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Research Article
Antioxidant and Antidiabetic Activity of Aqueous Ethanolic Extract of *Momordica charantia* L. (*Cucurbitaceaevar Guti*) Fruit in Streptozotocin (STZ) Induced Diabetes Rats

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Abstract

Background and Objective: Type 2 diabetes is caused by the failure of beta cells (β-cells) to compensate for insulin resistance. This leads to hyperglycaemia, which can in turn exert deleterious effects on β cells. The current study aimed to assess the antioxidant activity and hypoglycemic effects of aqueous ethanolic extract of *Momordica charantia* L. fruit (MCE) in normal and streptozotocin induced diabetic rats. Moreover, the interacting effects of co-administration of MCE on the antidiabetic activity of metformin and glibenclamide were evaluated. **Materials and Methods:** Total phenolic content, DPPH radical-scavenging activity and oral glucose tolerance test (OGTT) for aqueous ethanolic extract of *Momordica charantia* L. fruit (MCE) were assayed. Albino rats were divided into five groups of six rats in each group: group 1 (NR): Normal rats, group 2 (DR): Diabetic rats, group 3 (DR administrated MCE at dose of 50 mg kg^{-1}), group 4 (DR administrated 600 μg kg^{-1} glibenclamide and) lastly group 5 (DR administrated MCE at dose of 50 mg kg^{-1} plus glibenclamide at dose of 600 μg kg^{-1}). The daily oral treatment was administered in between 08.00-09.00 AM for 4 weeks. Data were analyzed by using Excel (Microsoft Office 2007) and SPSS Version 18.0. **Results:** The total phenolic content of aqueous ethanolic extract of MCE was 673.9 mg GAE/100 g dry extract. The aqueous ethanolic extract of MCE exhibited 78.08% DPPH radical-scavenging activity at 500 μg mL^{-1} which was comparable to that of Butylatedhydroxytoluene (BHT) as positive standard at a concentration of 500 μg mL^{-1} (86.1%). The results of the main experiment indicate that the highest reductions (20.68, 35.54, 38.18 and 55.09) were observed for those diabetic rats treated with a combination of aqueous ethanolic extract of MCE (50 mg kg^{-1}) and 600 μg kg^{-1} of glibenclamide on 7th, 15th, 22nd and 30th day of treatment, respectively. Administration of MCE induced a significant protective effect reflected in the reductions of the serum levels of (AST and ALT), as well as kidney functions (urea and creatinine) in diabetic rats. **Conclusion:** These findings indicated that the hypoglycemic efficiency of the aqueous ethanolic extract of MCE at dose of 50 mg kg^{-1} was similar to the glibenclamide (600 μg kg^{-1}).

Key words: *Momordica charantia* L., type 2 diabetes mellitus, metformin, glibenclamide, streptozotocin, oral glucose tolerance test

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia resulting from disruption insulin secretion or action and is categorized into two main types, type 1 and type 2. Type 2 diabetes mellitus (T2DM) is the most common form of DM ,which make up nearly 90% of diabetics. The prevalence of DM is expected to rise to 4.4% in 2030 ((WHO)[1].

Egypt occupies the 9th country of the world in the incidence of diabetes. Recent changes in physical activities and food types have promoted incidence of diabetes and in case of failure to take preventive and corrective procedures, up to 13% of Egyptians will have diabetes by the year 2025[2,3].

T2DM is a metabolic disorder characterized by a weak response to the secretion of insulin or resistance to the action of insulin, or both of them, the causes of this disorder may be β-cell dysfunction, low sensitivity to insulin (IS), Insulin Resistance (IR) and hyperglycemia. Furthermore, IS and IR can appear five years before the diabetes disease becomes manifest[4].

Recently, diabetes medications include drugs acting on insulin-resistant or on the pancreas[5]. Metformin is the first-line pharmacological therapy for type 2 diabetes in parallel with the intervention of lifestyle, which is mended by the American Diabetes Association and the European Association for the Study of Diabetes[6]. Nevertheless, an annual failure rate of 17% for metformin treatment has been recorded , even though this was decreased to 12% (10.5-14.4%) in patients who initiated metformin therapy within 3 months of type 2 diabetes diagnosis[7].

