapy of *M. charantia* [22]. It is particularly noteworthy that *M. charantia* is an alternative herbal therapy that has been used primarily to reduce blood glucose level for thousands of years in patients with DM. Regarding adverse effects, some studies have reported hypoglycemic coma and convulsions in children, reduced fertility in mice, a fetish-like syndrome, increase in gamma glutamyltransferase and alkaline phosphatase levels in animals and some headaches [22].

1.6 Clinical and Basic Experimental Studies

Over the past 50 years, both basic and clinical studies have been done to determine the effect of *M. charantia* on the management of DM. Table 3 shows the effect of oral administration of *M. charantia* on both animal and human type 2 models. Today the literature contains over 40 studies employing adult human subjects and another 100

have employed animal models. They were administered with a hot water extract, concoction, the fruit, the fruit juice or the seeds [13]. Typically, of the twenty or more studies present in Table 3, only three have demonstrated no beneficial effects, one on T2DM patient [16], one in rabbit [23] and the other in rat [24]. However, the other seventeen studies have successfully demonstrated that the whole plant, ethanol extract, fruit juice, powder, concoction or seed can evoke hypoglycemic effects. The reason for the three unsuccessful findings may be due to the fact that the authors employed high doses of *M. charantia*. Previous studies have successfully demonstrated that *M. charantia* is more beneficial as a hypoglycemic agent only at therapeutic doses. Either pharmacological or high doses of *M. charantia* seem to exert inhibitory effects [17, 25]. To date, not much study have been done on purified components of *M. charantia* in both human and animal models.

Table 3 : Effects of Oral Administration of *M. charantia* on Diabetes Mellitus

Experimental Models	Parts of Plant used	Effects	References
Normal and diabetic rats	Whole plant	Beneficial	Leatherdale *et al*. [26] Jeevathayaparan *et al.* [27] Chandrasekar *et al.* [28]
Diabetic rats	Ethanol extract of whole plant	Beneficial	Chandrasekar *et al.* [29]
Normal and diabetic rats	Fruit juice and various extracts	Beneficial	Ali *et al.* [30, 31]
Human (NIDDM)	Fruit juice/leaves	Beneficial	William and Pickup [6]; Ahmad *et al.* [45]
Human (NIDDM)	Fruit juice	No effect	Patel *et al*. [12], Day and Bailey[8]
Human (NIDDM)	Fruit powder	Beneficial	Akhtar *et al*. [32],Ahmed *et al*. [33]
Normal rabbits	Fruit juice	No effect	Kulkarni *et al.* [23]
Diabetic rabbits	Fruit juice	Beneficial	Akhtar *et al*. [32]
Normal and diabetic rats	Fruit juice	No effect	Karunanayake *et al.* [24] Platel and Srinivasan [16, 17]
Normal and diabetic rats	Fruit juice	Beneficial	Srivastavaet *et al*. [49]: Karunanayake *et al*. [24]
Normal and diabetic rat	Fruit juice	Beneficial	Sharma *et al*. [37]; Day *et al*. [19]
Normal and diabetic rats	Seed	Beneficial	Kedar and Chakrabarti [35]
Normal and diabetic rats	Fruit juice	Beneficial	Sharma *et al*. [34]

1.7 Possible Modes of Action of *M. charantia* and Its Extract

M. charantia and its various extracts and components are believed to exert their hypoglycemic effects via different physiological, pharmacological and biochemical modes [13, 15, 17, 33]. Table 4 lists some possible modes of the hypoglycemic actions of *M. charantia* and its various extracts and compounds. Today over 140 different studies worldwide [13] have investigated anti-hyperglycemic and hypoglycemic effects of the different extracts and ingredients of *M. charantia* in both human and animal models. These include the fruit extracts with either hot water, ethanol, lyophilized, benzene, chloroform, acetone, fruit juice and powder. Of all the different studies, about 120 have reported active and beneficial effects, whereas the remaining 20 have reported inactive or no beneficial effects. There are several reasons for these discrepancies in the activity of *M. charantia* and its various extracts and isolated compounds. These may be due to the duration (short time period) of the experiments, doses of the compounds (high doses seem to evoke inhibitory or toxic effect) [32], the animal models and gender employed in the studies, the method of application/administration, and in some cases the laboratories and countries where the work was done and also the extracts administered. These discrepancies may also be due to seasonal variations [13].

Table 4 : Possible modes of hypoglycaemic actions of M. charantia

Possible Modes of Action		Reference
1.	Insulin secretagogue effect	Karunanayake *et al.* [24], Kedar and Chakrabarti [35], Jeevathayaparan *et al.* [27]
2.	Stimulation or peripheral and skeletal muscle glucose utilisation	Day *et al.* [19]; Bailey and Day [8], Cummings *et al.* [25]
3.	Inhibition of intestinal glucose uptake	Meir and Yaniv [47]; Ahmed *et al*. [33], Ahmed *et al.* [40]
4.	Inhibition of hexokinase activity	Meir and Yaniv [47]
5.	Suppression of key gluconeogenic enzymes	Shibib *et al.* [48]
6.	Stimulation of key enzyme of HMP pathway	Shibib *et al.* [48]
7.	Preservation of islet beta cells and their functions	Ahmed *et al*. [39], Ahmed *et al.* [40]

M. charantia, its extracts and isolated components are believed to exert their hypoglycaemic effects via different physiological and biochemical processes. These include insulin secretagogue like effect, stimulation of skeletal muscle and peripheral cell glucose utilization, inhibition of intestinal glucose uptake, inhibition of hexokinase activity, suppression of key gluconeogenic enzymes, stimulation of key enzymes, HMP pathway and preservation of pancreatic islet cells and their functions (see Table 4 for relevant references).

1.8 Preservation of Pancreatic β Cells and Insulin Secretion

It was previously demonstrated by Jeewathayaparan et al. [25] that oral administration of *M. charantia* could lead to the secretion of insulin from endocrine pancreatic beta cells. This observation was further confirmed by Ahmed et al. [33, 39, 41] who investigated the effect of daily oral administration of *M. charantia* fruit juice and the distribution of α, β and δ cells in the pancreas of streptozotocin (STZ)-induced diabetic rats using immunohistochemical methods. In these studies, they observed that *M. charantia* significantly increased the number of β cells compared to untreated diabetic rats. However, the number of β cells was significantly less than that obtained in normal and *M. charantia*-treated control rats. This may be due to the fact that the study was only done for a period of 10 weeks and moreover, the STZ probably destroyed some of the beta cell completely one week prior to the administration of the fruit juice to the animals. From these studies, the authors concluded that *M. charantia* fruit juice may have a role in the renewal of β cells in treated diabetic rats or alternatively, the juice may permit the recovery of partially destroyed β cells [33, 39, 41]. Physiological experiments have also shown that *M. charantia* can stimulate insulin secretion from the endocrine pancreas [42] and elicit glucose uptake in the liver [43]. Current evidence therefore indicates that the recovery and subsequent increase in the number of insulin producing cells followed by the release of insulin may be part of the several pathways by which *M. charantia* exerts its hypoglycemic effects. In addition to the properties mentioned above, *M. charantia* and its extracts may possess cell-like proliferation and growth-like properties similar to that of insulin [5, 25]. Nevertheless, further experiment are required, at least at the molecular level, to determine the precise mechanisms whereby *M. charantia* can either repair damaged β cells or prevent their death.

1.9 M. charantia and Glucose Metabolism

Insulin plays a major biochemical role in stimulating the uptake of glucose by different cells of the body for the production of energy [4, 5, 25]. Since *M. charantia* and its various extracts and components have been reported to exert hypoglycemic effects, and then it is important to understand whether *M. charantia* may have a direct effect in inducing a reduction in blood glucose level [13]. Previous studies have shown that both the aqueous and alcoholic extracts of the fruit of *M. charantia* can inhibit the activities of fructose 1, 6-diphosphatase and glucose-6-phosphatase and at the same time stimulating the action of glucose-6-phosphatase dehydrogenase [17, 19, 34, 36]. It was previously reported that *M. charantia* and its various extracts can stimulate peripheral cell glucose uptake [17, 19, 25]. A number of studies have investigated the effect of the powder and chloroform extract of *M. charantia* in comparison with insulin on glucose and amino acid uptakes by skeletal L6 myotubes and Na+ and K+ glucose uptakes by jejunum brush border membrane vesicles in both age-matched control and STZ-induced diabetic rats. The results show that either the lyophilized fruit juice or chloroform extract at 5-10 µg ml-1 can stimulate 3H-deoxyglucose and 14C–Me AIB (N-methyl-amino-a-isobutyric acid) uptakes by L6 myotubes. These effects were similar in magnitude to the effects obtained with 100 nM insulin. Incubation of either insulin or *M. charantia* juice in the presence of wortmannin (a phosphatidylinositol 3-kinase inhibitor) resulted in a marked inhibition of 3H-deoxyglucose uptake by L-6 myotubes [25, 44]. Together, the results have clearly demonstrated that *M. charantia* contains insulin like properties, similar to one phytochemical component of *M. charantia* called V-insulin [13].

In addition to its insulin-like effects on skeletal muscle cells, daily oral intake of *M. charantia* fruit juice over a period of 10 weeks significantly reduced the amount of Na+ and K+-dependent 14C-D-glucose absorbed by rat jejunum brush border membrane vesicle compared to vesicles obtained from STZ-induced diabetic rats [33,41]. Taken together, these results clearly demonstrated that *M. charantia* and its extracts can directly regulate blood glucose via two mechanisms. Firstly, it can regulate how much glucose is absorbed by the gut into the blood following a meal and secondly, it can stimulate glucose uptake into skeletal muscle cells just like insulin. Moreover, it seems to exert its effect via the same intracellular signaling pathways as insulin in regulating glucose metabolism in the body [44].

1.10 Anti Neurophatic Effect of *M. charantia* Fruit Juice

Human diabetic neuropathy is both cumbersome and complicated and it may result in severe disability [5, 6]. In addition, treatment of diabetic neuropathy is very expensive. The most cost effective way is to either prevent or delay the onset of this, long-term diabetic complication. The influence of *M. charantia* fruit extract and insulin on the ultra-structural abnormalities of myelinated fibers in experimental diabetes in rats was investigated in previous studies [33, 37, 40].

The results have shown that the mean cross-sectional myelinated fibre area, axonal area and myelin area including the mean maximum myelinated fibres area were significantly less in untreated diabetic rats when compared with age-matched controls. In the *M. charantia* treated diabetic animals, myelinated fibre area and myelin area were significantly greater than untreated diabetics and not significantly different from age-matched controls. The mean value for the maximum fibre area was also significantly greater than untreated diabetics and was not significantly different from age-matched controls. The axon to fibre ratio ('g' ratio) did not differ between any of the experimental groups. It was concluded that the administration of *M. charantia* fruit juice not only reduced blood glucose level but also corrected the structural abnormalities of peripheral nerves in experimental diabetes. These results have strongly indicated that M. charantia possesses growth factor-like properties just like insulin [5, 31, 37, 39].

To date, M. charantia has been extensively studies worldwide for its medicinal properties to treat a number of diseases [38]. It is described as a versatile plant worthy of treating almost any disease inflicted on mankind. This may be due to the fact the plant possesses over 225 different medicinal constituents [13]. These different compounds may act either separately or together to exert their medicinal effects. In relation to diabetes [46], only charantin, insulin-like peptide and alkaloid-like extracts possess hypoglycemic properties similar to the plant itself or its crude extracts. These different compounds seem to exert their beneficial effects via several mechanisms to control and treat diabetes mellitus.

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Extraction of Insulin like Compounds from Bitter Melon Plants

[1]Saad Mohamed El-Said and [2]Ali S. Al-Barak
[1]Buraydah, Buraydah College of Food and Environment Technology, Saudi Arabia
[2]Faculty of Science, Haiil University, Saudi Arabia

Abstract

This study was focused on the insulin-like compound, charantin, as alternative drugs in order to reduce the blood. As such, the anti-diabetic compound, charantin was extracted from leaves and fruits of bitter melon. Liquid extraction charantin, from leaves and fruits of bitter melon was proposed using ethyl alcohol under special conditions of temperature and at a suitable pH values. Experiments were conducted to determine the effects of several pH values including (2-12) using different buffer systems on extraction efficiency. In addition, the extraction efficiency was found to be highly influenced by temperature, ethanol/water ratio and time of contact. A purification step was carried out using methanol-water mixture to remove interfering components such as chlorophylls and sugars from the analyte. Chemical analyses as well as identification of charantin were carried out by High Performance Liquid Chromatographic. The results indicate that Charantin were found in the fruit and leaf samples of Bitter Melon Plants. It was observed that bitter melon leaves plants contained higher concentrations of charantin analyzed as compared to fruits. The optimized conditions were 50 and 70% ethanol, 70.24 and 80.34°C and a time of contact of 60 and 70 h at pH is 5.4 for fruit and leaves, respectively. The corresponding predicted values were 55.27 mg charantin equiv. g-1 dry fruit and 144.58 mg charantin equiv. g-1 dry leaves. This experiment will help to highlight the importance of these valuable organic compounds found in these plant species and their demand in the market will be increased in the future.

1. Introduction

Medicinal plants are a major source of drugs for the treatment of various health disorders. Nowadays huge number of allopathic medicines also contains plant based ingredients that are used for their preparation by different companies. There are about 400,000 species of higher plants in the world, as compared to animal's species that are

about 5-10 million. The plant materials contain thousands of chemicals which act against diseases and infections of humans and animals when properly used (Shinwari and Khan, 1998).

Plants contain different types of compounds such as resins, rubbers, gums, waxes, dyes, flavors, fragrances, proteins, amino acids, bioactive peptides, phyto hormones, sugar, flavonoids and bio pesticides (Khattak et al., 1985). Furthermore according to assessment of WHO about 80% of world population depend on medicinal plants for their health care needs and more than 30% of the pharmaceutical preparations are based on plants (Shinwari and Khan, 1998). Where as some reports indicated that there are 90 popular medicinal plants and different Pharmaceutical companies are using extracts of these plants in various drugs. Scientists throughout the world are trying to explore the precious assets of medicinal plants to help the suffering humanity (Edward, 2001). However, the developed countries mostly import raw material from developing countries and after processing export it back as high priced prepared medicines to developing countries (Shinwari and Khan, 1998).

Bitter melon (*Momordica charantia*) or commonly as Ma-ra-khee-nok, is an herbal plant grown in Thailand and other tropical regions. It is traditionally known for its medicinal properties such as antidiabetic, antitumorous, anticancer, anti-inflamatory, antiviral and cholesterol lowering effects etc. (Ahmed et al., 2001; Taylor, 2002). The main constituents of bitter melon which are responsible for these effects are such as triterpene, proteid, steroid, alkaloid, inorganic, lipid and phenolic compounds. The protein in bitter melon including protein MAP-30, alpha-momorcharin and beta-momorcharin were shown to have the ability for fighting against HIV (Luetrakul, 1998). A steroid, charantin, contained mainly in the aerial parts, has been proven for its antidiabetic activity (Chanchai, 2003).

Conventionally, isolation of this compound involves extraction Charantin could be used to treat diabetes and can potentially replace treatment by injection of insulin which has not been successful in stimulating the pancreas of the diabetic patients to lower blood sugar to the desired level (Belinda, 2000). In some cases, the injected patient shows signs of side effects. A molecule of charantin consists of aglycone or a steroidal portion, which is highly soluble in relatively non-polar solvent such as chloroform and dichloromethane. However, the glucosides attached to its molecules make it slightly soluble in polar

organic solvent such as ethanol or methanol. Conventionally, isolation of this compound involves extraction with mixtures of these solvents.

In this study, we investigated the charantin contents of bitter melon obtained by water ethanol extraction. The effect of extraction temperature and pH values was considered also. Moreover, the quantitative analysis of these extracts was determined.

2. Materials and Methods

2.1 Materials and chemicals

The fruits and leaves of bitter melon were obtained from the market in Al Qassiem Saudia in the period between March and May 2004. Ethanol was obtained from Sigma Chemical Co. (St. Louis, Mo, USA). A heater model 21V50-2, serial number 1291A39968, manufactured in Germany it set to work with Natural Gas only. A Beckman Model 332 (Germany) liquid chromatographic system was employed, consisting of a Model 110A pump and a Model 420 system controller. Charantin was detected using a Beckman 155 variable wavelength detector fitted with a 20-p1, I cm optical path cell.

2.2 Sample preparation

The cleaning of Bitter Melon was made using distilled water before cutting them into small pieces and then oven dried at 50°C for a day. The dried sample was then pulverized into fine powder in a grinder, which was then stored at 4°C until use. The leaves fragments were further ground under liquid nitrogen using mortar and pestle. A sub sample was weighed, dried at 50°C and reweighed to obtain the dry weight/wet weight ratio.

2.3 Water extraction

In order to extract and purify charantin from leaves and fruits samples, following procedures were adopted: About 100 g (each of fruits and leaves) samples were soaked in water/ethanol solvent at different contact time from 1 to 72 h The effect of temperature also carried out from 30 to 80°C. The pH of the mixture was adjusted to several values from 2-13 with buffer solution. Buffers solution at pH 3.0-6.0 is 10 mM citrate-phosphate); pH 7.0 (10 mM sodium-phosphate); pH 8.0 (10 mM Tris-HCl); pH 9.0-10.0 (10

mM carbonate-sodium bicarbonate); pH 11.0-12.0 (10 mM glycine-NaOH). The extract was cooled in a coil immersed in a water bath and the extract was collected in fractions in sample collecting vials every 3 h in a first day and then was collected every 6 h in the second and third days. These extracts were evaporated under vacuum to remove water ethanol solvent. The mixture was run through a column using silica gel to separate the charantin, which were further identified on High Performance Liquid Chromatographic using reference standards whereas for protein, sephadex (G 20 and G 50) was used. The concentration level of charantin was determined with the help of spectrophotometer at 734 nm.

