

Original Research Article

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The Role of *Momordica charantia* in Reducing the Level of Glucose in Mice

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ABSTRACT

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The effects of aqueous and alcoholic extract (*Momordica charantia*) on the level of serum glucose were studied on 40 mature males distributed into 5 equal groups and fed basal diets. Group (1) was kept as control negative (fed on basal diet), while the other 4 groups were subcutaneously administered a single dosage of alloxan to induce experimental diabetes. Group (2) was left as a control positive group (diabetes) while the other groups (3), (4) and (5) were orally given *Momordica charantia* once a day at a dosage of 0.10, 0.20 and 0.40 mg/ml respectively, for 10 days. At the end of the experiment, blood samples from all mice were collected for a biochemical analysis. The results had an effect on the glucose level of the serum, at 0.30mg/ml of water extract of *Momordica charantia*. On the other hand, there are no effects on the glucose level at the higher dosages of Methanolic *Momordica charantia* extract.

Introduction

Momordica charantia is a herbal plant that belongs to The family cucurbitaceous (Whitaker *et al.*, 1990). It has been commonly consumed as a vegetable and used as a medicinal herb in India, China, Africa and in various parts of wounds, ulcer, eczema, jaundice, kidney stone, leprosy and scabies (Basch *et al.*, 2003). The main constituents of *Momordica charantia* which are responsible for medicinal effects are triterpenes, steroids, alkaloids, in organics, lipids and phenolic compounds) Grover *et al.*, 2004). Phenolic compounds are categorized as secondary metabolites essential for growth and reproduction of plants.

They are known as hydrophilic antioxidants, and are produced as a response for defending injured plants against pathogens. They potentially show antioxidant, anti mutagen, antitumor, anti-inflammatory and anti carcinogenic properties (Lee *et al.*, 2003). Free radicals are known to be the major cause of various chronic and degenerative diseases, including aging, coronary heart disease, inflammation, diabetes mellitus and cancer (Cheng *et al.*, 2003). Recently, natural foods and food derived antioxidants such as vitamins and phenol phytochemicals have received growing attention because they are known to function as chemo protective agents against the oxidative damage (Wu *et al.*, 2008).

Natural products and many active principles identified from plant species are known to play an important role in pharmaceutical biology (Joseph and Jini, 2011). *Momordica charantia* has a significant antidiabetic activity so that it can be used to treat diabetes as well as to delay the late complications of diabetes. In the present review, the possible antidiabetic activity of *Momordica charantia* has been elucidated in addition to and its medicinal potency responsible for the hypoglycemic activity.

The aim of the present study to elucidate the possible antidiabetic activity *Momordica charantia* extract in relieving symptoms and conditions of diabetes in experimental animals.

What is *Momordica charantia*?

Momordica charantia is a tall-growing annual fruit originated in South-East Asia. It can also be cultivated in Africa, South America, and India. The plant belongs to the Cucurbitaceae family in the *Momordica* genus of climbing vines and it is considered a member of the same family as squash, watermelon, cantaloupes, cucumber, etc (Kar *et al.*, 2003). *Momordica charantia* are characterized by lobed leaves, yellow flowers, and edible fruit pod with bitter-taste. The fruit color is green with cucumber-shaped (as shown in figure 1.1), soft lengthwise ridges and surface bumps. As the fruit begin to mature gradually it become hard, turn yellow or brown in color. All parts of *Momordica charantia* (fruit, leaves, seeds, seed oil, and roots) are used (Der-Marderosian and Beutler, 2010). The genus *Momordica* contains about 60 species from the old world tropics (Stevens, 2012). The generic name apparently derives from the Latin *mordeo* (to bite), perhaps a reference to the jagged edges of the seeds; *charantia* is from the ancient Greek for