Traditional medicines produced mainly from plants who play a great role in the therapy of diabetics[8,9]. *Momordica charantia* Linn belongs to the family of Cucurbitaceae and called bitter melon, bitter gourd, karela and grows in the tropical and subtropical areas of the world.

The *Momordica charantia* plant has been modified to grow in different climatic environments, however the best production is in warm regions[10]. *Momordica charantia* plant contains several bioactive compounds, mainly momordicin I, momordicin II and cucurbitacin B. The plant also contains reasonable amounts of glycosides (momordin, charantosides, goyaglycosides, momordicosides) and terpenoid compounds (momordicin-28, momordicinin, momordicilin, momordenol and momordol)[11-14]. Fruits and seeds of *Momordica charantia* have pharmaceutical characteristics such as, anti-inflammatory, anti-leukemic, antimicrobial, anti-HIV, anti-ulcer , antitumor and antidiabetic feature[15].

However, there is no sound of published data/evidence about the interacting effects of co-administration of aqueous ethanolic extract of *Momordica charantia* L. fruit (MCE) on the antidiabetic activity of metformin and glibenclamide; therefore, the current investigation was performed to explore the antioxidant and antidiabetic activities of aqueous ethanolic extract of *Momordica charantia* L. (*Cucurbitaceae*) fruit. In addition, to assess the interacting effects of co-administration of aqueous ethanolic extract of *Momordica charantia* L. (Cucurbitaceae) fruit on the antidiabetic activity of metformin and glibenclamide using male Albino rats.

MATERIALS AND METHODS

Chemicals: Streptozotocin (STZ) [2-Deoxy-2-(3- (methyl- 3-nitrosoureido)-D-glucopyranose)], BHT and polyoxy-ethylene-sorbitan monolaurate (Tween 80), were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Metformin HCl kindly supplied by CID pharmaceuticals, Cairo, Egypt. All other reagents were of analytical grade.

Source of *Momordica charantia* L. fruits: Ripe fruits of *Momordica charantia* variety of Guti (*Momordica charantia* L., Cucurbitaceae) used in the current investigation were collected in the year 2014 from a garden in Hofuf, Saudi Arabia. The fruits were identified by department of Botany, National Research Centre, Giza, Egypt.

Animals: Male Albino rats (Rattus CFT-Wister strain), weighing 230-240 g were purchased from the animal house of Food Technology Research Institute; Agricultural Research Centre, Giza, Egypt. Rats were accommodated in polypropylene cages lined with husk in standard conditions (temperature 25±2°C and 12:12 light: dark cycle). The animal experiments were conducted in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health[16]. The procedures of animal handling have been approved by committee of experimental animals ethics of the Food Technology Research Institute; Agricultural Research Centre, Giza, Egypt.

Reagent methodology kits: The kits used for determination of the activities of alanine transaminase (ALT) and aspartate transaminase (AST) were supplied by Reactivos GPL, Barcelona, Spain. Whereas, the kits used for determination of bilirubin and urea were obtained from Biosystems, Barcelona, Spain.

Preparation of the extracts: *Momordica charantia* L., (*Cucurbitaceae*) fruits were carefully dried at 40°C for 48 h in

an air convection oven, ground and passed through a 60 µm mesh sieve. Dried material (100 g) was extracted with 1000 mL of aqueous ethanol (ethanol: water, 70:30 v/v) by steeping at room temperature for 72 h. The extract has been filtered by using filter paper (Whatman No. 1). The solvent has been removed under vacuum at 45°C by using a rotary evaporator. Dry extract yield was approximately 11% (w/w) of crude material.The resulting extracts were kept in light-protected containers at -18°C until further use. The frozen extract has been reconstituted with a normal saline solution for preparation the final concentration as needed[17]. Based on preliminary studies to determine the best type of extracts, it has been shown that aqueous ethanol (ethanol: water, 70:30 v/v) extract had the highest efficiency and was selected for further studies.