2.4 Sample purification

To purify the crude extract, the protocol was carried out (Chanchai, 2003). Briefly, 5 mL of 50:50 (v/v) methanol-water was added to the crude extract. The mixture was then sonicated for 15 min and centrifuged at 3500 rpm for 15 min to separate the supernatant from the precipitate. The precipitate was then added with 5 mL of 70:30 (v/v) methanol-water and the mixture was again sonicated and centrifuged. The precipitate from this step was added with 3 mL of hexane and the step was repeated. The precipitate from this step was re-dissolved in 200 L of 1:1 (v/v) chloroform-methanol mixture and then adjusted to volume with methanol (to 1 mL volume for extracts obtained with PLE and to 2 mL volume for that obtained with Soxhlet extraction). The purified solution was filtered through a 0.45 m nylon membrane filter (Millipore, USA) before being analyzed by an HPLC.

3. Results

A chemical analysis of various compounds found in leave and fruits of Bitter melon were detected expressed in percentage (%) (Table 1). The results obtained from Table 1 indicated that concentration of charantin (9.65%) and (3.41%) was found in leaves and fruit, respectively. The utilization of pH effects on extraction of charantin from bitter melon plants was successfully applied using suitable ethanol/water solvent ratio and temperature. Although, Fig. 1 demonstrate that the maximum extraction of charantin (55.27 mg charantin equiv. g-1 dry fruit and 144.58 mg charantin equiv. g-1 dry leaves) was found to be at pH 5.4 with at 50 and 70% ethanol, 70.24 and 80.34°C and a time of contact of 60 and 70 h for fruit and leaves, respectively.

Present results on the extractability of charantin at pH 4.5 agree with those of Smith and Circle (1938), Wolf et al. (1964). The effect of temperature on extraction efficiency of charantin was examined over the range of 30-150°C at optimum pH (4.5) and ethanol/water ratio, the results show that 70.24 and 80.34°C is the optimum temp for fruit and leaves respectively (Fig. 2).

Table 1: The percentage of various chemical compounds analyzed from leaves and fruit of bitter melon

Constituent in leaves	Percentage	Constituent in fruit	Percentage
Chrantin	9.65	Chrantin	3.51
Protein	7.50	Protein	15.60
Fat	6.5	Fat	14.50
Sugar	2.10	Sugar	27.50
Fiber	13.50	Fiber	6.50
Palmatine	0.32	Palmatine	0.80
Calcium	1.80	Calcium	0.90
Sulphur	2.70	Sulphur	0.80
Celleulose	40.25	Celleulose	20.20
Berberine	5.60	Berberine	2.50
Vitamin C	1.20	Vitamin C	14.58

Also the results of extraction of charantin from bitter melon fruits at different ethanol/water ratio illustrated as shown in Fig. 3. The mixtures of ethanol and water were tested to determine the effect of mixture compositions: 0, 20, 50, 70, 80 and 100% ethanol on the extraction of charantin from leaves and fruits. The conditions used in each experiment were pH = 4.5, at 70.24 and 80.34°C and a time of contact of 60 and 70 h for fruit and leaves respectively. The extraction efficiency increased with increasing percentage of ethanol in the solvent mixture until up to 50 and 70% for fruits and leaves this result. These results were supported also by Pitipanapong et al. (2007). Also the effect of contact time are studied and the results are shown in Fig. 4 which show that high concentration of charantin observed in leaves and fruits after 70 and 60 h at the same previous conditions.

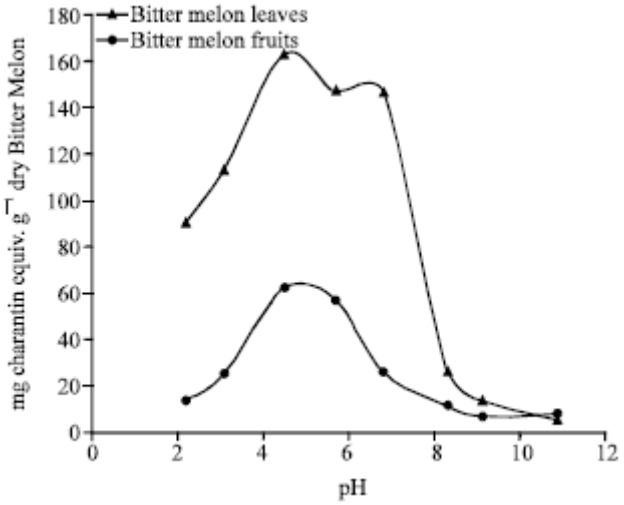

Fig. 1: Effect of pH on charantin extraction at 50 and 70% ethanol, 70.24 and 80.34°C and a time of contact of 60 and 70 h for fruit and leaves, respectively

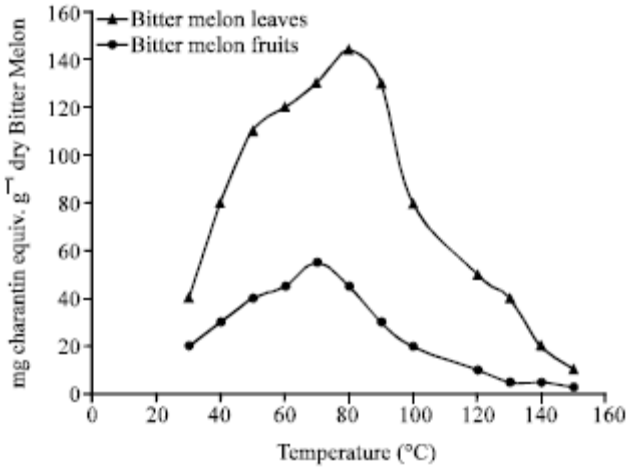

Fig. 2: Effect of temperature on extraction of charantin from bitter melon leaves and fruits at optimized conditions 50 and 70% ethanol, time of contact of 60 and 70 h at pH is 5.4 for fruit and leaves, respectively

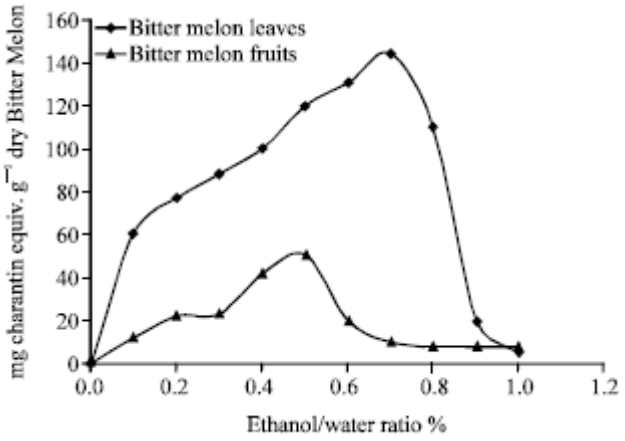

Fig. 3: Effect of solvent composition on extraction of charantin from bitter melon leaves and fruits at time of contact of 60 and 70 h and 70.24 and 80.34°C for fruit and leaves, respectively at pH is 5.4

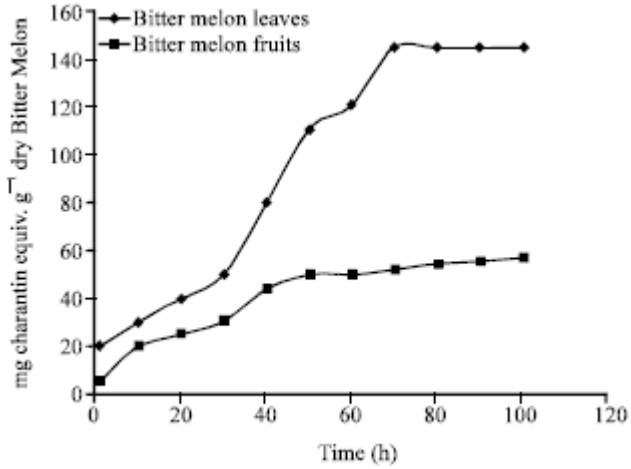

Fig. 4: Effect of contact time on extraction of charantin from bitter melon leaves and fruits at optimized conditions 50 and 70% ethanol and 70.24 and 80.34°C for fruit and leaves, respectively at pH is 5.4

4. Discussion

The major determining factors on charantin solubilization in ethanol/water solvent at a definite temperature are the pH influence. The pH of the aqueous phase was varied between 3.0 and 12.0, using different buffer systems according to their pKa value (Fig. 1). The results showed a high extraction of chrantin from bitter melon within the pH range of 3.0-6.0, with a maximum of value at pH 5.4. A similar situation has been reported for Cratylia mollis seed lectin (Nascimento et al., 2002), with a maximum extraction at pH 5.0. As the pH of aqueous phase increased from 5.5 to 12, the extracted protein decreased, probably due to the proximity of the isoelectric point of charantin compound. This phenomenon was observed for different proteins at distinct isoelectric points (Goklen and Hatton, 1987). Therefore, the selected pH to be used in further experiments was 5.4.

Figure 2 reveals that extraction efficiency was greatly influenced by temperature. As described earlier, the increase in temperature decreases solvent polarity as a result of reduced polar forces and hydrogen bonding, making the solvent more suitable for extraction of charantin. Moreover, at elevated temperatures, the solvent density and viscosity decrease, resulting in increased mass transfer of the solvent into the matrix of plant sample. However, when temperature increases from 80 to 100°C, the percent charantin extracted did not change until the end of extraction. This suggests that there appears to be an optimal temperature between 60 and 80°C. It is possible that beyond this temperature, further decrease in ethanol polarity may be disadvantageous for extraction of charantin. However the influence of temperature on solvent polarity and thus solubility at subcritical condition is not completely understood as the experimental data near critical temperature are not available. More detailed study is needed to completely understand the behavior of solvent and the solute solubility under these conditions these results were supported by (Pitipanapong et al., 2007).

Figure 3 show the effect of solvent composition on extraction of charantin where as increasing ethanol/water ratio (from 0 to 70%) the extraction of chrantin from bitter melon leaves and fruits increase to certain values. Further increase in ethanol composition (up to 70%) did not further increase the extraction efficiency. It is worth noting that when pure water was used, the solubility of charantin was low because water polarity was extremely high. However, the addition of some amount of water to as high as 70% into ethanol was found not to hinder the extraction efficiency. A possible

explanation was that the lower viscosity of water allows it to more easily penetrate into the pores of the sample matrix, thus causing the swelling of the plant materials. This increases the contact volume and area between the solvent and the plant porous matrix, thus the internal mass diffusion is increased (Rostagno et al., 2003; Li et al., 2005). Rostagno et al. (2003) has indeed reported that addition of water enhances the solubility of some glucoside compounds and thus improves the extraction efficiency (Li et al., 2005). In this study, the extraction efficiency of pure ethanol and ethanol-water mixture up to 50% ethanol are comparable. However, the ethanolic mixture with too high water content has lower dissolving power for charantin as a result of increased polarity.

5. Conclusion

Extracts of charantin obtained by water ethanol solvent at optimum pH had higher total contents from leaves and fruits of Bitter melon. So, it can be used by people to diabetes type 2. Therefore it is recommended that extraction and purification of such charantin are very valuable in the preparations of drugs of diabetes of various types. The assessments of various effects of such compounds on human health are required in the future studies.

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Antidiabetic Activities of Triterpenoids Isolated from Bitter Melon Associated with Activation of the AMPK Pathway

Min-Jia Tan[1, 5], Ji-Ming Ye[2, 5], Nigel Turner[2, 5], Cordula Hohnen-Behrens[2], Chang-Qiang Ke[1], Chun-Ping Tang[1], Tong Chen[1], Hans-Christoph Weiss[3], Ernst-Rudolf Gesing[4], Alex Rowland[2], David E. James[2], ,Yang Ye[1],

[1] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201203, China

[2] Diabetes and Obesity Research Program, Garvan Institute of Medical Research, University of New South Wales, Sydney, NSW 2010, Australia

[3] Bayer Industry Services GmbH & Co., Leverkusen 51368, Germany

[4] Bayer CropScience AG, R & D, Agricultural Center, Monheim 40789, Germany

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Abstract

Four cucurbitane glycosides, momordicosides Q, R, S, and T, and stereochemistry-established karaviloside XI, were isolated from the vegetable bitter melon (*Momordica charantia*). These compounds and their aglycones exhibited a number of biologic effects beneficial to diabetes and obesity. In both L6 myotubes and 3T3-L1 adipocytes, they stimulated GLUT4 translocation to the cell membrane—an essential step for inducible glucose entry into cells. This was associated with increased activity of AMP-activated protein kinase (AMPK), a key pathway mediating glucose uptake and fatty acid oxidation. Furthermore, momordicoside(s) enhanced fatty acid oxidation and glucose disposal during glucose tolerance tests in both insulin-sensitive and insulin-resistant mice. These findings indicate that cucurbitane triterpenoids, the characteristic constituents of *M. charantia*, may provide leads as a class of therapeutics for diabetes and obesity.

1. Introduction

Currently, there are 150 million people with diabetes worldwide, and this figure is expected to increase to over 300 million by 2025. This global pandemic is driven by type 2 diabetes (T2D) (Zimmet et al., 2001). Since insulin resistance is a major metabolic abnormality of T2D, there has been considerable interest in insulin-sensitizing agents to counteract insulin resistance for the treatment of this disease (Moller, 2001). Currently, pharmacological treatment of insulin resistance mainly targets two mechanisms: peroxisome-proliferator-activating receptors (PPARs) (Smyth and Heron, 2006) and AMP-activated protein kinase (AMPK) (Ye et al., 2005). The two most popular agents now in use are the thiazolidinediones (TZDs) and the biguanides. The TZDs are widely used but can have undesirable side effects (weight gain, fluid retention, and heart failure). The biguanide metformin does not cause weight gain but mainly acts in liver rather than muscle and thus on its own is not a complete therapy. There is a worldwide search for better agents ([Moller, 2001] and [Smyth and Heron, 2006]).

Traditional medicines (TM) or complementary and alternative medicines are a fruitful source of future drugs to counteract insulin resistance, consistent with a resurgence of interest in drug discovery from natural products (Koehn and Carter, 2005). For example, metformin was a biguanide derivative of guanide, originated from the plant Goat's Rue (*Galega officinalis*) as a structure-modified natural product to vastly improve its efficacy. A major advantage of TM is that they have been used to treat human diseases for many years and so there is considerable knowledge concerning in vivo efficacy and safety, two of the confounding problems facing other new chemical entities. However, in most cases there is little rigorous scientific evidence proving their efficacy and the mode of action is generally not known. To overcome these problems, it is essential to identify the active ingredients or molecules and investigate their specific effects in well-defined biological systems and animal models relevant to humans.

In this study, we have taken a targeted approach to investigate the active chemical molecules in *Momordica charantia* L. (Cucurbitaceae), also known as bitter melon, bitter gourd, or balsam pear. This plant is widely cultivated as a vegetable and medicinal herb in many Asian countries and has been shown to exert hypoglycemic effects in animal models and humans (Grover and Yadav, 2004). Although major chemical constituents of

M. charantia include cucurbitane triterpenoids ([Okabe et al., 1980], [Okabe et al., 1982a],[Okabe et al., 1982b], [Miyahara et al., 1981], [Murakami et al., 2001], [Harinantenaina et al., 2006],[Nakamura et al., 2006], [Matsuda et al., 2007] and [Zhu et al., 1990]), the precise active compounds responsible for the antidiabetic activity of this plant have not been clearly identified. In this work, we investigated the chemical constituents of *M. charantia* for the purpose of identifying the antidiabetic principles of this medicinal vegetable.

Four novel cucurbitane glycosides, momordicosides Q, R, S, T, (2, 3, 5, 6); the absolute-stereochemistry-established karaviloside XI (1); a spectroscopic-data-revised glycoside, momorcharaside B (7); and two known triterpenoids, momordicoside A (9) and momordicoside B (10), were isolated (Figure 1A). These compounds, particularly 1 and 5, and their aglycones (4 and 8), stimulated the translocation of the insulin-responsive glucose transporter GLUT4 to the plasma membrane in muscle and adipocyte cell lines. Intriguingly, these compounds also activated the AMP-activated protein kinase (AMPK) pathway, a major regulatory pathway for GLUT4 translocation (Huang and Czech, 2007). In vivo studies in mice showed a significant enhancement of glucose disposal and increases in fatty acid oxidation after acute administration of compound 5. These results suggest that cucurbitane triterpenoids from *M. charantia* may provide novel leads for the development of a new class of AMPK-activating agents.

2. Results

2.1 Structural Identification

Compound 1 was established as $C_{36}H_{60}O_{10}$ by HRESIMS (*m/z* 675.4064 $[M+Na]^+$). The plane structure of compound 1 was established as the recently reported karaviloside XI (Matsuda et al., 2007) on the basis of their identical NMR data. Mild acid hydrolysis of 1 furnished aglycone 4 and allose. The absolute configuration of aglycone 4 was established by X-ray diffraction analysis (Figure 1B) and its biogenetic relationship. Thus, the structure of compound 1 was elucidated as 3-O-β-D-allopyranosyl-5β,19-epoxycucurbita-6-ene-23(*R*),24(*S*),25-triol.

