RESEARCH ARTICLE

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HYPOGLYCEMIC EFFECT OF MOMORDICA CHARANTIA (KARELA) ON NORMAL AND ALLOXAN DIABETIC ALBINO MICE

ABSTRACT:
The effects of Bitter Melon (Momordica charantia) on the normal and onset of alloxan-induced diabetes in male and female Swiss albino mice were examined. It was observed that the mice given orally juice extract of Bitter melon (10 ml/kg BW for 12 weeks) showed a significant decrease in the blood glucose level and glycosylated hemoglobin A1c of diabetic mice induced by intraperitoneal injection of alloxan (50 mg/kg, BW i.p.) and significantly improved the glucose tolerance test. Histological examinations were also done on pancreas. Oral administration of juice extract of Bitter melon (10 ml/kg BW) three times weekly for 12 weeks showed dramatic regeneration in the pancreatic islets of alloxan diabetic mice. The present results suggest that orally given of fruit juice, M. charantia may have a role in the renewal of β cells in alloxan-diabetic mice or alternately may permit the recovery of partially destroyed β cells. Momordica charantia may effectively normalize blood AST, ALT, creatinine and cholestrol in alloxan induced-diabetic group. These findings revealed that juice extract of bitter melon may have a potential benefit in the treatment of diabetes, play a role in its management and reduces the risk of diabetic complications.

KEY WORDS:
Karela, Momordica charantia, bitter melon, hyperglycemia, diabetes mellitus.

INTRODUCTION:
Diabetes mellitus (DM) is a group of metabolic disorders that result in hyperglycemia as a result of a relative or absolute lack of insulin, or the actions of insulin on its target tissues or both (Srividya and Shailendra, 2008). It is the most common endocrine disorder and by the year 2010, it is estimated that more than 200 million people worldwide will have DM and 300 million will subsequently have the disease by 2025 (Bastaki, 2005). Insulin is the mainstay for patients with type 1 diabetes and it is also important in type 2 when blood glucose levels cannot be controlled by diet, weight loss, exercise and oral medications. Previous to the use of insulin, dietary measures are the major form of this treatment especially in many developing countries; this includes the traditional medicines derived from plants (Ayyanar et al., 2008). Also the high cost of diabetes, both in healthcare and quality of life, has led to a growing interest in alternative therapies for diabetes management. A multitude of plants have been used for the treatment of diabetes throughout the world. One of such plant is Momordica charantia (Family: Cucurbitacea), whose fruit is known as Karella or bitter gourd (Duke et al., 2002; Rahman et al., 2006).

Bitter melon Fruits (BM) is the English name of Momordica charantia. Bitter melon grows in tropical areas, including parts of East Africa, Asia, the Caribbean, and South America, where it is used as a food, as well as a medicine. The fruit should be firm, like a cucumber, and it tastes very bitter. Although the seeds, leaves, and vines of Bitter Melon have all been used, the fruit is the safest and most prevalent part of the plant used medicinally.

Multiple clinical studies have clearly established the role of bitter melon in people with diabetes. Scientists have now identified three groups of constituents that are thought to be responsible for its blood sugar lowering action. One of these, a compound called charantin, which is composed of mixed steroids, was found to be more effective than the oral hypoglycemic drug, tolbutamide, in reducing blood sugar (Basch et al., 2003;
Patel et al., 2006). Another, an insulin-like polypeptide called polypeptide P, appears to lower blood sugar in type I (insulin dependent) diabetics, while alkaloids present in the fruit have been noted also to have a blood sugar lowering effect. As yet, researchers are unclear as which of these compounds is most effective or if it is the synergistic effect of all three (Murray, 1995; Basch et al., 2003). Compounds known as oleanolic acid glycosides have been found to improve glucose tolerance in Type II (maturity onset) diabetics by preventing the absorption of sugar from the intestine (Duke, 1989). It is still unclear which of these is most effective or if all three work together. At least 32 active constituents have been identified in bitter melon, including beta-sitosterol-d-glucoside, citrulline, GABA (gamma-aminobutyric acid), a neurotransmitter of the central nervous system that inhibits excitatory responses, lutein, lycopene and zeaxanthin (Handa et al., 1990; Raman and Lau, 1996). Nutritional analysis reveals that bitter melon is also rich in potassium, calcium, iron, beta-carotene, phosphorus, good dietary fiber and vitamins B1, B2, B3 and C (Abascal and Yarnell, 2005). It has been proven by western scientists to contain proteins or glycoproteins that act as an anti-tumor agent and inhibit HIV-1 infection (Sylvia et al., 1995; Taylor, 2002).