beautiful flower. The species *Momordica balsamina* has edible fruit, and is widely distributed as crops becoming naturalized throughout the tropics. The scientific name of this fruit is *Momordica charantia* L. *Momordica charantia* has also different common names including *Momordica charantia*, Bitter gourd, Karilla fruit, Balsam pear, Karolla, Cearasee and carillac undeamor. *Momordica charantia* in some English texts may be called by its local names. In Arabic, it is known as Khyar Karillash, in South Asia, as karela, in Kannada, paavayka, in Bengali, kerela, in Island Southeast Asia, ampalaya, in Filipino, parya, and peria in Malaysian, and pare in Javanese and Indonesian (Englberger, 2009; I3N-Brazil, 2014). In East Asia, it is known as kugua, in Chinese, yeaju in Korean, caraille or carilley in Latin America, Panama and some parts of central America it is known as balsamino, cerasee or sorosi in Jamaica and in some regions of South America and elsewhere, it is known as kudhrethnarhyas in Turkey (Lim, 2013).

Bio-active compounds of *Momordica charantia* in relation to its physiological functions

Previous studies have shown that water extracts of the leaf and fruit of *Momordica charantia* have a strong antioxidant activity and that *Momordica charantia* fractions are rich in phenolics (Kubola and Siriamornpun, 2008). The fruit of *Momordica charantia* have been reported to have significant compounds such as saponins (Matsuda *et al.*, 1998) and peptides (Yuan *et al.*, 2008); cucurbitane-type triterpenoid that is like the Charantin, an antidiabetic substance promising for the treatment of diabetes (Lee *et al.*, 2009). Many extracts studies of *Momordica charantia* have been investigated to possess antitumor activity, through the inhibition of mouse spontaneous

mammary tumorigenesis (Nagasawa *et al.*, 2002). Besides, anti-tumor activities, the antiviral (Lee *et al.*, 1995), and immunomodulating properties (Cunnick *et al.*, 1990) of this plant have also been reported. Recently, More than 50 triterpenoids have been isolated from *Momordica charantia* with different biological activities, some triterpenoids have antiproliferative and anti-invasive activities (Chen *et al.*, 2009). Moreover, two types of Flavonoids such as (rutin, naringin) extracted from the leaves of *Momordica charantia* successfully show growth inhibition of leukemia and ovarian carcinomas, with anti-invasive effects on melanoma. Such flavonoids might be promising components with critical roles against cancer cell progression (Yasuda *et al.*, 2009). The phenolic acids such as gallic acid, benzoic acid, coumaric acid, and *t*-cinnamic acid, from *Momordica charantia* are reported to exhibit an antioxidant activity (Horax *et al.*, 2005).

Anti-diabetic effect of *Momordica charantia*

There are many old herbal remedies that have been used to relieve symptoms or treat diabetes in many developing countries (Singh, 2011). *Momordica charantia* is one of most important plants that have been traditionally used for the treatment of diabetes (Hasan and Khatoun, 2012). Many studies have shown anti-hyperglycemic and hypoglycemic effects of the different extracts of *Momordica charantia* in both human and experimental animals (Fuangchana *et al.*, 2011; Wehash *et al.*, 2012).

Various extracts and components of *Momordica charantia* are attributed to the increasing glucose utilization in the liver through different physiological,

pharmacological and biochemical modes (Bhushan *et al.*, 2010). The hypoglycemic actions of *Momordica charantia* have many modes including the hypoglycemic effect (Ragasa *et al.*, 2011), stimulating insulin release from isolated beta cells (Ahmed *et al.*, 1998) stimulating utilization of peripheral and skeletal muscle glucose (Akhtar *et al.*, 2011), inhibiting the glucose uptake of intestinal (Abdollah *et al.*, 2010), inhibiting the differentiation of adipocyte (Nerurkar *et al.*, 2010), suppressing many gluconeogenic enzymes (Singh *et al.*, 2011), stimulating the key enzyme of HMP pathway (Shibib *et al.*, 1993) and inducing glucose uptake in liver (Welihinda *et al.*, 1986).