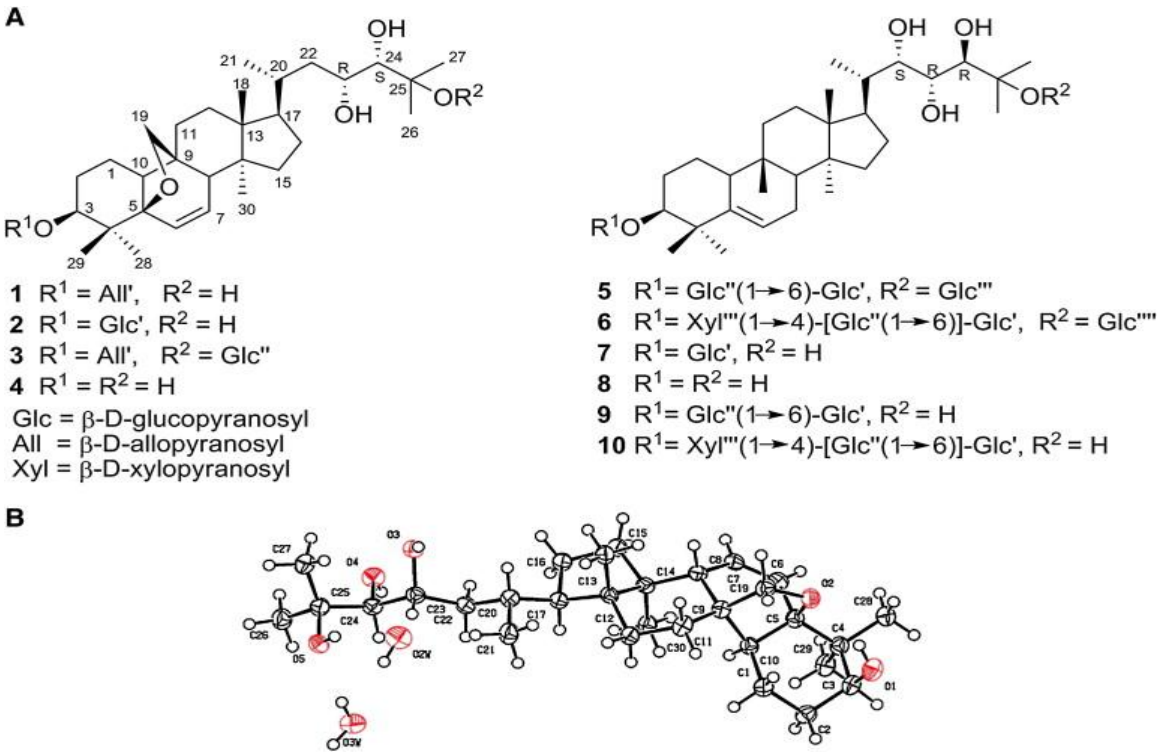

Figure 1: Chemical Structures of Cucurbitane Triterpenoids from Bitter Melon
(A) Chemical structures of cucurbitane triterpenoids from bitter melon.
(B) Perspective ORTEP drawing for compound 4.

Momordicoside Q (2) showed $[M+Na]^+$ at *m/z* 675.4113 ($C_{36}H_{60}O_{10}Na$) in the HRESIMS. Acid hydrolysis of compound 2 yielded aglycone 4 and glucose. ^1H NMR and ^{13}C NMR signals of compound 2 were superimposable on those of karaviloside XI (1) except for the variation of signals of the sugar moiety, which was inferred as β-D-glucose from its anomeric proton (δ_H 4.95, d, *J* = 8.1). According to these data, compound 2 was identified as 3-O-β-D-glucopyranosyl-5β,19-epoxycucurbita-6-ene-23(*R*),24(*S*),25-triol.

The molecular formula of momordicoside R (3) was $C_{42}H_{70}O_{15}$ revealed by HRESIMS (*m/z* 837.4632, $[M+Na]^+$), which was supported by ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) data. Allose and glucose were obtained by acid hydrolysis of compound 3. ^{13}C NMR and DEPT spectra indicated the existence of six tertiary methyls, one secondary methyl, two olefinic carbons, six quaternary carbons, and ten methenes, three of which bore a hydroxyl group. The above ^{13}C NMR data as well

as ^{1}H NMR data bore a resemblance to those of karaviloside XI (1) with the exception of the appearance of six additional carbon signals for a monosaccharide moiety. Two anomeric proton signals, H-1′ (δ_H 5.34, d, *J* = 7.7) and H-1″ (δ_H 5.21, d, *J* = 7.8), were assigned to β-D-allopyranosyl and β-D-glucopyranosyl moieties, respectively. ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) experiment disclosed the long-range correlations between H-1′ and C-3, and H-1″ and C-25, which established the linkage of each sugar moiety. The above evidence and analysis of heteronuclear single-quantum coherence (HSQC), HMBC, total correlated spectroscopy (TOCSY) spectra confirmed compound 3 as3-O-β-D-allopyranosyl-25-O-β-D-glucopyranosyl-5β, 19-epoxycucurbita-6-ene-23(*R*),24(*S*)-diol.

HRESIMS of momordicoside S (5) presented the molecular formula of $C_{48}H_{82}O_{20}$ (*m*/*z* 1001.5253 [*M*+Na]$^{+}$).^{1}H NMR, ^{13}C NMR, and DEPT data showed the presence of seven tertiary methyls, one secondary methyl, a trisubstituted double bond, six quaternary carbons, seven aglycone methylenes, and three sugar methylenes, as well as three anomeric carbons, which suggested a triterpenoid glycoside with three sugar moieties. Enzymatic hydrolysis of compound 5 liberated aglycone 8 and glucose. Aglycone 8 was identical to the aglycone of momordicoside A (^{1}H NMR, ^{13}C NMR, DEPT, ESIMS, [a]), which was secured by the same hydrolysis procedure (Okabe et al., 1980). Thus, the absolute configuration of the aglycone 8 was established. The signals of three anomeric protons, H-1′ (δ_H 4.75, d, *J* = 7.8), H-1″ (δ_H 5.12, d, *J* = 7.4), and H-1‴ (δ_H 5.09, d, *J* = 7.3), indicated to three β-D-glucopyranosyl moieties. In addition, the HMBC correlations between C-3 and H-1′, C-6′ and H-1″, and C-25 and H-1‴ were also observed. Based on the comprehensive analysis of ^{13}C NMR, DEPT, HSQC, HMBC, and TOCSY spectra, the structure of momordicoside S (5) was formulated as 3-O-[β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl]-25-O-β-D-glucopyranosyl-22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbit-5-ene.

HRESIMS afforded a possible molecular formula of momordicoside T (6) as $C_{53}H_{90}O_{24}$ (1113.5714, [*M*+Na]$^{+}$), which suggested a triterpenoid glycoside with four monosaccharide moieties. Xylose and glucose were released by acid hydrolysis of compound 6. Carbon signals from ^{13}C NMR and DEPT data were in good agreement with those of momordicoside S (5), except that five extra signals due to a xylopyranosyl moiety were observed. Four anomeric proton signals were assigned as β-D-

glucopyranosyl moiety [H-1′ (δ_H 4.71, d,*J* = 7.8)], β-D-glucopyranosyl moiety [H-1″ (δ_H 5.29, d, *J* = 6.6)], β-D-xylopyranosyl moiety [H-1‴ (δ_H 5.30, d,*J* = 7.2)], and β-D-glucopyranosyl moiety [H-1⁗ (δ_H 5.10, d, *J* = 7.8)] from its ^{1}H NMR spectrum, respectively. The oligoglycoside structure of compound 6 was characterized by HMBC correlation: H-1′ and C-3, H-1″ and C-6′, H-1‴ and C-4′, H-1⁗ and C-25. Based on the above spectroscopic evidence, together with TOCSY spectrum, structure of compound 6 was determined to be 3-O-{β-D-xylopyranosyl(1→4)-[β-D-glucopyranosyl(1→6)]-β-D-glucopyranosyl}-25-O-β-D-glucopyranosyl-22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbit-5-ene.

The spectroscopic data of momorcharaside B (7) reported by Zhu et al. were not consistent with the described structure (Zhu et al., 1990). Herein, we revised the spectroscopic data of momorcharaside B. Its molecular formula $C_{36}H_{62}O_{10}$ was derived from HRESIMS (*m/z* 677.4224, $[M+Na]^+$). Acid hydrolysis of momorcharaside B with 2% aqueous HCl-dioxane (1:1, v/v) gave glucose and aglycone 8. Six glucopyranosyl carbon signals disappeared by contrast with those of momordicoside A. β-D-configuration of the sugar moiety was discerned from the coupling constant of anomeric proton (*J* = 7.7). Consequently, its structure was formulated as 3-O-β-D-glucopyranosyl-22(*S*),23(*R*),24(*R*),25-tetrahydroxycucurbit-5-ene by ^{1}H NMR, ^{13}C NMR and DEPT spectra.

2.2 Assessment of Antidiabetes Properties

In order to assess the potential activity of these compounds on glucose metabolism and insulin action, we first examined their effects on the translocation of the glucose transport GLUT4 to the plasma membrane, because this is an essential step for inducible glucose uptake into muscle and fat cells. The results showed that most compounds exhibited the same pattern of biologic activity to stimulate GLUT4 translocation in L6 muscle cells (Figure 2A) and 3T3L1 adipocytes (Figure 2B), and among them 1, 4, 5, and 8 increased GLUT4 translocation by 3- to 4-fold, an efficacy close to the maximum effect of insulin (5-fold) and AICAR (~4-fold). The dose-response curves (Figure 2C) showed that compounds 1, 4, 5, and 8 exhibited this biological activity at concentrations as low as 0.1 nM and reached their maximal effects at between 10 and 100 nM.

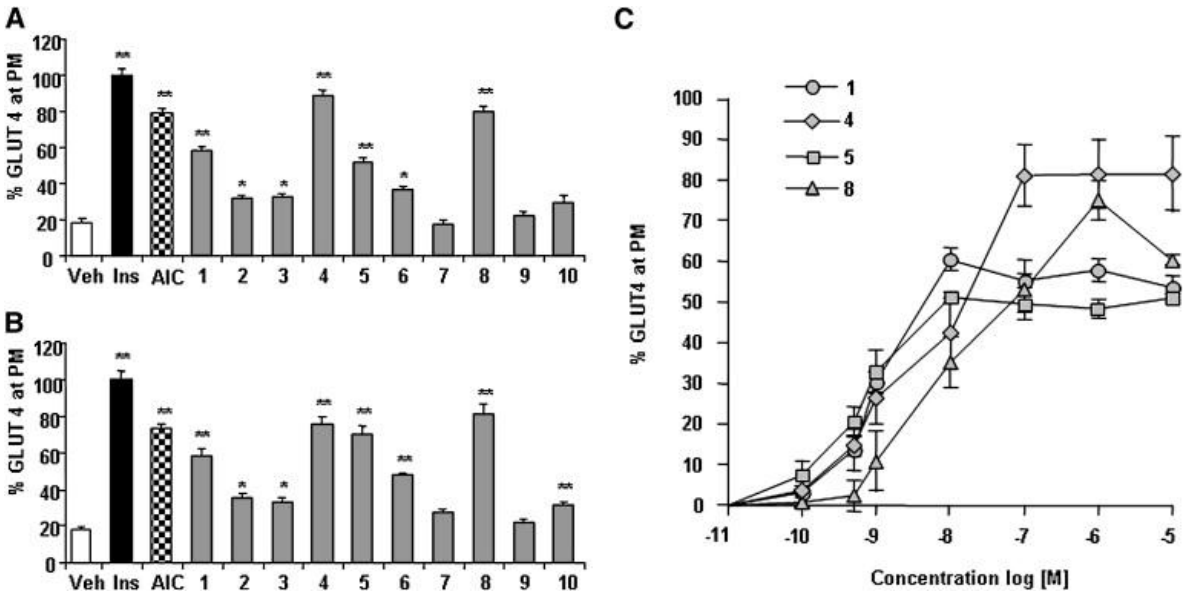

Figure 2 : Effect of Cucurbitane Triterpenoids on GLUT4 Translocation in CellsL6 myotubes (A) or 3T3-L1 adipocytes (B) were incubated with vehicle (Veh, DMSO containing saline, final concentration of DMSO: 0.2%), 100 nM insulin (In), 2 mM AICAR (AIC), or test compounds (10 μM) for 30 min, and GLUT4 translocation from the cytosol to plasma membrane (PM) was measured as described in the Experimental Procedures. The dose-response curve was constructed in 3T3-L1 adipocytes for each compound at concentrations from 0.1 nM to 10 μM (C) The results were quantified as a percentage of the maximum effect of insulin (100%) and expressed as means ± SE. Three to four independent experiments were performed for each compound. $*p < 0.05$, $**p < 0.01$ versus Veh control.

GLUT4 translocation is mainly regulated by two independent pathways: the insulin signaling pathway and the AMP-activated protein kinase (AMPK) pathway (Huang and Czech, 2007). The phosphatidyl inositol 3′ kinase (PI3K)/Akt pathway is known to play a major regulatory role in the insulin action pathway, and PI3K inhibitors such as wortmannin, inhibit insulin stimulated GLUT4 translocation. The stimulatory effects of cucurbitane triterpenoids on GLUT4 translocation were not affected by wortmannin (Figure 3A). Moreover, in contrast to that observed with insulin, these compounds had no significant effect on phosphorylation of Akt phosphorylation, a downstream substrate of PI3K (Figure 3B).These data indicate that the stimulatory effects of cucurbitane triterpenoids on GLUT4 translocation are not likely to be mediated via the PI3K/Akt pathway.

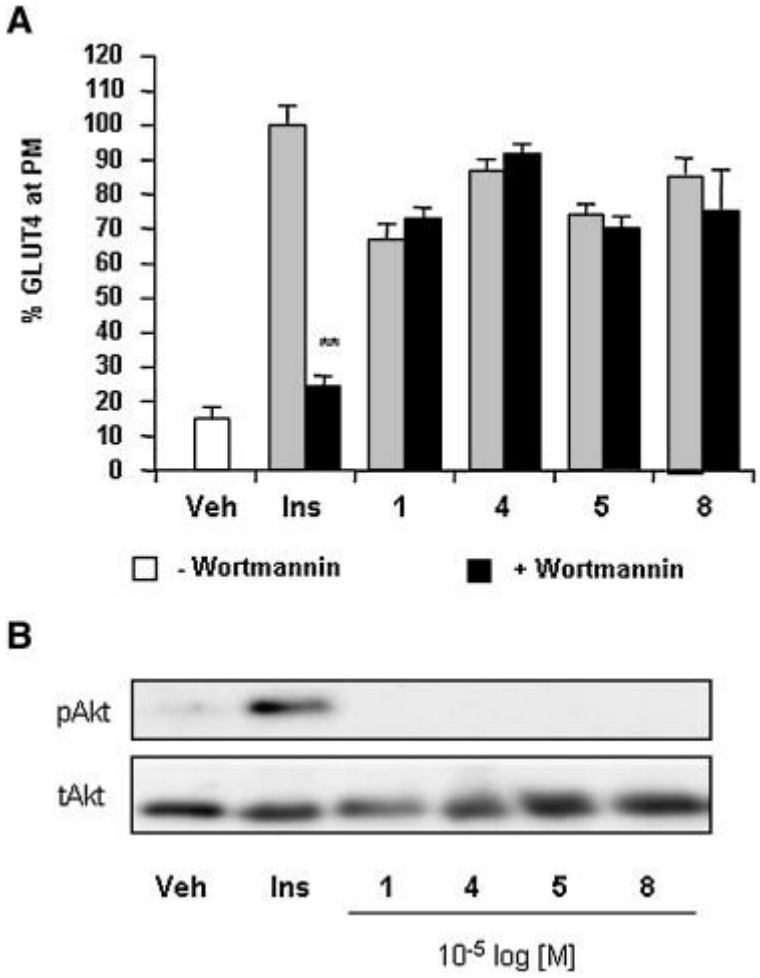

Figure 3 : Effects of Compounds 1, 4, 5, and 8 on Insulin Signaling PathwaysExperiments were performed in 3T3-L1 adipocytes and values represent means ± SE. Cells were incubated with 100 nM insulin, 10 μM test compounds in the presence of vehicle (Veh, DMSO containing saline, final concentration of DMSO: 0.2%) or 10 μM wortmanin.(A) GLUT4 transduction during inhibition of insulin signal transduction from three to four independent experiments (*p < 0.05, **p < 0.01 versus Veh).(B) Immunoblots for phospho-Akt (Ser473) and Akt in 3T3-L1 adipocytes (representative blots of three repeats). Cells were incubated with Veh, 100 nM insulin or test compounds (at 10 μM each) for 30 min.