Glycosylated (or glycated) hemoglobin, measured as HbA1c is used as an index of mean glycemia in diabetes over the preceding two to three months i.e. life span of erythrocyte (Schwartz, 1995; Thevarajah, et al. 2008). The percentage of glycosylated hemoglobin in human blood depends on the concentration of glucose, the duration of glucose exposure to hemoglobin and the turnover of erythrocytes. Measuring glycated hemoglobin assesses the effectiveness of therapy by monitoring long-term serum glucose regulation (Larsen et al.,1990). The American Diabetes Association (ADA, 2008) recommended keeping the HbA1c less than 7%. The ADA (2008) reported that there is a 10% decrease in relative risk of microvascular complications, such as diabetic nephropathy or diabetic neuropathy, for every 1% reduction in HbA1c.

The present work aimed to study the possible hypoglycemic effect of bitter melon as a natural and safe anti-diabetic agent for normal and diabetic mice previously treated with alloxan by measuring the blood glucose levels, some blood parameters related to diabetes, glucose tolerance test and glycosylated HbA1c.

**MATERIAL AND METHODS:**
**Preparation of bitter melon fruits (BM) juice:**

Bitter melon fruits (BM) or *Momordica charantia* (Fig. 1) were obtained from the local market, washed thoroughly, and fresh juice was prepared on a juicer (Crompton, India) eliminating seeds. It was centrifuged at 1000 rpm on a tabletop refrigerated centrifuge for 10 min at 4°C. The clear supernatant was considered as 100% BM juice (BMJ) which was diluted with autoclaved distilled water. The 100% juice was stored at 4°C.

![Fig. 1. A picture showing bitter melon fruits (*Momordica charantia*)](image)

**Animals:**

40 male and 40 female Swiss albino mice (2 months old, 20-25 g) were obtained from an inbred strain in the College of Veterinary Medicine, King Faisal University, Al-Hassa, Saudi Arabia. Males and females were housed separately in stainless steel cages containing hard wood chips, five animals/cage. Mice were housed at room temperature (20-22°C).

Animals in all groups were given a basal diet composed of 60 % of ground corn meal, 15% ground beans, 10% wheat bran, 10% corn oil, 3% casein, 1% mineral mixture, and 1% vitamin mixture (Nelson and Halberg, 1986). Water was given ad libitum.

**Induction of experimental diabetes (control diabetic group):**

Each animal in this group (10 males and 10 females) was injected intraperitoneally with a single dose of 50 mg/kg BW of alloxan (Takeshi et al., 1998; Alercon-Aguilar et al., 2000; Shenoy, 2000). Alloxan (C4 H2 N2 O4) also named 2, 4, 5, 6 (1H, 3H)-Pyrimidinetetron. Random plasma glucose levels were measured to ascertain the diabetic status in different groups of mice.

**Experimental design:**

Animals were divided into 4 groups (10 males and 10 females each).

Group 1 was used as control mice (without alloxan & without BMJ).

Group 2 was used as control diabetic mice (alloxan without BMJ).

Group 3 was used as control BMJ (without alloxan with BMJ).

Group 4 was used as treated diabetic mice with BMJ (alloxan and BMJ).
Distilled water was used as a negative control for groups 1 and 2, control BMJ diabetic groups were administrated orally three times weekly 10 ml BMJ /kg BW, for a period of three months for groups 3 and 4. Mice were sacrificed at the end of three months to remove pancreas for histological study.

**Hematological studies:**

Blood collection using the saphenous puncture (Hem et al., 1998) for blood sampling of the mouse (superficial veins of the hind limb). As a general rule, a blood volume equivalent to about 0.5% of the animal’s body weight may be safely drawn, as a single sample (Wolfensohn and Lloyd, 1994), and this can usually be repeated at fortnightly intervals without disturbances to the animal’s hematological status. Fasting blood (12 hours) was collected from 5 males and 5 females weekly from each group for estimation of glucose and blood parameters.

**Determination of blood parameters:**

Blood glucose levels and other blood parameters were determined using a dry chemistry blood analyzer (Reflotron plus, Roche, Germany) using Reflotron kites; Roche, Diagnostic GmbH D 196424001 Mannheim, Germany (James et al., 1988; Phillips et al., 1988; Wadaan, 2006) and hemoglobin A1C meter; NycoCard® Reader II, Oslo, Norway (Jeppsson et al., 2002). Glucose tolerance test was carried out using method of Fischbach and Dunning (2004).