Methods

This research was conducted in the period from January 2016 until March 2016. Animal maintenance was performed at the Animal Hospital of Biotechnology Research Center at Al-Nahrain University, while the sample testing was carried out in the Laboratory of Enzymology Medical Department at Applied Biotechnology College.

Plant collection

The plant (*Momordica charantia*) was bought from the Malaysian market by Dr. Raghda Saad, Molecular and Medical Biotechnology Department, of Applied Biotechnology College/ AL-Nahrain University. The plant fruit were air dried at room temperature and crashed to be extracted.

Plant extraction

The manufacture of aqueous extract of *Momordica charantia* dry fruit were milled to obtain dry powder. Dry powder of

Momordica charantia fruit as much as 15g, half amount was then extracted with 1 L of water (1:5) for 2 hours at 100°C as reflux, then filtered with filter paper. The extract was dried using a rotary evaporator with a temperature of 50 °C to obtain crude extract of *Momordica charantia* fruit. The other half amount was extracted with 70% methanol (1:5) according to Fua *et al.* (2010) by using shaker incubator 25 c for 24hr. Buchner funnel was used to filter the extracted solution. Then, the rotary evaporator was used at 40°C to concentrate the filtered solution, finally the filtered solution was evaporated by lyophilize, and the resultant crude powder was kept at -20 °C until use.

Experimental design

Animals male mice with an age of 6 weeks were divided into five groups (5 each): control positive, *Momordica charantia* dose 10 mg/ml, and *Momordica charantia* dose 20 mg/ml and *Momordica charantia* dose 40 mg/ml. Mice in the control negative only fed the standard until the end of the treatment, while the remaining (20 rats) were fed basal diet and injected with 100µl of aloxan/kg of body weight three times during the same week then the glucose level were examined after one week.

The period of treatment was restricted for 10 days. The water and alcoholic extract of *Momordica charantia* were injected subcutaneously (100 µl) every day for 10 days. At the end of treatment, blood samples were collected (600-1000µl).

The measurement the glucose level was done by using the glucose kit from bio System Company. The color that appeared was measured by a spectrophotometer and the absorbance was read at the wavelength 500 nm.

Determination of glucose oxidase (GOD) activity

A colorimetric method using 4-aminoantipyrine- in a glucose oxidase-peroxidase system was proposed to determine the glucose level in the biological system. The principle involved in the estimation of glucose level was firstly the β-D glucose to be oxidized into gluconic acid and hydrogen peroxide in the presence of glucose oxidase (GOD). The hydrogen peroxides the reacts with phenol and 4-amino antipyrine by the action of peroxidase to form a pink colored quinoamine dye complex. In the present work, the level of β-D glucose was measured before and after the exposure of the glucose oxidase to radiation, the absorbance of the samples and standards was read against the blank at 500 nm. All the working steps were done according to the procedure of Sigma Company.

Sample preparation

Reagent 1 includes:

A: (Buffer solution): 50 mM sodium acetate buffer, then adjust to pH 5.1 at 35°C with 1 M HCl.)

B: *O*-Dianisidine Dihydrochloride (ODD): This reagent was prepared immediately by dissolving 10 mg in 4 ml of distilled water to obtain 0.21 mM of final concentration.

C: Peroxidase enzyme solution (POD)

D: Glucose Oxidase enzyme solution (GOD): This solution was prepared immediately before use. It contains 0.2 mg/ml of Glucose oxidase.

Reagent 2 (Standard solution): glucose solution (10% (w/v). standard solution. pipette the following reagents into a test tube as follow:

Reagent /serum	Volume (µl)Test	Volume (µl) blank	Volume (µl) Standard
Serum	10	-----	-----
Reagent 1	1000	1000	1000
Reagent 2	-----	-----	10

Table.1 Comparision between methanol extract and water extract of *Momordica charantia*

Treatment (Dosage)	Mean ± SD		LSD value
	Methanol extract	Water extract	
0.075 (mg/ml)	273.00 ± 83.16	260.67 ± 28.08	28.913 NS
0.15 (mg/ml)	266.00 ± 65.73	173.67 ± 12.23	33.612 *
0.30 (mg/ml)	247.67 ± 25.46	155.00 ± 7.81	27.053 *
* (P<0.05), NS: Non-significant.			