The stress kinase, AMPK, has also been shown to regulate GLUT4 translocation (Huang and Czech, 2007), and hence we investigated whether the cucurbitane triterpenoids activate AMPK. In 3T3-L1 adipocytes we observed increased phosphorylation (Thr172) of AMPK with compounds 1, 4, 5, and 8 (Figure 4A) to a level comparable with the well-

described AMPK agonist AICAR (Figure 4B). Consistent with our data for GLUT4 translocation, AMPK phosphorylation was not induced by these compounds at a concentration of 0.1 nM, but was elevated at 100 nM to 10 µM (Figure 4B). We also observed increased AMPK phosphorylation in L6 myotubes by compounds 1, 4, 5, and 8, again to a relatively similar level to AICAR (Figures 4C and 4D)

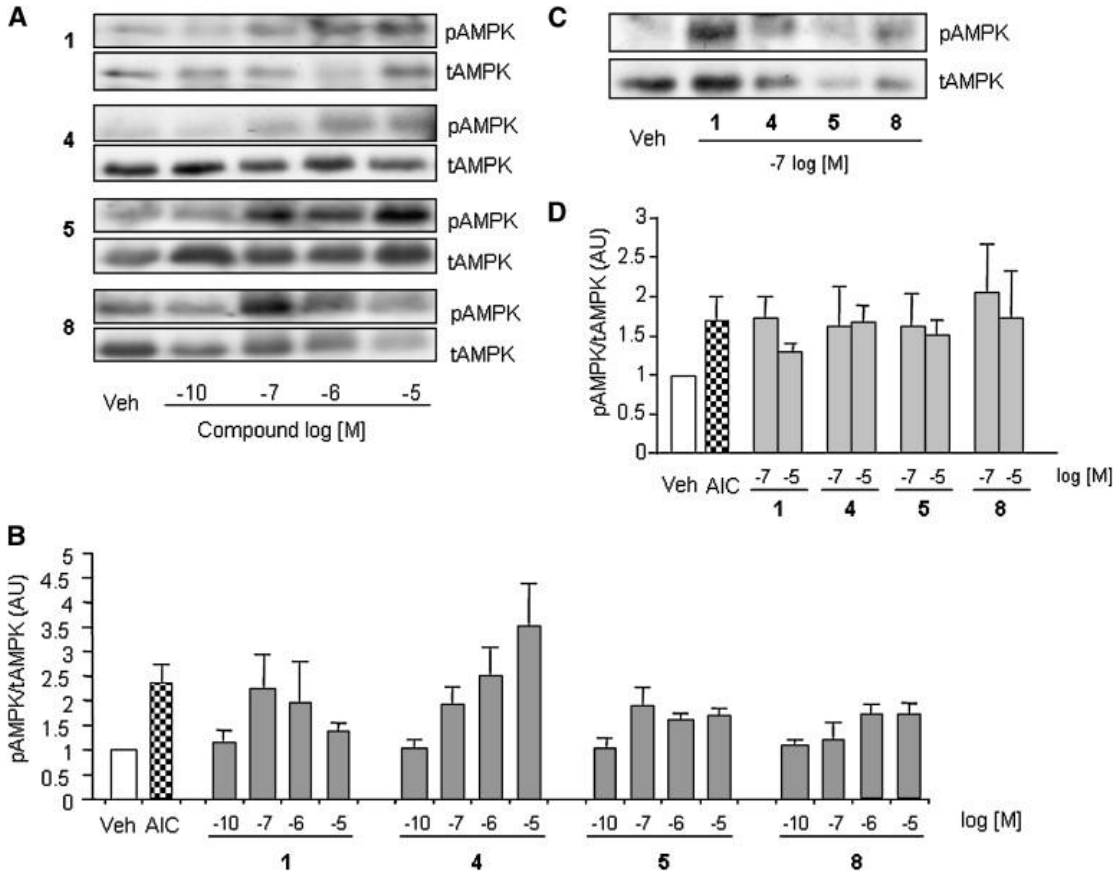

Figure 4 : Effects of Compounds 1, 4, 5, and 8 on AMPK Phosphorylation3T3-L1 and L6 cells were treated with test compounds as described in the Experimental Procedures and equal amounts of lysates were resolved by SDS-PAGE and immunoblotted for AMPK and phospho-AMPK (Thr172).(A) Representative blots for compounds 1, 4, 5 and 8 in 3T3-L1 adipocytes.(B) Quantification of the ratio of phospho-AMPK to total AMPK from three independent experiments (means ± SE).(C) Representative blots for compounds 1, 4, 5 and 8 in L6 myotubes at 0.1 µM.(D) Quantification of the ratio of phospho-AMPK to total AMPK from three independent experiments in L6 myotubes (means ± SE).

2.3 Assessment of Antidiabetes Properties in Animals

On the basis of these in vitro data, we examined whether this class of compounds had any beneficial effects on glucose and fuel metabolism in vivo. For these studies, we restricted our analysis to compounds 5 and/or 6 because we were not able to isolate the other compounds in sufficient quantity for in vivo administration. We first examined whole-body energy expenditure and fat oxidation in the resting state. The results (Figure 5) showed a small increase in energy expenditure (indicated by increased oxygen consumption, VO_2) at around 30 min and 7.5 hr after the administration of compound 5 (100 mg/kg) as compared to vehicle (0.9% saline). Strikingly, there was a substantial increase in fat oxidation (indicated by decreased respiratory exchange ratio, RER) with compound 5 during this entire period of time. These effects are comparable to those induced by AICAR at 250 mg/kg.

We further examined the effects of compounds 5 and 6 on whole-body glucose metabolism. Compound 5 had no significant effect on basal blood glucose levels (Figure 6A). However, we did observe a significant increase in glucose clearance during an intraperitoneal glucose tolerance test. Under the same conditions, AICAR at a dose of 500 mg/kg (but not at 250 mg/kg; data not shown) had a modest effect on glucose tolerance. Thus, these data indicate that the cucurbitane triterpenoids are significantly more potent in vivo than a traditional AMPK agonist. Although metformin had a small effect to reduce blood glucose (from 6.30 ± 0.17 to 5.27 ± 0.21 mM, $p < 0.05$, $n = 8$) at the tolerable dose (200 mg/kg), it did not have any significant effect on glucose disappearance during the ipGTT. In order to examine whether cucurbitane triterpenoids also acutely improve glucose tolerance in the insulin-resistant state, we tested the effect of compound 6 (10 mg/kg) in high-fat-fed mice. As shown in Figure 6B, compound 6 resulted in a significant improvement in glucose tolerance in high-fat-fed animals.

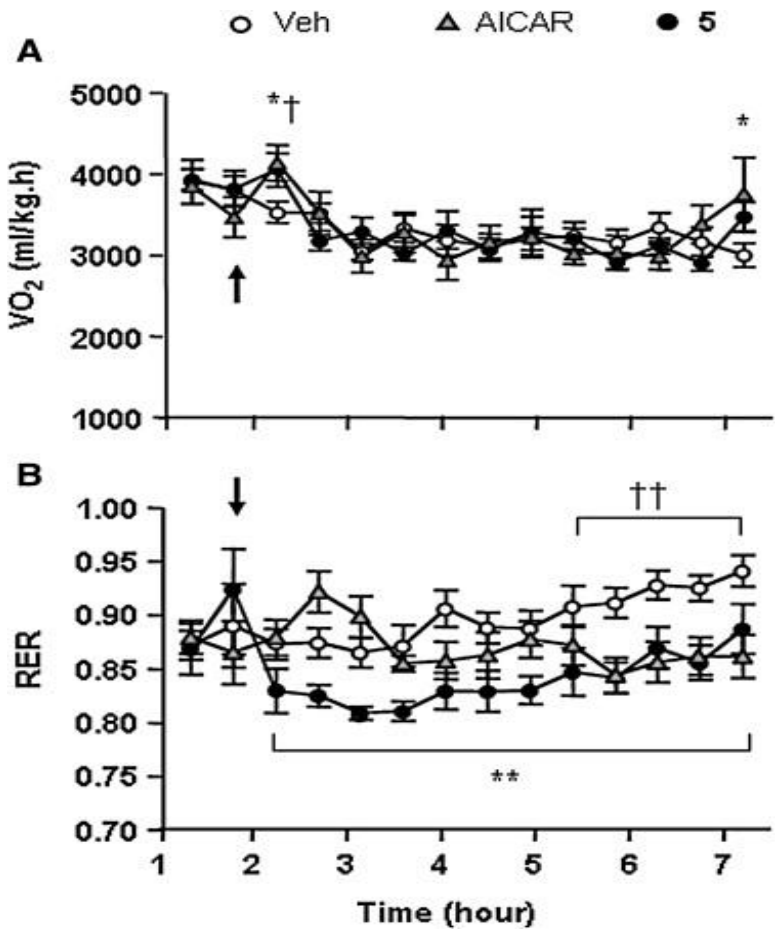

Figure 5 : Effects of Compound 5 on Whole-Body VO_2 and RER in Mice(A and B) Mice were placed in a metabolic chamber at 9:00 a.m. and after 2 hr of rest compound 5 (100 mg/kg), AICAR (250 mg/kg), or saline (Veh) was injected subcutaneously into the mice. *p < 0.05, **p < 0.01% versus Veh; † p < 0.05, †† p < 0.01 5 versus Veh (n = 6–8/group).

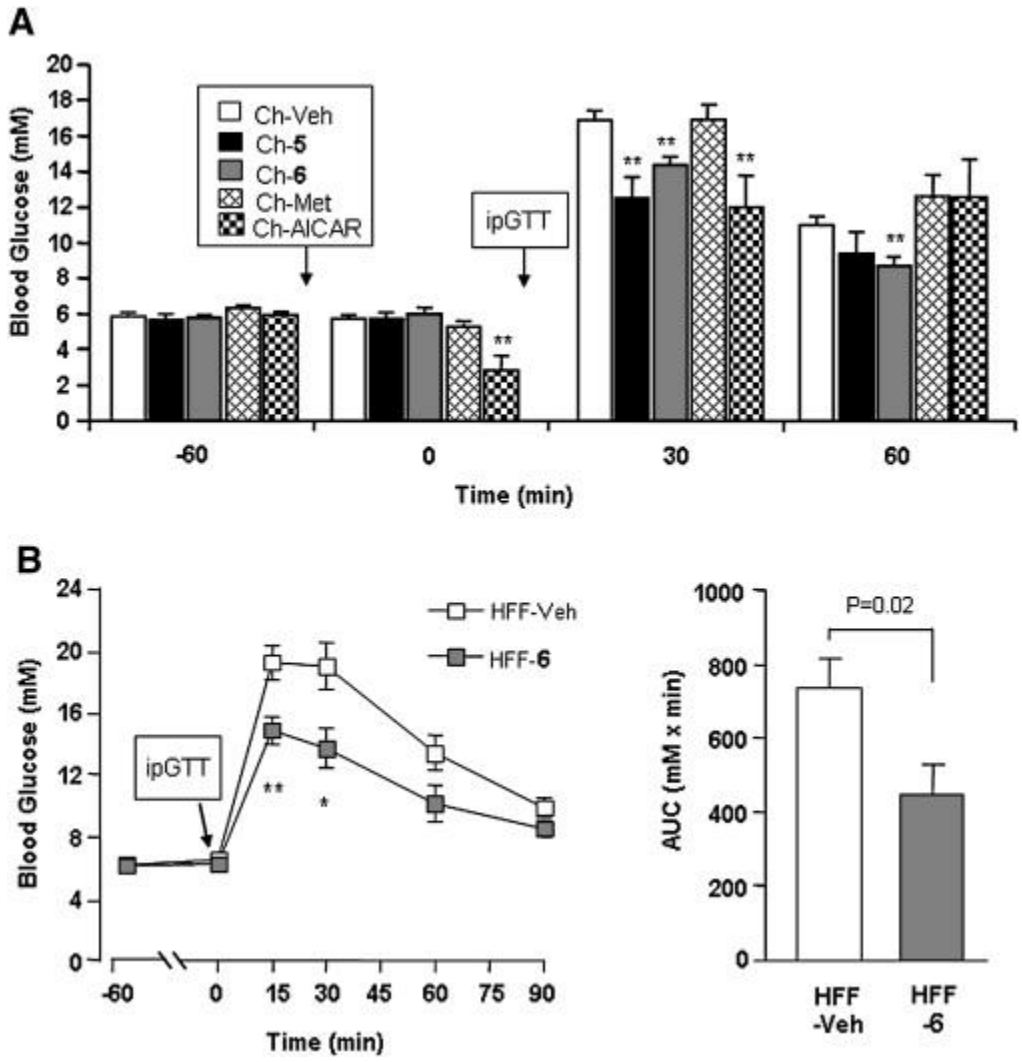

Figure 6 : Acute Effects of Compounds 5 and 6 on Blood Glucose in Mice(A) Standard chow-fed (Ch) mice fasted for 5-7 hr were used for the experiments. Following a basal blood sample at −60 min, vehicle (100 µl of 15% glycerol, 5% ethanol and 80% saline), compound 5 (100 mg/kg), compound 6 (at a lower dose: 10 mg/kg), AICAR (500 mg/kg), or metformin (200 mg/kg) was injected intraperitoneally (ip). A second sample of blood was taken around 0 min to assess followed an immediate ip glucose tolerance test (ipGTT) at a glucose load of 3.0 g/kg in insulin sensitive Ch-fed mice.(B) The effect on blood glucose in insulin-resistant, high-fat-fed (HFF) mice. The ipGTT was performed at a glucose load of 2.0 g/kg. *p < 0.05, **p < 0.01 versus corresponding vehicle controls (n = 7–9/group).

3. Discussion

Bitter melon is one of the most popular dietary botanicals for the treatment of diabetes mellitus, though the responsible active components are not clearly elucidated. It has been widely reported that its antidiabetic metabolites are the mixture of polypeptides, glycosides, alkaloids, and sterols. Earlier studies appear to indicate that polypeptide-p of *M. charantia* has hypoglycemic effects in gerbils, langurs, and human (Khanna et al., 1981). Recent studies have suggested the antidiabetic properties of bitter melon extracts in insulin-target tissues such as skeletal muscle and adipose tissues (Grover and Yadav, 2004). However, the antidiabetic activity of cucurbitane triterpenoids, the characteristic chemical constituents of *M. charantia*, is not well defined. Only two cucurbitane triterpenes were reported to show blood hypoglycemic effects in the alloxan-injected mice at 400 mg/kg (Harinantenaina et al., 2006). However, it should be noted that the diabetic animal model used in this study was very mild. Moreover, the biologic mechanisms involved in their antidiabetic properties are not clear. Particularly, our study focused on the structure identification, and biological evaluation of four novel cucurbitane glycosides, momordicosides Q, R, S, T (2, 3, 5, 6), karaviloside XI (1), and their aglycones.

We have revealed two classes of cucurbitane triterpenoids (1–10), both of which stimulate GLUT4 translocation in adipocytes and muscle cells and both of which stimulate the activity of the AMPK pathway. Among these compounds, we have identified two glycosides, karaviloside XI (1) and momordicoside S (5), which shows the most significant potency. Interestingly, aglycones of these two compounds also show similar biologic properties, which suggest that cucurbitane aglycones may be the important pharmacophore for the antidiabetic activity.

To screen the antidiabetic properties of these compounds, we chose GLUT4 translocation in muscle cells and adipocytes as the primary system. This screen measures the movement of the insulin responsive glucose transporter GLUT4 to the cell surface, an essential step for insulin-responsive glucose transport in muscle and adipose tissue that becomes defective in insulin resistance (Huang and Czech, 2007). We found that most of the tested compounds in the cucurbitane triterpenoid class, particularly 1, 4, 5, and 8, exhibited a strong effect to stimulate GLUT4 translocation by several fold in both cell

types to a level that was comparable to maximal insulin and AICAR stimulation. Of particular note, the concentration required for compounds 1, 4, 5, and 8 to reach their maximal effect was approximately 10,000 times lower compared to AICAR (0.1 uM for 1, 4, 5, and 8 versus 1–2 mM for AICAR). These results indicate that cucurbitane triterpenoids are highly potent and efficacious in stimulating GLUT4 translocation in insulin responsive cells.

To investigate the mechanism responsible for the stimulated GLUT4 translocation by these compounds, we examined their effects on the cellular signaling pathways that are known to mediate this process. Our findings clearly demonstrate that in contrast to previous studies (Grover and Yadav, 2004), these compounds do not activate the PI3K/Akt pathway in cells and their ability to stimulate GLUT4 translocation was insensitive to the PI3K inhibitor wortmannin. We further investigated whether the AMPK pathway is involved because this pathway is another major regulator of GLUT4 translocation during exercise or in response to some antidiabetic agents such as AICAR and metformin (Huang and Czech, 2007). Indeed, these triterpenoids were able to increase the phosphorylation of AMPK to a relatively similar level to AICAR, suggesting that the AMPK signaling pathway is likely responsible for the stimulation of GLUT4 translocation by this class of compounds.

The identification of the AMPK pathway as a likely mechanism for the stimulation of GLUT4 translocation by triterpenoids from *M. charantia* is particularly interesting in relation to diabetes and obesity because activation of AMPK increases fatty acid oxidation, inhibits lipid synthesis, and can improve insulin action ([Ye et al., 2005] and [Iglesias et al., 2002]). Based on a number of studies showing that AMPK regulates a variety of different metabolic pathways, it is widely recognized as a useful and safe target for the treatment of metabolic disorders such as T2D and dyslipidemia ([Ye et al., 2005] and [Musi, 2006]). Hence, our findings of the activation of the AMPK pathway by these compounds may implicate these triterpenoids as a novel class of molecules with therapeutic potential for insulin resistant states by targeting AMPK.

One of the difficulties in pursuing the mechanism of action of these isolated compounds at the present stage is their limited availability due to the complicated secondary metabolites from *M. charantia*. Despite this, we were able to scale up

isolation of S (5) and T (6) to produce amounts sufficient for limited acute studies in animals. Consistent with the in vitro studies implicating the AMPK pathway, we observed that momordicoside S (5) was able to stimulate whole-body fat oxidation in mice, with a minor increase in energy expenditure. The AMPK activator AICAR has been shown to acutely lower plasma glucose and ameliorate insulin resistance in high-fat-fed rats (Iglesias et al., 2002). Based on our findings described above, we predicted that cucurbitane triterpenoids may have similar effects on glucose metabolism in vivo. Indeed, we found that both momordicoside S (5) and T (6) significantly enhanced glucose tolerance in normal mice, and the efficacy was comparable to that produced by AICAR at a 5- to 50-fold higher dose. Interestingly, neither a tolerable intraperitoneal dose of metformin nor a reduced dose of AICAR (250 mg/kg; data not shown) had any detectable effect on glucose tolerance. These findings are consistent with the results on GLUT4 translocation and AMPK activity in vitro and show that cucurbitane triterpenoids identified in the present study have increased potency to improve glucose tolerance in mice compared with commonly used AMPK activators AICAR or metformin. Compound 6 was also able to acutely improve glucose tolerance in high-fat-fed mice, suggesting therapeutic potential of these cucurbitane triterpenoids for the treatment of insulin resistance. Based on their in vitro effects, we suspect that other compounds of this class may have similar effects.