**Preparation of histological slides:**

Mice were observed regularly throughout the experiment. Any moribund or dead animals were removed and autopsied.

All organs were examined and tumours or other pathological changes were collected. The pancreas was fixed in 1% neutral buffered formalin followed by processing in the routine technique of paraffin embedding and blocking. Paraffin sections of 3-5 micrometers thick were prepared by microtomy and then routinely stained with Eherlich’s hematoxylin and counterstained with eosin (Bancroft and Stevens, 1990) and examined on light microscope for the histopathological studies.

**Statistical analysis:**

All observations were first recorded in a notebook and entered into PC computer and verified by another person for accuracy of data entry. Values were expressed as mean ± SD (of 10 animals, 5 males and 5 females). The statistical analysis was performed using a Student’s two tailed-test software program according to Flower and Cohen (1997). P values less than 0.05 were considered statistically significant.

**RESULTS:**

The blood glucose levels are recorded in table 1. The blood glucose levels (BGLs) of diabetic mice treated with BM juice were reduced in comparison with those of control diabetic group. Fasting BGLs (12 hours) were lowered significantly (p<0.05) after giving 10 ml BMJ/kg BW, 3 times weekly for 12 weeks. Also, results indicate that BM improved postpradinal blood glucose level as BM caused a significant decrease (P<0.05) in the blood glucose level at the end of the experiment as compared to control diabetic group.

**Table 1. Effect of Bitter melon juice (BMJ) on the blood glucose level in normal and alloxan diabetic mice during 12 weeks.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>1 w</th>
<th>2 w</th>
<th>4 w</th>
<th>6 w</th>
<th>8 w</th>
<th>10 w</th>
<th>12 w</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Control mice group</td>
<td>96 ±11</td>
<td>102 ± 5.2</td>
<td>99 ± 7.3</td>
<td>101 ± 6.4</td>
<td>98 ± 9.8</td>
<td>104 ± 4.6</td>
<td>106 ± 2.5</td>
</tr>
<tr>
<td>2- Control diabetic group</td>
<td>233 ±18.9</td>
<td>242 ± 12.4</td>
<td>247 ±14.5</td>
<td>251 ± 16.6</td>
<td>249 ± 19</td>
<td>254 ± 21.7</td>
<td>257 ± 17.2</td>
</tr>
<tr>
<td>3- BMJ group</td>
<td>88 ± 7.3</td>
<td>92 ± 5.8</td>
<td>86 ± 4.2</td>
<td>82 ± 6.4</td>
<td>96 ± 7.1</td>
<td>84 ± 5.9</td>
<td>85 ± 3.7</td>
</tr>
<tr>
<td>4- BMJ diabetic group</td>
<td>90 ± 4.4</td>
<td>99 ± 3.5</td>
<td>89 ± 4.8</td>
<td>86 ± 8.9</td>
<td>85 ± 2.8</td>
<td>87 ± 1.7</td>
<td>81 ± 5.4</td>
</tr>
</tbody>
</table>

The data represent the mean ± SD of 10 animals (5 males and 5 females).
Oral administration of 10 ml/kg body weight 3 times weekly of BM juice for a period of 12 weeks significantly improved the performance of alloxan diabetic mice in the glucose tolerance test (Table 2) and significantly decreased hemoglobin A1c ratio (Table 3).

Table 2. Effect of treatment with BMJ (10 ml/kg body weight 3 times weekly for 12 weeks) on blood glucose levels (mg/dl) in alloxan diabetic mice during glucose tolerance test

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Pre-treatment* (mg/dl)</th>
<th>Post-treatment** (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>280 ± 13.8</td>
<td>210 ± 10.5</td>
</tr>
<tr>
<td>60</td>
<td>385 ± 22.4</td>
<td>250 ± 8.9</td>
</tr>
<tr>
<td>90</td>
<td>420 ± 19.4</td>
<td>310 ± 14.8</td>
</tr>
<tr>
<td>120</td>
<td>400 ± 15.8</td>
<td>270 ± 17.5</td>
</tr>
</tbody>
</table>

The data represent the mean ± SD of 10 animals (5 males and 5 females).

** P < 0.05 as compared with the corresponding value in the 1st week control group.

Momordica charantia extract caused a significant decrease of serum or plasma AST (Aspartate aminotransferase), and ALT (Alanine aminotransferase activities), creatinine and total cholesterol levels in the blood (Table 3). Also, these results suggested that BMJ possesses anti-diabetic effect in alloxan-induced diabetic mice.