All samples were mixed, then incubated for 5 mn at 37°C. The increase in A_{500nm} was recorded and the $A_{500}/time$ (minute) was obtained by using the maximum linear rate for both tests $\Delta 7.5$ unit /ml - ΔA_{500nm} . The colour was stable for 30 mn. Then, the results were recorded immediately (Bergmeyer *et al.*, 1974).

Equation: absorbance of test/ absorbance of stander X 100

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to affect the difference factors in the study parameters. The least significant difference –LSD test was used to significantly compare between the means in this study.

Result and Discussion

The main target of the present study was to test the *Momordica charantia* in reducing the glucose level in mice suffering from type 2 diabetes after treatment with 100 µl of water and alcoholic extract of *Momordica charantia* for 10 days at different dosages of both extracts.

Yield of Crude Extracts

One species of *Momordica charantia* was extracted using two solvents systems namely, 70% methanol and water and the results were illustrated as bellow:

Various studies assessed the effects of *Momordica charantia* on blood glucose-level in healthy and diabetics individuals. The results found no significant changes in random blood glucose concentration following treatment with alcoholic extraction at different dosages in comparison to the water extraction of plants. The significant reduction was seen in the level of glucose in the serum of mice after treatment with 100 and 150 mg/ml of *Momordica charantia* in comparison to the a standard control group. A similar study by Lim *et al.*, (2010) showed that the *Momordica charantia* ingested the capsule of either fruit or leaves did not significantly reduce the blood glucose levels compared to when it was consumed orally, or injected subcutaneously under skin or in juice form. A study by Dans *et al.*, (2007) has shown no significant effect on fasting blood glucose from capsules of *Momordica charantia* isolated from Philippines. Therefore, further

studies are required to determine the efficacy of different species of *Momordica charantia* in modulating the level of glucose in serum in humans, particularly in a raw or fresh form together with rice-based meals, as commonly consumed in Malaysia (Mohd *et al.*, 2014).

Many animal studies have shown hypoglycaemic effects of different parts of *Momordica charantia* in normal animals (Mohammady *et al.*, 2012). Another animal study on albino rats by Singh *et al.*, (2008) has shown the effect of the alcoholic extract of *Momordica charantia* in lowering blood sugar levels feeding for 15 days. Another study on albino rats by Singh, N. and Gupta, (2007) confirmed the anti hyperglycemic effect of 0.25, 0.50 and 0.75 mg/kg body weight of acetone extract from whole fruit of *Momordica charantia* in lowering the blood glucose from 13.3% to 50.0% after treatment with alloxan. *In vitro* studies have indicated that *Momordica charantia* has powerful properties.

Many different substances including antidiabetic properties such as charantin, vicine, and polypeptide-p, and other unspecific bioactive components such as antioxidants phenolic components may negate the effects of oxidative stress caused by hyperglycaemia (Michael *et al.*, 2006). Metabolic and hypoglycemic effects have been demonstrated in cell culture, animal, and human studies. The oxidative stress has been linked to mechanisms that lead to the dysfunction of both beta and endothelium cells with insulin resistance which can lead to diabetes (Avogaro, 2011).

In conclusion, *Momordica charantia* has been widely studied for its medicinal properties to treat many diseases. This may be due to the fact that *Momordica charantia* possesses about 225 different medicinal

compounds (Taylor, 2002). All these compounds may act either separately or together to exert their beneficial effects via several mechanisms to control and treat many diseases.

The aqueous extract of *Momordica charantia* fruit dose of 0.75 mg has an effect on diabetes through reducing the level of glucose in the serum. To date, there is insufficient evidence to investigate the effectiveness of traditional Malaysian vegetables *Momordica charantia* in modulating blood glucose concentrations.

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