It is intriguing that many compounds that appear to have beneficial effects in the treatment of insulin resistance do so at least in part via activating AMPK activity. In particular, metformin (Zhou et al., 2001) and berberine (Lee et al., 2006), two plant-derived compounds that have been described to increase insulin sensitivity and reduce body weight, both activate AMPK. Interestingly, both metformin and berberine have also been described as weak mitochondrial poisons, and so it is believed that elevated intracellular AMP levels ensuing as a function of reduced mitochondrial respiration may trigger increased AMPK activity ([Brunmair et al., 2004] and [Pereira et al., 2007]). In this context, it is interesting that the upstream AMPK kinase, LKB1, is required for AMPK activation by metformin and its subsequent therapeutic affects in vivo (Shaw et al., 2005). We have observed no activation of AMPK by compounds 1, 4, 5, and 8 in HeLa cells (data not shown) which lack LKB1, suggesting that these triterpenoid compounds may activate AMPK in a similar fashion to metformin. However, it is important to mention that we have not observed any toxic effects of these compounds in either cell in culture

or in animals, suggesting that if they do affect mitochondrial integrity, these effects are likely to be quite mild.

In view of the potency of the cucurbitane triterpenoids on glucose metabolism, it will be of great interest to determine the primary targets of these compounds leading to the activation of AMPK. One possibility might be that they act at intracellular target(s) after entering cells by some active transport system as the structural features of glycosides may render them as substrates for naturally occurring transporters (Lipinski et al., 2001). For example, cardiac sodium pumps are the receptors for classical cardiac glycosides such as digitalis, and there is a report which shows that ginsenoside Rg1 can be transported into epithelial cells with peak concentration of 1.28 mg/10^5 cells at 0.5 hr (Meng et al., 2007). Alternatively, cucurbitane triterpenoids may bind to cell-surface receptors, initiating an intracellular signaling pathway analogous to that seen with other biological molecules such as leptin and adiponectin. Future studies will be required to distinguish between these possibilities.

4. Significance

The structures and absolute configurations of four novel cucurbitane glycosides, momordicosides Q, R, S, and T, and karaviloside XI (1) were elucidated on the basis of spectroscopic data, chemical degradation, and X-ray diffraction analyses. Momordicoside S (5), karaviloside XI (1), and their aglycones (4 and 8), exhibited a number of biologic effects in cells. In both L6 myotubes and 3T3-L1 adipocytes, they stimulated GLUT4 translocation from cytosol to the cell membrane. This effect was associated with an increase in the activity of AMP-activated protein kinase. Consistent with these in vitro effects, administration of momordicoside S (5) into mice significantly enhanced glucose disposal from the circulation and promoted fatty acid oxidation. Additionally, we have demonstrated significant amelioration of glucose intolerance by momordicoside T (6). Based on our review of the literature, there are approximately 70 reported cucurbitane triterpenoids, and our UPLC/ESIMS analyses of the extracts of bitter melon suggest their high abundance in this plant. Along with this, their extremely high potency indicates that cucurbitane triterpenoids are likely to be major contributors to the antidiabetic effects of bitter melon. Importantly, we have identified AMPK as a potential mediator of the cucurbitane triterpenoids for their stimulation of GLUT4 translocation in

muscle and fat cells. The present study provides an important basis for further analysis of structure-activity relationship to develop optimized leads from cucurbitane triterpenoids for the treatment of insulin resistance and obesity.

5. Experimental Procedures

5.1 General Experimental Procedures

Optical rotations were taken on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on Nicolet Magna FT-IR 750 spectrophotometer using KBr disks. NMR spectra were recorded on Bruker AM-400 and INVOR-600 NMR spectrometers. The chemical shift (δ) values are given in ppm with TMS as internal standard, and coupling constants (*J*) are in Hz. EIMS and HREIMS spectra were recorded on Finnigan MAT-95 mass spectrometer. ESIMS and HRESIMS spectra were recorded on Micromass LC-MS-MS mass spectrometer. Column chromatographic separations were carried out by using silica gel H60 (300–400 mesh, Qingdao Haiyang Chemical Group Corporation), MCI GEL CHP20P (75–150 μm, Mitsubishi Chemical Industries), and Sephadex LH-20 (Pharmcia Biotech AB) as packing materials. HSGF254 silica gel TLC plates (Yantai Chemical Industrial Institute) were used for analytical TLC. The Analytical HPLC system was composed of Waters 2690 separations module, Waters 996 diode array detector (Waters), and All-tech 2000 ELSD. A LinChrospher 100 RP-18e (Merck) column (125 × 4 mm i.d.; particle size 5 μm) was used for the separation. The Preparative HPLC system composed of two PrepStar SD-1 solvent delivery modules, a ProStar UV-Vis 320 detector, and a ProStar 701 Fraction Collector (Varian). A LinChrospher 100 RP-18 (Merck) column (220 × 25 mm i.d.; particle size 12 m) was used for isolation. Gas chromatography was carried out on a Shimadzu GC 14-BPF apparatus equipped with a 5% OV225/AW-DMCS-Chromosorb W (80–100 mesh) column (2.5 m × 3 mm) as well as a hydrogen-flame ionization detector.

5.2 Plant Materials

Fruits were purchased from a cultivation plant in Guangxi Province. Representative samples were deposited at the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China.

5.3 Extraction and Isolation

The freeze-dried fruit powder (75 kg) was extracted by maceration with 80% EtOH. After filtration and evaporation of the solvent under reduced pressure, the alcohol extract was partitioned successively with CH_2Cl_2 and *n*-BuOH. The *n*-BuOH-soluble extract (800 g) was subjected to macroporous resin column chromatography eluting with H_2O, 25% EtOH, 60% EtOH, and 95% EtOH to yield four fractions: KG5 (600 g), KG6 (59 g), KG7 (50 g), and KG8 (30 g). KG8 was subjected to normal phase silica gel column chromatography with gradient elution [$CHCl_3$-MeOH-H_2O 40:3:1 low layer (10 l), $CHCl_3$-MeOH-H_2O 20:3:1 low layer (10 l), $CHCl_3$-MeOH-H_2O 10:3:1 low layer (10 l), $CHCl_3$-MeOH-H_2O 8:3:1 low layer (8 l), $CHCl_3$-MeOH-H_2O 65:35:10 low layer (6 l)] to give six fractions (Fr1-6). Compound 1 (150 mg), compound 2 (80 mg), and compound 7 (150 mg) from Fr6 (5.5 g) were purified by repeated column chromatography over MCI gel, silica gel, and Sephadex LH-20, respectively. Further fractionation of KG7 by normal phase silica gel column chromatography with gradient elution [$CHCl_3$-MeOH-H_2O 20:3:1 low layer (10 l), $CHCl_3$-MeOH-H_2O 10:3:1 low layer (10 l), $CHCl_3$-MeOH-H_2O 8:3:1 low layer (8 l), $CHCl_3$-MeOH-H_2O 65:35:10 low layer (10 l), $CHCl_3$-MeOH-H_2O 6:4:1 (6 l)] gave six fractions (Fr7-12). Compound 5 (160 mg) and compound 6 (220 mg) from Fr11 (6.5 g) were isolated by repeated column chromatography over MCI gel, silica gel and Sephadex LH-20. Compound 3 (18 mg) from Fr8 (6.5 g) were separated by repeated column chromatography over MCI gel, silica gel, Sephadex LH-20, and preparative HPLC (MeOH–H_2O, 60:40-85:15). Momordicosides A (350 mg) and B (250 mg) from Fr9 (4.3 g) were isolated by repeated normal phase silica gel.

5.4 Acid Hydrolysis of Compounds 1, 2, 3, 5, 6 and 7

Compound 1 (100 mg) in 2% HCl-dioxane (1:1, 25 ml) were heated at 40°C for 7 days in a water bath. The reaction mixtures were neutralized with Ag_2CO_3, filtered, and then extracted with $CHCl_3$. Aglycone 4 (25 mg) was obtained from $CHCl_3$ layer by column chromatography and crystallized in MeOH. Compounds 2, 3, 5, 6 and 7 (3 mg each) were refluxed with 2% HCl-dioxane (1:1, 25 ml) at 90°C for 4 hr. The reaction mixtures were neutralized with Ag_2CO_3, filtered, and then extracted with $CHCl_3$. The aqueous layer was evaporated, and then the residue was treated with L-cysteine methyl ester hydrochloride (4 mg) in pyridine (0.5 ml) at 60°C for 1 hr. After reaction, the solution was treated with

acetic anhydride (3 ml) at 60°C for 1 hr. Authentic samples were prepared by the same procedure. The acetate derivatives were subjected to GC analysis to identify the sugars (column temperature 210°C; injection temperature 250°C; carrier gas N_2 at a flow rate of 25 ml/min). D-allose (t_R 5.0 min) was observed from 1 and 3; D-glucose (t_R 1.8 min) was observed from 2, 3, 5, 6 and 7; D-xylose (t_R 4.0 min) was observed from 6.

5.5 Enzymatic Hydrolysis of Compound 5

Compound **5** (60 mg) and momordicoside A (40 mg) were treated with cellulose in 0.1 M acetate buffer solution at 37°C for 7 days. The reaction mixtures were then extracted with $CHCl_3$. Aglycone **8** was obtained from both $CHCl_3$ layers by preparative TLC.

5.6 Spectroscopic Data of Compounds 2, 3, 5, 6, 7

Momordicoside Q (2)

Amorphous powder; $[\alpha]D^{23}$ = −76 (c 0.1600, MeOH); IR ν_{max} (KBr) 3396, 3165, 2935, 2783, 1612, 1514, 1454, 1417, 1277, 1263, 1223, 1165, 1122, 1039, 814, 689 cm^{-1}; ^1H NMR and ^{13}C NMR data see Table 1; HRESIMS *m/z* 675.4113 (calcd for $C_{36}H_{60}O_{10}Na$ $[M+Na]^+$, 675. 4084).

Table 1 : ^{13}C and ^1H NMR Data of Compounds **2**, **3**, **5–7** in C_5D_5N

	δ_C					δ_H				
	2[a]	**3**[b]	**5**[b]	**6**[b]	**7**[a]	**2**[c]	**3**[d]	**5**[d]	**6**[d]	**7**[c]
1	18.9	18.5	22.6	22.5	22.6	1.30 1.78	1.31 1.72	1.52 1.78	1.46 1.78	1.58 1.76
2	27.6	27.2	29.1	29.0	29.1	1.76 2.36	1.74 2.32	1.90 2.50	1.20 2.38	1.92 2.50
3	85.4	85.2	87.3	87.3	87.7	3.70 br s	3.64 br s	3.53 br s	3.66 br s	3.86 br s
4	39.1	38.6	41.6	41.6	41.8					
5	85.8	85.8	143.2	143.1	143.2					

	δ_C					δ_H				
	2[a]	**3**[b]	**5**[b]	**6**[b]	**7**[a]	**2**[c]	**3**[d]	**5**[d]	**6**[d]	**7**[c]
6	134.2	133.8	118.6	118.7	118.7	6.21 (10.5)	6.14 dd (1.3 8.3)	5.37 d (4.4)	5.42 d (4.2)	5.4 d (4.8)
7	130.0	130.1	24.5	24.5	24.6	5.54 dd (3.7 9.0)	5.5 dd (3.3 9.8)	1.62 2.18	1.70 2.24	1.64 2.27
8	52.3	51.9	43.7	43.7	43.8	2.25	2.26	1.58	1.66	1.60
9	45.3	44.9	34.6	34.6	34.7					
10	40.1	39.7	38.4	38.3	38.5	2.25	2.24	2.16	2.22	2.18
11	23.9	23.5	32.4	32.4	32.5	1.30 1.58	1.29 1.58	1.28 1.52	1.34 1.56	1.32 1.54
12	31.2	30.7	30.7	30.6	30.8	1.521.56	1.44 1.54	1.46 1.54	1.52 1.60	1.48 1.58
13	45.6	45.2	46.7	47.3	46.8					
14	48.9	48.6	49.0	49.1	49.2					
15	33.3	32.9	35.2	35.2	35.3	1.101.15	1.09 1.14	1.04 1.14	1.12 1.22	1.15 1.23
16	28.5	28.1	27.6	27.7	27.8	1.381.92	1.36 1.86	1.48 2.34	1.56 2.42	1.58 2.48
17	51.6	51.2	47.8	47.7	47.7	1.48	1.44	1.95	2.00	2.02
18	15.0	14.6	15.4	15.3	15.3	0.77 s	0.74 s	0.82 s	0.83 s	0.89 s
19	80.1	79.8	28.1	28.0	28.2	3.57 d (8.3) 3.7 d (8.3)	3.56 d (7.7) 3.74 d (7.7)	0.80 s	0.81 s	0.85 s
20	32.7	32.3	42.8	42.8	43.1	2.09	2.04	2.14	2.20	2.22

	δ_C					δ_H				
	2[a]	**3**[b]	**5**[b]	**6**[b]	**7**[a]	**2**[c]	**3**[d]	**5**[d]	**6**[d]	**7**[c]
21	18.9	18.5	14.9	14.9	14.8	1.12 d (6.1)	1.06 d (6.0)	1.38 d (6.6)	1.37 d (6.6)	1.44 d (6.6)
22	43.3	42.3	71.3	71.3	72.5	1.22 2.38	1.14 2.26	4.59 d (3.6)	4.66	4.60
23	67.7	66.6	71.4	71.3	71.2	4.59	4.48	4.18	4.24	4.14
24	79.9	79.7	74.1	74.1	75.4	3.56	3.61	4.28	4.36	4.46
25	73.6	81.0	81.5	81.5	74.4	1.65 s	1.72 s	1.81 s	1.82 s	1.70 s
26	28.0	23.0	23.2	23.3	24.2	1.67 s	1.73 s	1.70 s	1.71 s	1.68 s
27	27.0	24.6	24.0	24.1	29.1	1.56 s	1.45 s	1.43 s	1.44 s	1.53 s
28	21.1	20.5	25.8	25.7	26.0	0.87s	0.86 s	1.00 s	1.03 s	1.09 s
29	25.6	25.2	28.4	28.4	28.5	0.93 s	0.83 s	0.78 s	0.78 s	0.83 s
30	20.2	19.8	18.1	18.0	18.1	1.12 d (6.1)	1.06 d (6.0)	1.38 d (6.6)	1.37 d (6.6)	1.44 d (6.6)
1′	106.7	104.0	106.9	106.8	107.4	4.95 (8.1)	5.34 d (7.7)	4.75 d (7.8)	4.71 d (7.8)	4.9 d (7.7)
2′	75.8	72.7	75.2	76.2	75.5	4.02	3.92	3.84	4.12	3.98
3′	78.3	72.3	78.4	74.8	78.7	4.03	4.67	3.89	3.81	4.00
4′	71.9	68.9	71.5	80.0	71.8	4.25	4.14	4.05	4.28	4.22
5′	78.4	75.8	77.3	75.0	78.2	4.026	4.44	4.02	4.00	4.24
6′	63.1	62.9	70.1	68.4	63.1	4.45 4.58	4.32 4.50	4.24 4.76	4.62 4.84	4.4 4.59

	δ_C					δ_H				
	2[a]	**3**[b]	**5**[b]	**6**[b]	**7**[a]	**2**[c]	**3**[d]	**5**[d]	**6**[d]	**7**[c]
1″		98.5	105.1	105.1			5.21 d (7.8)	5.12 d (7.4)	5.29 d (6.6)	
2″		75.0	75.2	75.0			3.96	3.98	4.00	
3″		78.5	78.4	78.4			4.22	3.90	3.96	
4″		71.5	71.5	71.4			4.16	4.18	4.14	
5″		78.0	78.3	78.4			3.93	4.18	4.18	
6″		62.5	62.6	62.6			4.32 4.52	4.30 4.47	4.34 4.54	
1‴			97.6	105.1				5.09 d (7.3)	5.30 d (7.2)	
2‴			75.1	75.0				4.00	3.94	
3‴			78.4	78.4				3.79	4.24	
4‴			71.4	70.8				3.91	4.16	
5‴			78.7	67.1				4.10	3.85 4.17	
6‴			62.6					4.20 4.40		
1⁗				97.6					5.10 d (7.8)	
2⁗				75.2					4.00	
3⁗				78.4					3.84	
4⁗				71.4					4.14	
5⁗				78.7					4.16	
6⁗				62.6					4.28 4.46	

ppm, *J* in Hz., [a]100 MHz. , [b]150 MHz. , [c]400 MHz. , [d]600 MHz.

Momordicoside R (3)

Amorphous powder; $[\alpha]_D^{23}$ = −64 (*c* 0.188, MeOH); IR ν_{max} (KBr) 3415,2928, 2874, 1643, 1466, 1377, 1155, 1082, 1032 cm^{-1}; ^1H NMR and ^{13}C NMR data see Table 1; HRESIMS *m*/z 6837.4632 (calcd for $C_{42}H_{70}O_{15}Na$ [*M*+Na]$^+$, 837.4612).

Momordicoside R (5)

Amorphous powder; $[\alpha]_D^{23}$ = −8 (*c* 0.2245, MeOH); IR ν_{max} (KBr) 3406, 2933, 2873, 1646, 1551, 1452, 1383, 1306, 1076, 1038, 534; ^1H NMR and ^{13}C NMR data see Table 1; HRESIMS *m*/z 1001.5253 (calcd for $C_{48}H_{82}O_{20}Na$ [*M*+Na]$^+$, 1001.5297).

Momordicoside R (6)

Amorphous powder; $[\alpha]_D^{23}$ = −1 (*c* 0.1385, MeOH); IR ν_{max} (KBr) 3408, 2931, 2857, 1639, 1468, 1381, 1308, 1163, 1076, 1039 cm^{-1}; ^1H NMR and ^{13}C NMR data see Table 1; HRESIMS *m*/z 1113.5714 (calcd for $C_{53}H_{90}O_{24}Na$ [*M*+Na]$^+$, 1113.5720).