The histological examination of the pancreases showed the following:-

1) - There is a generalized reduction in size of pancreatic islets in the diabetic group (Fig. 2B) as compared to normal control one (Fig. 2A). We observed a generalized shrinkage in size of pancreatic islet in the control diabetic group. This observation supports the fact that an increase in blood glucose level of diabetic mice was due to the damage done to pancreatic islets.

2) - A pronounced regeneration of pancreatic islets especially in BMJ treated diabetic group (Fig. 2C) as compared to control diabetic group. The BMJ treated group nearly had returned the size of the pancreatic islet to the same size of the normal control mice and nearly normalized fasting blood glucose after 12 weeks.

Fig. 2. Hematoxylin-eosin stained pancreatic section showing: A- morphology and large number of cells in a pancreatic islet of control mouse, X 1000, B- morphology of a pancreatic islet of alloxan-diabetic group mouse, X 1000, C-morphology of a pancreatic islet of BMJ extract treated group mouse, X 1000 L: an islet of Langerhans

These results suggested that BMJ possesses anti-diabetic, hepato-renal protective effect and lowered total cholesterol level in alloxan-induced diabetic mice.
DISCUSSION:

Finding that BMJ ameliorate the fasting and postprandial blood glucose levels of alloxan diabetic mice and significantly decreased HbA1c ratio as compared with diabetic control results. These results reveal the beneficial effect of BMJ in the diabetic patients and this agrees with previous studies of Welihinda et al. (1982), Shibib et al. (1993), Sarkar et al. (1996), Ahmed et al. (1998), Blumenthal et al. (1998), and Yibchok-Anun et al. (2006). Bitter melon preparations have shown to significantly improve glucose tolerance and fasting blood glucose levels. This is in concordance with the study of Welihinda et al. (1986) who reported that M. charantia fruit juice increased both glucose uptakes by tissues in vitro and liver glycogen storage. Decreases in fasting blood glucose, HbA1c and postprandial blood glucose ranged between 15-42% was recorded also by Akhtar (1982) and Ahmad et al. (1999).

Bitter melon may block the release of glucose in the blood stream and breaks down the barrier that prevents cells from using their own natural insulin and this anti-diabetic effect of BMJ has been shown to increase the number of β cells by the pancreas, thereby improving ability to produce and release insulin, while at the same time it may increase the number and activity of insulin receptors.

The hypoglycemic effect of bitter melon may be mediated through stimulating synthesis and / or release of insulin from the beta cells of Langerhans or due to regeneration of pancreatic islet cells. This agrees with previous studies (Wang et al., 1982).

The relative distribution of pancreatic islet cells in the control diabetic animals is similar to the results of previous studies in the rat (Adeghate et al., 1991). Bitter melon has been reported also to increase the number of β cells in the pancreas (Singh et al., 2004), thereby improving capability to produce insulin.

Physiological experiments have shown that M. charantia can stimulate insulin secretion (Welihinda et al., 1982) and induce glucose uptake in liver (Welihinda et al., 1986). 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 bitter melon. The reason for this action 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.

The present results revealed that Momordica charantia extract caused a significant decrease of AST and ALT activities, creatinine, and cholesterol levels in the blood. Bitter melon juice lowered cholesterol levels in alloxan diabetic mice. The juice may exert rapid protective effects against lipid peroxidation by scavenging of free radicals there by reducing the risk of diabetic complications. This agrees with the previous studies as follows. Animal studies demonstrated that BM juice, particularly the saponin fraction, have lipid-lowering effects resulting from inhibition of pancreatic lipase activity and subsequently decreased lipid absorption (Oishi et al., 2007). Another study demonstrated that BM juice has an inhibitory effect on membrane lipid peroxidation(Ahmed et al., 2001).

The conclusions of the present study are summarized as follows:-

1) One or more constituents of BMJ may be responsible for reducing blood glucose level in the alloxan diabetic mice.
2) The hypoglycemic effect of BMJ may be due to regeneration of pancreatic islets, which may increase insulin secretion and / or may be related to the action of BMJ in reducing the intestinal glucose absorption thereby helping to control the increase in postprandial blood sugar levels.
3) BMJ significantly improved the performance of alloxan diabetic mice in the glucose tolerance test and hemoglobin A1c ratio.
4) BMJ extract caused a significant decrease in AST and ALT activities, creatinine and cholesterol levels in the blood of alloxan diabetic group. These findings revealed that bitter melon may have a potential benefit in the treatment of diabetics and plays a role in the management of diabetes and reducing the risk of diabetic complications.

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