Momordicoside R (7)

Amorphous powder; $[\alpha]_D^{23}$ = −20 (*c* 0.1600, MeOH); IR ν_{max} (KBr) 3406, 2949, 2875, 1633, 1468, 1381, 1167, 1076, 1036, 951, 619 cm^{-1}; ^1H NMR and ^{13}C NMR data see Table 1; HRESIMS *m*/z 677.4224 (calcd for $C_{36}H_{62}O_{10}Na$ [*M*+Na]$^+$, 677.4241).

5.7 X-ray Crystallographic Data for Compound 4

$C_{30}H_{56}O_8$ + 3 H_2O, mol. wt. = 544.75, orthorhombic space group $P2_12_12_1$, *a* = 6.33550(12) Å, *b* = 13.7125(2) Å, *c* = 35.1964(7) Å, *V* = 3057.71(18) $Å^3$, *Z* = 4, *d* = 1.183 g/cm^3. *F*(000) = 1200, μ = 0.676 mm^{-1}. A single crystal of dimensions 0.12 × 0.03 × 0.02 mm was used for X-ray measurements. The data collection was performed on a Gemini R Ultra diffractometer using Cu-Ka-radiation. Data were collected up to θ = 65.60° at 100 K. A total of 5059, thereof 4908, independent reflections were measured, giving a Rint of 0.0312. Programs used were Data collection and reduction Crysalis Version 1.171.35. Crystal structure solution and refinement was achieved using direct methods as implemented in SHELXTL Version 6.12 and visualized using XP program. 359 Parameters were refined using 4908 reflections with $F_0 > 4\sigma(F_o)$ giving R1 = 0.0469, wR2 = 0.1050,

Goodness of Fit 1.144, remaining electron density 0.247 and −0.217 e^{-} $Å^{-3}$. The absolute structure could be determined with high probability giving a Flack × Parameter of −0.0505 (0.2077), the result is confirmed by the analysis of Bijvoet pais implemented in Platon. CCDC 654600 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge viahttp://www.ccdc.cam.ac.uk/conts/retrieving.html.

5.8 Cell Culture

L6 myoblasts up to passage 15 were cultured in a-minimal essential medium (a-MEM) supplemented with 10% heat-inactivated fetal calf serum (FCS) at 37°C in 5% CO_2. For differentiation into myotubes, cells were cultured in a-MEM supplemented with 2% heat-inactivated FCS at 37°C in 5% CO_2 and were maintained in this medium postdifferentiation. Myotubes were used for experiments 5–7 days after differentiation. 3T3-L1 cells were cultured at 37°C in Dulbecco's modified Eagle's medium (DMEM) containing 10% bovine calf serum (BCS) in an atmosphere of 10% CO_2. The differentiation of 3T3-L1 cells was induced as described previously (Govers et al., 2004). Briefly, the confluent cells were incubated for 2 days in DMEM that was supplemented with 10% fetal bovine serum (FBS), 0.5 mM 3-isobutyl-1-methylxanthine, 1 µM dexamethasone, and 5 µg/Ml insulin. Thereafter, the medium was replaced every other day with DMEM containing 10% FBS and 5 µg/Ml insulin.

5.9 GLUT4 Translocation Assay

HA-GLUT4 translocation to the PM was measured as previously described (Govers et al., 2004) with minor modifications. Briefly, cells were grown in black, clear-bottom, 96-well plates and starved for 2 hr in serum- and bicarbonate-free DMEM containing 20 mM HEPES (pH 7.4) and 0.2% BSA (DMEM/BSA) at 37°C before starting the experiment. Plates were then transferred to 19°C, and vehicle, 100 nM insulin, 2 mM AICAR, 10 µM wortmanin, or test compounds were added for 30 min. At given time points, paraformaldehyde was added to the wells to a concentration of 3%. After 15 min, the paraformaldehyde was quenched by the addition of glycine (final concentration, 50 mM). The cells were washed extensively and incubated for 20 min with 5% normal swine serum (NSS) in the absence or presence of 0.1% saponin to analyze the amount of HA-

GLUT4 at the PM or the total HA-GLUT4 content, respectively. Cells were incubated for 60 min with anti-HA or, as a control, a nonrelevant antibody (mouse IgG1-MOPC21) in PBS containing 2% NSS. Cells were extensively washed and incubated for 20 min in 5% NSS in the presence or absence of 0.1% saponin. Cells were then incubated with ALEXA488-conjugated goat-antimouse in PBS containing 2% NSS. After washing, fluorescence was measured using the bottom-reading mode in a fluorescence microtiter plate reader (FLUOstar Galaxy, BMG Labtechnologies).

5.10 Determination of Phosphorylation of Akt and AMPK

Differentiated 3T3-L1 and L6 cells were serum-starved in DMEM/BSA (2 hr at 37°C) prior to incubation either with test compounds (1, 4, 5, 8) or vehicle (DMSO containing saline, final concentration of DMSO: 0.2%) for 30 min, or with 100 nM Insulin or 2 mM AICAR for 25 min. Following treatment, cells were washed three times with ice-cold PBS and subsequently lysed in 1× RIPA buffer (50 mM Tris HCl [pH 8], 150 mM NaCl, 1% NP-40, 0.5% sodium Deoxycholate, 0.1% SDS) supplemented with Complete protease inhibitor cocktail (Roche) and phosphatase inhibitors [2 mM Na_3VO_4, 1 mM $Na_4P_2O_7$, 1 mM $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 10 mM NaF], then passed through a 22-gauge needle 10× and centrifuged at 20,000 × g for 20 min. Supernatant protein concentration was determined via BCA assay (Pierce). Equal amounts of protein were then diluted 4× in SDS sample buffer (62.5 mM Tris-HCl, 20% glycerol, 2% SDS, 75 µM DTT, and 0.05% bromophenol blue), subjected to SDS PAGE and immunoblotted with antibodies specific for Akt, phospho-Akt (Ser473), AMPK, and phospho-AMPK-a (Thr172) (Cell Signaling Technology). Immunoblots were quantified using Image J software (NIH) and expressed at a ratio of phosphorylation to total.

5.11 Experimental Animals

C57BL/6 mice (10 week old males) supplied by the Animal Resources Center (Perth, Australia) were acclimatized in communal cages at 22°C, with a 12 hr light, 12 hr dark cycle (lights on at 0700) for 1 week and had access to a standard chow diet (Gordon's Specialty Stock Feed) or a high (lard) fat diet for 7 weeks ad libitum to generate glucose intolerance similarly to a previous report (Iglesias et al., 2002). All experimental procedures were approved by the Garvan Institute Animal Experimentation Ethics

Committee, following guidelines issued by the National Health and Medical Research Council of Australia.

5.12 Determination of Whole-Body Energy Expenditure and Fat Oxidation

The oxygen consumption rate (VO_2) and CO_2 production rate was measured using an eight chamber indirect calorimeter (Oxymax series; Columbus Instruments) as described previously (Molero et al., 2006). The animals were placed in the metabolic chamber (20 cm × 10 cm × 12.5 cm) at 9:30 a.m. After 2 hr of acclimation, momordicoside S (**5**) (100 mg/kg), AICAR (250 mg/kg) or normal saline (vehicle) was injected subcutaneously. VO_2 was measured in individual mice at 27 min intervals over a 24 hr period under a consistent environmental temperature (22°C) and its values are proportional to energy expenditure. During the study, mice had ad libitum access to food and water. Respiratory exchange rate (RER) was calculated from VO_2 and CO_2 production and its values are in reverse proportion to whole-body fat oxidation.

5.13 Measurement of Blood Glucose Tolerance Test

The experiment was performed in mice after 5–7 hr of fasting. Blood glucose taken from the tail tip was measured using a glucosemeter (Accu-Chek, Roche). Following the measurement of basal blood glucose concentration at 0 min, vehicle solution (100 µl of 15% glycerol, 5% ethanol, and 80% saline), momordicodise S (**5**) (100 mg/kg), momordicodise T (**6**) (at a low dose: 10 mg/kg), AICAR (500 mg/kg), or metformin (200 mg/kg) was injected into the peritoneal cavity. Around 60 min, a sample was taken to assess the direct effect on blood glucose and this was immediately followed by an intraperitoneal glucose tolerance test (ipGTT). The glucose load was 3.0 g/kg for normal, chow-fed mice and 2.0 g/kg for insulin-resistant, high-fat-fed mice. These different glucose doses for different insulin sensitivity states were chosen to maximize the detection of treatment effects based on pilot experiments.

5.14 Data Analyses

An unpaired student t test was used for the comparison between treatments or groups and a p value ≤ 0.05 is considered statistically significant.

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Hypotriglyceridemic and hypocholesterolemic effect of anti-diabetic Momordica charantia (karela) fruit extract in streptozotocin-induced diabetic rats

I.Ahmed, M.S. Lakhani, M. Gillett, A. John, H. Raza*

Department of Anatomy and Biochemistry, Faculty of Medicine and Health Sciences, UAE University, P.O. Box 17666, Al Ain, United Arab Emirates

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Abstract

Momordica charantia (karela) is commonly used as an antidiabetic and antihyperglycemic agent in Asian, Oriental and Latin American countries. This study was undertaken to investigate the effects of long term feeding (10 weeks) of *M. charantia* fruit extract on blood plasma and tissue lipid profiles in normal and streptozotocin (STZ) – induced Type 1 diabetic rats. The results show that there was a significant ($p<0.05$) increase in plasma non-esterified cholesterol, triglycerides and phospholipids in STZ-induced diabetic rats, accompanied by a decrease in high density lipoprotein (HDL)–cholesterol. A moderate increase in plasma (LPO) product, malonedialdehyde (MDA), and about two-fold increase in kidney LPO was also observed in STZ-induced diabetic rats. The treatment of diabetic rats with *M. charantia* fruit extract over a 10-week period returned these levels close to normal. In addition, karela juice also exhibited an inhibitory effect on membrane LPO under in vitro conditions. These results suggest that *M. charantia* fruit extract exhibits hypolipidemic as well as hypoglycaemic effects in the STZ-induced diabetic rats.

1. Introduction

Treatment of hyperlipidemia in diabetes involves improving glycemic control, exercise and use of lipid lowering diets and drugs [1]. *Momordica charantia* (family, *Cucurbitaceae*), commonly known as karela or bitter melon, is used as a vegetable in the Indian subcontinent, South America and the Orient and the extract of the fruit pulp

and seed have been reported to have various medicinal properties, including antitumour and antimutagenic activities [2-4]. However, in Chinese and Ayurvedic traditional medicine, the plant is usually used as a hypoglycaemic and antidiabetic agent [5] and many components have been identified from *M. charantia* which posses hypoglycaemic properties [6]. Karela fruit juice has also been shown to stimulate, significantly, both glycogen storage by the liver [7] and insulin secretion by isolated β-cells of islets of Langerhans [8]. The hypoglycaemic activity of karela fruit has been shown in both spontaneous and chemically-induced diabetes mellitus in experimental animals as well as in human patients [6,9,10]. It has recently been shown that there is a significant increase in the number of β-cells in the pancreas of streptozotocin (STZ) – induced diabetic rats after 8 weeks of karela fruit juice treatment [11].

There are several reports that in alloxan- or STZ-induced diabetes mellitus, hyperglycemia is accompanied by increases in serum cholesterol and triglyceride levels [12-16]. *M. charantia* has been reported to significantly reduce serum cholesterol levels in normal rats [13] and a related plant, *M. cymbalaria*, has also been shown to reduce, significantly, serum cholesterol and triglycerides in alloxan diabetic rats [16]. In similar studies, another related plant, *Tinospora cordifolia*, was also found to reduce serum and tissue cholesterol, phospholipids and free fatty acids significantly in the alloxan-diabetic rat [15]. In the present study, we have investigated the effect of karela fruit extract on serum and tissue lipid profiles and lipid peroxidation (LPO) in normal and STZ-induced diabetic rats.

2. Materials and methods

2.1 Induction of diabetes and administration of karela fruit extract

Male Wistar rats, aged 12 weeks and weighing between 250-300g were used. Diabetes was induced by a single intraperitoneal injection of a buffered (0.1 M citrate, pH 4.5) solution of STZ at a dosage of 60 mg/kg body weight, essentially as described earlier [11,17]. The animals were considered diabetic, if their blood glucose values were above 16.65 mMol/l on the 3rd day of STZ injection. The animals were divided into four groups with six animals in each group. These comprised STZ-induced diabetic group, diabetic-karela juice (10 ml 100% fruit extractper kg body weight daily for 10 weeks) fed group,

karela juice fed control group treated as in diabetic group and untreated control group [17].

2.2 Isolation of tissues and preparation for biochemical analysis

At the end of the experimental period, i.e on the 11th week after karela fruit extract treatment, the animals were sacrificed after an overnight fast and EDTA blood samples from all four groups of animals were collected. Following perfusion of the animal with physiological saline, the liver, kidney, testis and brain were removed. The tissues were washed with ice cold saline (0.9% NaCl) and portions weighing about 1g were homogenized in 4ml of 100mM potassium phosphate buffer 9pH 7.4), containing 150mM KCl and 0.1mM EDTA. For microsome preparations, normal rat liver was similarly homogenized followed by centrifugation at 3000g for 10 min. The pellet was discarded and the supernatant was further centrifuged at 15000 x g for 15 min. The supernatant from this was, then, spun at 100000 x g for 60 min and the microsomal pellet was suspended in homogenizing buffer containing 20% glycerol. The protein concentration in blood plasma, tissue homogenate and microsomes was measured by the method of Bradford [18].

2.3 Measurement of lipid profiles

Triglycerides, total, non-esterified and high density lipoprotein (HDL) – cholesterol were measured in plasma from all animals, using appropriate enzyme-colorimetric kits (Boehringer Mannheim, Germany). Plasma total phospholipids were determined as described by Bartlett [19]. Total and non-etherified cholesterol and triglycerides were extracted from tissue homogenates of all the four groups of animals and their concentration estimated as above.

2.4 Measurement of LPO

Thiobarbituric acid reactive substances (TBARS) were measured in tissue microsomes as malonedialdehyde (MDA) formation during NADPH-dependent LPO, according to the method described previously by Bhagwat and associates [20]. The total concentration of TBARS was also measured in blood plasma. To observe the direct in vitro effects of karela fruit extract and STZ on LPO, the rate of NADPH-dependent TBARS formation was

measured in normal rat liver microsomes with and without the addition of different amounts of STZ (up to 4.5mg) were added to1.0ml of an assay system containing 10mM potassium phosphate buffer, pH 7.4, 1-2mg mircosomal protein, 1mM Mg-ADP and the formation of TBARS was measured as described above.

2.5 Statistical analysis

All values are reported as mean ± S.E.M and the statistical significance of differences from control rats were assessed using Student's t-test. A value of P≤0.05 was considered significant.

3. Results

3.1 Plasma lipids

The plasma lipid profiles of control and STZ-induced rats are shown in Table 1. There was about a two-fold increase in plasma non-etherified cholesterol and phospholipids in STZ-induced diabetic rats, when compared with control animals. Simultaneously, triglyceride levels were increased about four-fold and HDL-cholesterol levels were decreased by about 50% in the same animals. In STZ-induced diabetic rats fed with karela juice, the increases in non-etherified cholesterol, phospholipids and triglycerides were abolished, whilst total and HDL-cholesterol had increased when compared with STZ-induced rats. The treatment of karela fruit extract to control rats did not affect any of the parameters studied except for the total cholesterol levels, which were reduced significantly.

3.2 Tissue lipids

Table 2 compares the level of total and non-etherified cholesterol and triglycerides in liver, kidney, brain and testis of STZ-induced diabetic and control animals with and without treatment with karela juice. In STZ-induced diabetic rats, testis alone showed an increase (46%) in total cholesterol. This increase was abolished in diabetic animals treated with karela fruit extract. Karela administration to control animals selectively reduced the level of total cholesterol in liver (30%) and kidney (25%), but not in brain and testis. Non-etherified cholesterol levels in the tissues of diabetic animals were not affected, except in brain and testis, where they were moderately increased (30-44%).

Feeding karela fruit extract to STZ-induced diabetic animals did not have any marked effect on non-etherified cholesterol levels in liver, kidney, testis and brain, but in control animals, it led to a moderate increase (16-20%) in the non-etherified cholesterol level in liver and kidney with a slight decrease in the brain.

In STZ-induced diabetic rats, triglycerides levels were decreased by 34 and 40%, respectively, in liver and kidney and increased by about 40% in testis whilst no changes were seen in brain. Feeding of karela fruit juice to STZ-induced diabetic animals did not reverse any of these changes in tissue triglyceride levels and it also had little effect in control animals, except to decrease liver triglycerides.

3.3 LPO

The levels of TBARS in blood plasma and tissues are shown in Table 3. There was a moderate increase (22%) in the plasma of STZ-induced diabetic rats compared with controls. The rate of NADPH-dependent enzymatic LPO was increased (77%) in the kidney of diabetic animals and reduced (36%) in the liver. Feeding of karela fruit juice to diabetic rats abolished these changes.

The direct effect of both STZ and karela juice on microsomal LPO was also studied after in vitro addition at different concentrations to normal liver microsomes. There was a dose dependent microsomal LPO following the addition of karela extract, but no significant changes with STZ (Fig.1).

Table 1 : Plasma lipid profiles in diabetic and *M. charantia* treated rats[a]

	Control	CK	STZ	SK
Total Cholesterol	2.04 ±0.14	1.66*± 0.06	2.08 ± 0.23	1.55* ± 0.14
Non-esterified cholesterol	0.77 ± 0.12	0.68 ± 0.08	1.49* ± 0.40	0.84 ± 0.09
HDL-cholesterol	1.38 ± 0.06	1.18 ± 0.03	0.65* ± 0.03	0.99*± 0.12
Triglyceride	1.27 ± 0.15	1.08 ± 0.17	4.98* ± 0.82	1.22 ± 0.22
Phospholipids	1.44 ± 0.16	1.26 ± 0.05	2.38* ± 0.23	1.49 ± 0.12

[1] Plasma cholesterol, triglycerides and phospholipids in control, karela treated control rats (CK), diabetic (STZ) and diabetic rats treated with karela extract (SK) were measured by kits as described in section 2. Values are expressed as mmol/l and are mean of a minimum of 3 determinations. *P<0.05 compared with the control.

Table 2 : Tissue lipid profiles in diabetic and M. charantia treated rats[1]

		Control	CK	STZ	SK
Total Cholesterol	Liver	2.48 ± 0.13	1.76*± 0.15	2.72 ± 0.30	2.74 ± 0.40
	Kidney	2.10 ± 0.11	1.50*± 0.10	2.20 ± 0.25	2.40 ± 0.30
	Brain	1.55 ± 0.10	1.45 ± 0.08	1.57 ± 0.13	1.42 ± 0.10
	Testis	0.78 ± 0.05	0.85 ± 0.03	1.17*± 0.09	0.92 ± 0.09
Non-esterified	Liver	1.37 ± 0.10	1.65 ± 0.08	1.29 ± 0.13	1.85 ± 0.16
Cholesterol	Kidney	1.98 ± 0.15	2.31 ± 0.30	1.82 ± 0.20	2.20 ± 0.20
	Brain	0.89 ± 0.08	0.73 ± 0.10	1.28*± 0.09	1.05 ± 0.15
	Testis	0.69 ± 0.07	0.62 ± 0.00	0.9*± 0.05	1.07*± 0.10
Triglyceride	Liver	3.07 ± 0.27	2.01*± 0.18	2.03*± 0.20	2.28*± 0.20
	Kidney	4.31 ± 0.79	4.16 ± 0.37	2.57*± 0.30	2.88*± 0.27
	Brain	0.45 ± 0.01	0.46 ± 0.04	0.39 ± 0.04	0.55 ± 0.10
	Testis	0.47 ± 0.02	0.31 ± 0.02	0.63*± 0.05	0.65*± 0.15

[1]Liver, kidney, brain and testis were analyzed for cholesterol and triglycerides levels in control, karela treated control rats (CK), diabetic (STZ) and diabetic rats treated with karela extract (SK) by kits as described in materials and methods. Values are expressed as mmol/l homogenate (25% w/v) and are mean of a minimum of three determinations. *P<0.05 compared with control.

4. Discussion

Treatment of rats with STZ is an established model for Type 1 or insulin-dependent diabetes. Diabetes is associated with profound alterations in the plasma lipid and lipoprotein profile and with an increased risk of coronary heart disease [1]. The liver and some other tissues participate in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol and phospholipids and secretion of specific classes of plasma lipoproteins. Lowering of serum lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease and related complications [21]. Many herbs and plant products have been shown to have hypoglycaemic and hypolipidemic properties [5]. We have also shown that the feeding of karela fruit extract to STZ-induced diabetic rats mimics insulin in its effect as observed by the lowering of blood glucose and the increase in plasma insulin level [17].

In the present study, the ability of karela juice to partially reverse the hyperglycemia of STZ-treated rats is confirmed. Evidence is presented to show that, in addition to the hypoglycaemic activity of karela fruit extract, it also posses lipid lowering properties in

diabetic animals. A 2-4-fold increase in plasma non-etherified cholesterol, triglyceride and phospholipid levels was observed in STZ-induced diabetic rats, but in karela juice-treated STZ induced rats, these levels were close to controls. We have also observed that in STZ-induced diabetic rats, the level of HDL-cholesterol was significantly lower whilst phospholipid concentrations were increased. Feeding karela juice normalized these effects, possibly by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues.

Table 3 : LPO in blood plasma and tissues of diabetic and M. charantia treated rats[1]

	Control	CK	STZ	SK
Plasma**	2.22 ± 0.06	2.10 ± 0.30	2.72 ± 0.06	2.46 ± 0.24
Liver	0.37 ± 0.01	0.36 ± 0.01	0.24* ± 0.01	0.38 ± 0.01
Kidney	0.14 ± 0.00	0.12 ± 0.00	0.25* ± 0.01	0.17 ± 0.01
Brain	0.35 ± 1.01	0.24 ± 0.00	0.31 ± 0.00	0.32 ± 0.01
Testis	0.22 ± 0.02	0.21 ± 0.00	0.23 ± 0.00	0.26 ± 0.01

[1]LPO of blood plasma, liver, kidney, brain and testis of control, karela treated control rats (CK), diabetic (STZ) and diabetic rats treated with karela extract (SK) was measured as TBARS using MDA as standard. Values are expressed as nmol of MDA formation per min/mg protein of tissue or **nmol MDA formed per mg plasma protein. *P<0.05 compared with the control.

A decrease in the triglyceride levels of liver and kidney in STZ-induced diabetic rats may indicate an increased mobilization of lipids from these tissues or a decrease in fatty acid uptake and storage capacity, which may have caused an increase in serum triglycerides, and phospholipids. The effect of karela on controlled mobilization of serum triglycerides, cholesterol and phospholipids is presumably mediated by controlling the tissue metabolism and improving the level of insulin secretion and action. Karela treatment increased the insulin output from pancreas in diabetic animals and insulin activates the enzyme lipoprotein lipase, which hydrolyses lipoprotein-bound triglycerides [8,22]. In insulin deficient subjects, non activation of this enzyme causes hypertriglyceridemia. The strong antihyperlipidemic effect of karela fruit could also be through its control of hyperglycemia, as this is a major determinant of total and very low density lipoprotein (LDL) and triglyceride concentration [23]. The marked hyperlipidemia

that characterizes the STZ-induced diabetes seems to be a consequence of uninhibited action of lipolytic hormones on the fat depots. Insulin inhibits adipose tissue hormone-sensitive lipase and, therefore, reduces lipolysis and mobilization of peripheral depots. Karela fruit may mimic the action of insulin or may have a synergistic effect on insulin activity. We have also reported an increase in the number of beta cells in pancreas of STZ-induced diabetic rats fed with karela juice [11].

Another important factor determining the level and composition of serum and tissue lipids is LPO associated with cellular membranes. During diabetes an increased oxidative stress in certain tissues may lead to a rise in the rate of LPO [24]. The formation of the lipid peroxide product, MDA, was measured in tissue and serum as an index for increased LPO in diabetic rats, but with the exception of kidney, there was no appreciable increase in the liver. MDA formation was actually decreased in diabetic rats. Our previous observations have also suggested that in chronic diabetic rats, the kidney is the main target for oxidative damage [25]. Nevertheless, as seen in this study, the increase in tissue LPO does not appear to be a significant factor causing hyperlipidemia, as no profound alterations in plasma MDA were recorded. Nevertheless, a moderate increase in plasma TBARS in STZ-treated rats was found to be reduced after karela feeding. However, our present study on in vitro addition of karela fruit extract to liver microsomal membrane showed that the NADPH-dependant peroxidation of membrane was inhibited by karela fruit extract in a dose-dependent manner. Thus, an additional benefit of karela feeding may be the prevention of membrane LPO, presumably, because of some antioxidant components present in the fruit extract. It has been reported that agents with ability to inhibit LPO (e.g plant phenols) also possess hypoglycaemic and hypolipidemic properties [26,27].

In summary, karela fruit extract has been shown to have, besides hypoglycaemic properties, strong hypolipidemic action on diabetic hypertriglyceridemia and hypercholesterolemia as well. In addition, it also has some antioxidative properties, which may contribute towards preventing lipid peroxidative damage. Further characterizations of active components in karela, such as phenolics or related analogues are warranted and studies are in progress to isolate, identify and characterize such active components.

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Effects of *Momordica charantia* powder on serum glucose levels and various lipid parameters in rats fed with cholesterol-free and cholesterol-enriched diets

Anura P. Jayasooriya [a] , Masanobu Sakono [a] , Chizuko Yukizaki [b] , Mikio Kawano [b] , Kyosuke Yamamoto [c] , Nobuhiro Fukuda [a,*]

[a] Department of Biochemistry and Applied Biosciences, Faculty of Agriculture, Miyazaki University, Miyazaki 889-2192, Japan

[b] Miyazaki Prefectural Food Development Center, Miyazaki 880-0303, Japan

[c] Department of Internal Medicine, Saga Medical University School of Medicine, Saga 849-8501, Japan

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Abstract

The effect of dietary bitter melon (*Momordica charantia*) freeze-dried powder on serum glucose level and lipid parameters of the serum and liver were studied in rats fed diets supplement with and without cholesterol. Rats were fed the diets for 14 days containing bitter melon freeze-dried powder at the level of 0.5, 1 and 3% without an added dietary cholesterol (experiment 1) and those containing bitter melon at the level of 1% with or without 0.5% cholesterol and 0.15% bile acid (experiment 2). No adverse effect of dietary bitter melon powder on growth parameters and relative liver weight were noted. Dietary bitter melon resulted in a consistent decrease in serum glucose levels in rats fed cholesterol-free diets, although no dose-response was noted. Addition of cholesterol to the diets as compared to those without added cholesterol caused hypercholesterolemia and fatty liver. Bitter melon had little effect on serum lipid parameters, except for high density lipoprotein (HDL)-cholesterol; HDL-cholesterol levels tended to decrease by dietary cholesterol, while they were consistently elevated by dietary bitter melon in the presence and absence of dietary cholesterol, indicating an antiatherogenic activity of bitter melon. In addition, bitter melon exhibited a marked reduction in the hepatic total cholesterol and triglyceride levels both in the presence and absence of dietary cholesterol; the reduction of triglyceride levels in the absence of dietary cholesterol was

in a dose-dependent manner. These results suggest that bitter melon can be used as a health food.

1. Introduction

In oriental countries, located in tropical and subtropical regions, there are many herbal resources which are regarded as health foods, and it is believed that the habit of consumption of these herbs renders a number of beneficial effects to human health (Dubick, 1986). These herbs therefore are consider to be useful means to prevent and/or ameliorate certain disorders, such as diabetes and atherosclerosis, which are partly relevant to the westernization of dietary habit of oriental countries in the recent past. Among these herbal resources, the bitter melon (*Momordica charantia*) is a vegetable which exhibits a hypoglycemia potential in normal and diabetic rats as well as in human subjects with type 2 diabetes mellitus (Karunanayake et al., 1984; Welihinda and Karunanayake, 1986; Higashino et al., 1992). Although the mechanism(s) responsible for the observed reduction in the serum glucose concentration remained to be clarified, some of the experimental evidence have shown that bitter melon exerts its hypoglycemic effect either through an inhibition of glucose absorption (Meir and Yaniv, 1985), increased utilization of glucose by the liver (Welihindaet al., 1986; Day et al., 1990), or an insulin secretagogue action (Higashino et al., 1992).

On the other hand, it has been known that the dietary ingredients affecting glucose metabolism may also influence lipid metabolism (Jenkins et al., 1995). However, little is known about the effects of bitter melon on lipid metabolism, although the hypocholesterolemic effect by this dietary ingredient has been reported in normal rats as well as in alloxan diabetic rats (Singh et al., 1989; Platel et al., 1993). It is therefore of interest to examine whether bitter melon influences lipid parameters. In the present study, a more detailed examination about the beneficial effects of dietary bitter melon freeze-dried powder on some lipid parameters in the serum and liver of rats fed with diets supplemented with and without cholesterol is dealt with, and it is demonstrated that this dietary ingredients has favorable effects on the concentration of serum and hepatic lipids.

2. Materials and methods

2.1 Animals and diets

An unripe bitter melon (*M. charantia*) purchased from a local market was washed thoroughly with water, and the seeds were removed; the edible portion was cut into a small pieces, and freeze-dried and powdered. A part of this sample (Lot: 11001) was stored in the Agriculture Museum, Faculty of Agriculture, Miyazaki University. Male Sprague-Dawley rats (weighing 85-100 g in experiment 1and 100-110 g in experiment 2, respectively) obtained from Kyudo (Kumamoto. Japan) were given a pellet-stoked chow (Type CE-2, Clea Japan, Tokyo, Japan) and were acclimated for several days in a temperature controlled room (22-24° C, light on 07:00-19:00 h). Rats were then divided into four groups with five animals in each group (experiment 1) or four groups with six animals in each group (experiment 2), and allowed free access for 14 days to the control or experimental diet. Control group was fed with the diets containing 20% casein, 15% corn starch, 5% fiber, 5% corn oil, 0.3% DL-methionine, 0.2% choline bitatrate, 3.5% mineral mixture, 1% vitamin mixture, and 50% sucrose, while experimental group was fed with the diets supplemented with freeze-dried bitter melon powder; in experiment 1, bitter melon powder at different levels of 0.5, 1, and 3% was added at the expense of sucrose to the control diet, and in experiment 2 the same bitter melon powder at 1% was added in the presence or absence of cholesterol at 0.5% plus sodium cholate at 0.15% at the expense of sucrose to the control diet, respectively. Food intake and body weight were recorded every day. Rats were killed by decapitation around 9:00-10:00 h, blood was collated, and the serum was harvested by centrifugation at 4° C.

2.2 Analytical procedures

Serum glucose was immediately analyzed using a commercial kit (HDL-test Wako, Wako, Osaka, Japan). Liver was isolated, weighed, and kept at -45° C until the analyses were done. In both experiments, serum and liver lipids were extracted according to Folch et al., 1957). Analysis of high density lipoprotein (HDL)-cholesterol was done using an available commercial kit (Glucose test Wako, Wako, Osaka, Japan). The serum and lipid parameters were measured as described elsewhere (Fukuda et al., 1995).

2.3 Statistical analysis

Data were expressed as mean ± S.E. and the statistical significance of the difference of means were evaluated by Duncan's multiple range test (Duncan, 1995) at the level of $P < 0.05$.

3. Results

3.1 Body weight gain, food intake and relative liver weight

There were no significant differences in the food intake, body weight gain or relative liver weight in the bitter melon supplemented groups in comparison with the respective control groups in experiments 1 and 2, although the incorporation of cholesterol in the diet resulted in a significant increase of relative liver weight as compared with the cholesterol-free dietary groups in experiment 2.

3.2 Serum glucose and lipid concentrations

In experiment 1, there was a significant reduction in the concentration of serum glucose in rats fed with diets supplemented with bitter melon powder at 0.5% as compared with those with no added bitter melon, although increasing amounts of this dietary ingredient did not cause any additional hypoglycemic effects. Further, the hypoglycemic effect of dietary bitter melon (1.0% level) on serum glucose was reproduced in 0experiment 2, the reduction being statistically significant ($P < 0.05$) when analyzed by Student's *t*-test. However, this effect was diminished when cholesterol was included in the diet, suggesting that dietary cholesterol may be an important variable in modulating serum glucose levels caused by bitter melon feeding.

The effects of increasing amounts of bitter melon in the diet on serum lipid parameters, such as triglyceride, total cholesterol and phospholipid, were marginal, as shown in Table 1. In experiment 2, no effect of dietary bitter melon was again observed on these serum lipid parameters. However, an addition of cholesterol to the diet as compared with no added cholesterol resulted in a significant increase in the serum concentration of total cholesterol, while it caused a tendency toward a reduction in HDL-cholesterol. On the other hand, dietary bitter melon consistently elevated HDL-cholesterol levels, in comparison with respective controls, both in the presence or

absence of dietary cholesterol, suggesting a possible anthiatherogenic effect of bitter melon.

3.3 Hepatic lipid concentrations

The concentration of hepatic total cholesterol and triglyceride were significantly lower in rats fed bitter melon than in those fed the control diet, as shown in Table 1; increasing amounts of bitter melon powder in the diet caused a tendency toward reduction, (46.2, 54.7 and 56.7% reduction in triglyceride, and 22.8, 27.6 and 29.6% reduction in total cholesterol levels, respectively). Free cholesterol levels remained unchanged. As a result, the cholesterol ester ratio was significantly lower in rats fed bitter melon than in the animals fed the control diet except for dietary levels of 0.5%.

Addition of cholesterol to the diet resulted in a significant increased concentration of hepatic total and free cholesterol; the increase were much greater in total- than free cholesterol, indicating a marked accumulation of cholesterol ester in the livers of rats fed a cholesterol-enriched diet. In experiment 2, the beneficial effect of bitter melon feeding was again reproduced, as shown in Table 2. Thus, bitter melon consistently lowered hepatic triglyceride and total cholesterol levels by 39.2 and 32.0% in the absence of dietary cholesterol, and 26.2 and 22.4% in the presence of dietary cholesterol, respectively; the reduction of these lipid components was statistically insignificant except for total cholesterol in the presence of cholesterol in the diet. Free cholesterol levels again remained unchanged by feeding of bitter melon; as a result, the percentage of cholesterol ester was significant lower in the livers of rats fed bitter melon in the absence of dietary cholesterol, but not in the presence of dietary cholesterol, when compared with respective control counterparts. The effects of dietary bitter melon or cholesterol on phospholipids levels were inconsistent; in experiment 2 concentration were significantly reduced in the bitter melon supplemented groups in the absence of dietary cholesterol.

4. Discussion

The present studies were conducted to evaluate the beneficial effects of dietary bitter melon. *(M. charantia*), which is a common vegetable and used as a traditional medicinal herb for the treatment of diabetes in oriental countries (Bever and Zahnd,

1979), on lipid parameters of the serum and liver in normolipemic and dietary cholesterol induced hypercholesterolemic rats.

Present studies clearly showed that feeding of bitter melon for 14 days consistently lowered blood glucose concentration in rats fed diets free of cholesterol, but not in rats fed diets enriched with cholesterol, although the hypoglycemic effect was not augmented by increasing levels of dietary bitter melon in the absence of dietary cholesterol. The reduction in the absence of dietary cholesterol is consistent with previous observations by Karunanayake et al., 1984); they found a significant improvement of glucose tolerance and hyperglycemia when the juice of bitter gourd *(M. charantia*) was administered orally to rats. Other studies with normal and alloxan-diabetic rabbits also demonstrated a hypoglycemic effect of bitter melon powder in a dose dependent manner following oral administration (Akhtar et al., 1981). Similar lowering-effects of some components isolated from bitter melon with water or organic solvents on blood glucose

Table 1 : Effects of increasing amounts of dietary bitter melon on serum glucose, serum and liver lipid parameters in rats fed the cholesterol-free diet (experiment 1)*

	Control	Bitter Melon		
		0.5%	1%	3%
Growth parameters				
Initial body weight (g)	135 ± 4	135 ± 2	134 ± 3	135 ± 2
Final body weight (g)	258± 8	255 ± 5	242 ± 5	250± 6
Food intake (g/day)	22.6± 0.9	22.2 ± 0.6	20.3± 0.6	21.7± 1.0
Relative liver weight (g/100 g BW)	5.2 ± 0.2	5.2 ± 0.1	5.1± 0.1	5.1± 0.3
Serum parameters (mg/dl)				
Glucose	172 ± 5[a]	134± 7[b]	135 ± 2[b]	139± 5[b]
Triglyceride	96 ± 11	105± 18	111 ± 13	100± 16
Cholesterol				
Total	100 ± 11	93.4 ±5.5	92.3 ±7.7	99.8 ±16.7
Free	42.2 ± 4.9	48.1 ±11.1	39.3 ± 3.7	51.9 ± 7.4
Ester (%)	56.5 ± 5.8	49.3 ± 9.5	57.2 ± 2.9	51.6 ±15.7
Phospholipid	204 ± 13	188 ± 7	159 ± 11	186 ± 10
Liver parameters (mg / g liver)				
Triglyceride	41.1 ± 10.2[a]	22.1 ± 4.1[b]	18.6 ± 4.5[b]	17.8 ±4.2[b]
Cholesterol				
Total	2.49 ±0.15[a]	1.92±0.10[b]	1.81 ± 0.03[b]	1.76±0.08[b]

Effects of Momordica charantia powder on serum glucose levels and various lipid parameters in rats fed with cholesterol-free and cholesterol-enriched diets

Free	1.22 ± 0.07	1.14 ± 0.03	1.25 ± 0.06	1.18 ± 0.06
Ester (%)	50.1 ± 5.8[a]	39.7± 5.1[a]	30.7 ± 3.0[b]	32.1 ± 5.4[b]
Phospholipid	26.8 ± 1.5	26.9± 0.5	29.0± 1.0	30.0 ± 0.9

* The values are means + S.E. for six rats per group. [ab] The values not sharing common superscript letters are significantly different at $P < 0.05$.

Table 2 : Effects of dietary bitter melon on serum glucose, serum and lipid parameters in rats fed cholesterol-free and cholesterol-enriched diets (experiment 2)*

	CHOL-free		CHOL-enriched	
	Control	Bitter melon (1%)	Control	Bitter melon (1%)
Growth parameters				
Initial body weight (g)	166 ± 4	166 ± 4	166 ± 4	166 ± 3
Final body weight (g)	279 ± 8	284 ± 6	293 ± 8	294 ± 7
Food intake (g / day)	21.8 ±0.8	22.7 ± 0.8	22.2 ± 1.0	22.3 ± 0.7
Relative liver weight (g/100 g BW)	5.3 ± 0.5[a]	5.5 ± 0.1[a]	7.0 ± 0.3[b]	6.9 ± 0.2[b]
Serum parameters (mg/dl)				
Glucose	157 ± 5	144 ± 4	148 ± 5	143 ± 6
Triglyceride	207 ± 20	214 ± 17	195 ± 10	228 ± 15
Cholesterol				
Total	72.1 ±12.5[a]	93.6 ± 1.2[a]	138 ± 7[b]	136± 5[b]
Free	24.7 ± 1.9	27.0 ± 0.5	27.6 ± 1.7	27.3±0.7
Ester (%)	61.1 ± 6.8[a]	71.1 ± 0.2[ab]	79.7 ± 1.6[b]	79.8 ± 0.9[b]
HDL-cholesterol	59.9 ± 6.6[ab]	77.6 ± 7.4[b]	47.4 ± 7.3[a]	64.9 ± 9.1[ab]
Phospholipids	243 ± 15	243 ± 8	242 ± 11	230 ± 12
Liver parameters (mg/g liver)				
Triglyceride	35.2 ±7.3[ab]	21.4 ± 2.5[a]	37.0 ± 4.8[b]	27.3 ± 3.2[ab]
Cholesterol				
Total	3.34 ±0.45[a]	2.27± 0.18[a]	31.2 ± 3.5[b]	24.2 ± 2.6[c]
Free	1.51 ±0.07[a]	1.49± 0.05[a]	3.44 ± 0.16[b]	3.19 ± 0.26[b]
Ester (%)	50.3 ± 7.4[a]	32.2 ± 5.2[b]	88.1 ± 2.1[c]	86.2 ± 1.7[b]
Phospholipid				

* The values are means ± S.E. for six rats per group. [abc] The values not sharing common superscript letters are significantly different at $P < 0.05$.

levels were also reported in the normal and either alloxan- or streptozotocin-diabetic rats (Singh et al., 1989; Higashino et al., 1992). In addition, it has been known in the oriental

countries that the bitter melon has been used empirically as a therapeutic mean in patients with diabetes, because of a potent antidiabetic efficacy (Bever and Zahnd, 1979). These observations therefore suggest that the hypoglycemic potential of bitter melon is , in part, attributed to some active compenent(s) present in the edible portion of this vegetable.

On the other hand, it has been suggested that the mechanisms responsible for the serum glucose-lowering effect of bitter melon are attributed to an inhibitory effect of glucose absorption (Meir and Yaniv, 1985), an increased incorporation of circulating glucose as hepatic glycogen (Welihinda et al., 1986), or an enhanced secretion of insulin (Welihinda et al., 1986; Higashino et al., 1992). It is known that the factors influencing glucose metabolism under various physiological conditions do influence lipid metabolism as well (Jenkins et al.,1995). However, little is known about the effects of bitter melon on lipid metabolism. The present studies demonstrate for the first time that bitter melon has favorable effects on serum and liver lipid parameters in rats fed cholesterol-free and cholesterol-enriched diets.

The addition of bitter melon to the cholesterol-free and cholesterol-enriched diets caused an elevated serum HDL-cholesterol level, although it did not modify serum lipid parameters. It is well known that HDL plays an important role in the transport of cholesterol from peripheral cells to the liver by a pathway termed 'reverse cholesterol transport', and hence the elevated serum HDL-cholesterol levels by dietary bitter melon are considered to be favorable since there is a highly negative correlation between serum HDL-cholesterol levels and incidences of atherosclerosis (Levy, 1978). These observations therefore suggest that bitter melon is a useful dietary component to prevent and/or ameliorate atherosclerosis.

There was a consistent reduction of hepatic triglyceride and total cholesterol concentrations following a feeding of bitter melon in comparison with respective control counterparts both in the presence and absence of dietary cholesterol; these lowering effects were evidently dose-dependent manner following feedings of increasing levels of bitter melon in the absence of dietary cholesterol. It has been revealed that there is a considerable increase of triglyceride accumulation in patients with diabetes mellitus (Pitsin et al., 1986). Similar observations were also reported in experimental diabetic

animals (Iams and Wexler, 1977), suggesting that the bitter melon may also be a useful vegetable for the treatment/prevention of dietary-induced fatty liver. These observations may suggest that some active components of the edible portion of bitter melon are incorporated into liver cells and influence metabolism of the serum and liver lipids, although the mechanisms responsible for the beneficial effects on lipid metabolism remain to be clarified. Further studies are being carried out in the laboratory to reveal those mechanisms.

In summary, bitter melon is a vegetable that contains active components for preventing and/or relieving certain diseases, such as diabetes, fatty liver, and atherosclerosis.

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Potentiation of Anti-HIV Activity of Anti-Inflammatory Drugs, Dexamethasone and Indomethacin, By MAP30, The Antiviral Agent from Bitter Melon

Aldar S. Bourinbaiar and Sylvia Lee-Huang*

Department of Biochemistry, New York University School of Medicine 550 First Avenue, New York, NY 10016

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Abstract

MAP30 is an antiviral protein from bitter melon (*Momordica charantia*). The enhancement of weak HIV antagonists, dexamethasone and indomethacin, by MAP30 has been examined by measuring the reduction in p24 expression in acutely infected MT-4 lymphocytes. In the presence of 1.5 nM MAP30 the IC_{50} dose of dexamethasone and indomethacin has been lowered, without concurrent cytotoxicity, at least a thousand-fold to 10^{-7} M and 10^{-8} M, respectively. This observation indicates that MAP30, a multifunctional antiviral plant protein capable of topological inactivation of viral DNA and specific cleavage of 28 S ribosomal RNA, may regulate HIV replication in concert with steroid and non-steroidal inhibitors of prostaglandin synthesis. The results suggest that use of MAP30 in combination with low pharmacological doses of dexamethasone and indomethacin may improve the efficacy of anti-HIV therapy. © 1995 Academic Press, Inc.

1. Introduction

We have reported the isolation and purification of MAP30, a 30 kD antiviral protein, from seeds of bitter melon (*Momordica charantia*). MAP30 exhibits potent anti-HIV activity with 50% inhibitory concentration (IC_{50}) in nanomolar range and lacks toxicity to uninfected cells [1]. Bitter melon is well known in Chinese pharmacopeia for anti-infective, anti-tumor and immunomodulating properties. Recently there has been intense community interest in the use of bitter melon, the source of MAP30, as an alternative therapy for AIDS. Encouraging results have been noted by HIV-positive patients [2].

2. Materials and Methods

2.1. Drugs

Purified preparation of MAP30 was derived from seeds of M. charantia as described earlier [1]. All other reagents were purchased from Sigma (St Louis, MO), Azidodeoxythymidine (AZT), synthetic glucocorticoids, i.e., dexamethasone (DEX), prednisone, prednisolone, and hydrocortisone, as well as various NSAIDS, including indomethacin (IND), indoprofen, naproxen, ibuprofen, and acethylsalicylic acid (aspirin) were prepared as 100 mM stock solutions in appropriate solvents [15].

2.2. Virus

The stock preparation of HIV was derived from filtered-supernatant of H9/IIIB lymphocytes and stored frozen at -70°C. Viral load was estimated by p24 ELISA and infectious viral titer was determined by limiting end-point dilution assay as described [16].

2.3. Antiviral assay

The assay is based on determination of p24 expression using ELISA kit from Coulter (Hialeah, FL). MT-4 CD4+ T lymphocytes, used as indicator cells, were cultured in 96-well culture plates, each well contained 200 ml RPMI 1640 medium with 10% FCS, L-glutamine (2mM), penicillin (100 units/ml) and streptomycin (100 ug/ml). Serial $\log_{10}$ dilutions of test drugs were added to wells containing 2 x 10^4 MT-4 cells and followed by addition of HIV. The total input multiplicity was equivalent to 100 infectious units per lymphocyte. In drug combination studies each test well contained 1.5 nM of MAP30. The cell cultures containing virus and drugs were incubated at 37°C in a humidified incubator at 5 % CO_2 for 2 days until p24 assay. The values of p24 corresponding to the original viral inoculum left for 2 days in wells without MT-4 were calibrated as blanks. This allows us to distinguish newly-synthesized virus from residual inoculum of HIV. Each drug was assayed three times in triplicate wells.

2.4 Cytotoxicity assay

The toxicity of the drugs was determined by colorimetric assay based on measuring the conversion of XTT tetrasolium salt into light-dense formazan product. The culture

conditions and number of mock-infected MT-4 cells were the same as in the antiviral assay. Serial 10-fold dilutions of drugs were added to MT-4, incubated for 2 days, and XTT (1 mg/ml) was added for 3-4 hours. The optical density was measured a microplate reader with 450/620 nm filters and the results were expressed as percent of untreated control cells.

3. Results and Discussion

As early as two days postinfection HIV-exposed MT-4 cells started to form syncytia. The cytopathic effect was correlated inversely with the concentration of MAP30 present in treated wells. The dose-effect was confirmed by quantitative p24 ELISA. Based on mean values of obtained results the 50% inhibitory concentration (IC_{50}) was equivalent to 1.5nM or 45 ng/ml of MAP30. This dosage had no adverse effect on the viability of MT-4 cells (Fig. 1).

(Please login at http://www.sciencedirect.com/science/article/pii/S0006291X85714052 to view **fig. 1**).

The anti-HIV activity of anti-inflammatory drugs was evaluated individually prior to combination with MAP30. Both DEX and IND demonstrated antiviral activity, but seen in Fig. 1, their potency was modest. Significant suppression of de novo p24 synthesis was observed at relatively high doses with IC_{50} of 10^{-4} and 10^{-5} M respectively. Quantitative cell viability XTT assay indicated that 10^{-4} M DEX and IND showed no toxicity to target cells. In contrast, at equimolar concentrations, other anti-inflammatory drugs, i.e., aspirin, ibuprofen, and naproxen as well as prednisone, prednisolone, and hydrocortisone, exhibited no anti-HIV activity [15]. The combination of nontoxic 1.5 nM dose of MAP30 either with DEX or IND has resulted in a reduced yield of HIV without accompanying toxicity for MT-4 cells. As a result, the IC_{50} of DEX and IND have shifted from 10^{-4} and 10^{-5} to 10^{-7} and 10^{-8} M respectively. This effect appears to be specific to this combination of drugs since no changes in dose response curves were observed upon combination of drugs since no changes in dose response curves were observed upon combination of MAP30 with AZT (Fig. 1).

The mechanism of antiviral action of MAP30 is currently under investigation. This antiviral agent is a multifunctional protein. We found that in addition to antiviral activity it also

possess other activities, including topological inactivation of viral DNA and ribosome inactivation [1, 17-19]. The DNA topological inactivation activity is specific for plasmid and viral DNA, and converts supercoiled DNA into topologically inactive forms and interrupts DNA functions [17-19]. The ribosomal inactivation activity is assayed by cell-free translation in a rabbit reticulocyte lysate system [1]. This property is common to plant-derived single chain ribosome inactivating proteins (SCRIPs). Ribosome inactivation is due to an N-glycosidase activity that acts specifically on the glycosidic linkage between the ribose and adenine or guanine at A4324 or G4323 of the 28S rRNA (20). This, in turn, interrupts the binding between elongation factor 2 and the 60S ribosomal subunit and thus inhibits *in vitro* eukaryotic protein biosynthesis. Whether both of these activities are involved in the antiviral activity of MAP30 remains to be elucidated.

MAP30 is a SCRIP by virtue of its action on ribosomal RNA. However, there is a great deal of heterogeneity among SCRIPs in their toxicity and in the presence of other bioactivities. We have found that the viral DNA topological activity of MAP30 does not appear to be a universal property of all SCRIPs. It was recently reported that saporin, a SCRIP from *Saponaria officinalis*, has a unique polynucleotide adenosine nucleosidase activity that releases adenine residues from viral RNAs, DNAs, polyA, and globin mRNA in vitro. No other SCRIPs examined possess this activity [21]. These results illustrate that individual members of the SCRIP family may have distinct additional activities independent from ribosome inactivation. The present findings seem to indicate yet another novel activity of SCRIPs.

The anti-inflammatory drugs operate mainly through inhibition of the prostaglandin (PG) synthesis pathway [22]. PGs are derived from arachidonic acid (AA) residing in the phospholipids of mammalian cell membranes. Synthetic glucocorticoids, i.e., DEX, inhibit phospholipase A_2, responsible for the liberation of free AA. IND, on the other hand, acts by suppressing the enzymatic activity of cyclooxygenase, which converts AA into PGs. The role of DEX in the expression of HIV is not clearly defined. It has been reported either to block or to stimulate inducible HIV expression via various mechanisms during viral transcription [5-11]. The effect of NSAIDs on HIV replication has not been thoroughly investigated, although two recent reports described the antiviral activity of aspirin *in vivo* [12] and *in vitro* through suppression of NF-κB translocation [13].

The above studies deal with chronically infected cells. In acute infection, as in the case of MT-4 cells, the situation is different, since IND is more potent than aspirin [12] and DEX has a suppressive effect on *de novo* HIV expression. In spite of the fact that these anti-inflammatory agents represent two separate classes of drugs antagonizing AA metabolism, both possessed anti-HIV activity. However, effective doses of these drugs were rather high, approaching the therapeutic range usually associated with adverse side-effects [22]. The addition of a nontoxic dose of MAP30 has resulted in a substantial gain in antiviral efficacy. As a result, effective antiviral doses of DEX and IND attained pharmacological levels essentially free of clinical side effects.

Glucocorticoids and NSAIDs have been used clinically to avert neurological reactions of α-trichosanthin, abortive SCRIP with anti-HIV activity [23, 24] administered to patients either for inducing abortion [25] or for treating HIV-1 infection [26-29]. Since α-trichosanthin does not cross the blood-brain barrier [30] it has been suggested that the neurological response may be mediated through secondary soluble factors like PGs [31-33].

In vitro enhancement of the antiviral potential of DEX and IND with MAP30, and the therapeutic use of anti-inflammatory drugs to relieve the neurological effect by α-trichosanthin may be unrelated phenomena. Whether these two discrete events share a common pathway in AA metabolism is not known. On the other hand, alternative modes may also be possible at the level of transcriptional regulation of HIV expression by these drugs. Further studies are in progress to elucidate the nature of this important observation, with the ultimate goal of establishing the therapeutic advantage of such a drug combination.

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HERBAL
HOME

Herbal Home Sdn. Bhd.
No.102, Jalan Gasing, 46000
Petaling Jaya, Selangor Malaysia.
Tel. +603 - 7960 7777
Fax. +603 - 7960 9908
Website. www.herbalhome.us
Email. sales@herbalhome.us