Determination of total polyphenols: Total phenolic content of the extract of *Momordica charantia* L. was determined using the Folin-Ciocalteu assay system[18]. The results were expressed as mg of GAL equivalent/100 g DW.

Analysis of phenolic compounds using HPLC: Phenolic compounds of aqueous ethanolic extract of *Momordica charantia* L. (*Cucurbitaceaevar Guti*) fruit have been identified by using the method of Wu *et al.*[19]. The results are expressed as mg/100 g of extract.

Evaluation of antioxidant activity by DPPH radical assay: The free radical scavenging capacity of aqueous ethanolic extract of *Momordica charantia* L. (*Cucurbitaceaevar Guti*) fruit was assayed by using 1,1-Diphenyl-2-picrilhydrazyl (DPPH) method[20]. Different concentrations of *Momordica charantia* L. extract (100, 200,300,400 and 500 µg mL^{-1}) were individually mixed with 5 mL of 6×10^{-3} M methanolic solution of DPPH radical. The mixtures were vigorously shaken and left to stand in the dark for a half-hour. The absorbance was measured at 517 nm against a blank. The control was prepared, as above, without extract and methanol was used for the base line correction.

The radical-scavenging activity was expressed as percentage of inhibition and calculated using the following Eq.[20]:

$$\text{Radical scavenging activity}(\%) = \left[\frac{(A_{\text{control}} - A_{\text{sample}})}{(A_{\text{control}})}\right]\times 100$$

Where:

$A_{control}$ = Absorbance of DPPH radical+methanol

A_{sample} = Absorbance of DPPH radical+sample extract/standard

Preparation of drugs

Preparation of metformin: The tablets of metformin were ground into fine powder. Metformin powder was dissolved in deionized water and orally administered at dose of 100 mg kg^{-1} day^{-1}, according to Morales *et al.*[21].

Preparation of glibenclamide: Five milligrams of glibenclamide (Sanofi-aventis , S.A.E. Cairo, Egypt) tablet was dissolved in 83.33 mL of deionized water to give 60 µg mL^{-1} solutions. This solution was orally administered at dose of administered at a dose of 600 µg kg^{-1} b.wt.[22].

Induction of experimental diabetes: After an overnight fasting (deprived of food for 16 h had been allowed free access to water), hyperglycemia was induced by intraperitoneal injection of 60 mg kg^{-1} of freshly prepared solution of STZ (0.1 g dissolved in 5 mL of freshly prepared sodium citrate buffer 0.1 M, pH 4.5)[23]. The STZ-injected animals were given 20% of glucose solution for 24 h to prevent mortality which occurs as a result of hypoglycemic effects of drugs. Diabetic status of animals was confirmed by monitoring Fasting Blood Glucose (FBG) using Accu check glucometer (Accu check, Roche,USA). Rats with FBG level of more than 200 mg dL^{-1} were considered as diabetic and selected for further study.

Experimental design

***In vitro* assay:** To determine the optimal dose of *Momordica charantia* L. extract, the effect of graded doses of aqueous ethanolic extract of *Momordica charantia* L. fruit on blood sugar concentrations during (OGTT) in normal rats was assayed. The OGTT was performed in overnight fasted normal rats. Normal rats were divided into six groups each consisting of five rats. The first one presents the control rats. The rats of the second to sixth groups were treated orally with aqueous ethanolic extract of *Momordica charantia* L. (*Cucurbitaceaevar Guti*) fruit at dose of 50, 75, 100, 125 and 150 mg kg^{-1}. Glucose (2 g kg^{-1}) was administered orally to each rat. Blood was withdrawn from the tail vein at 0, 30, 60 and 120 min. Glucose levels were measured immediately by Accu check glucometer (Accu check, Roche,USA).

The second experiment of *in vitro* assay, was conducted to evaluate the interacting effects of co-administration of 50 mg kg^{-1} of *Momordica charantia* L. extract and metformin or glibenclamide in diabetic rats by OGTT. Oral glucose tolerance test was performed in overnight fasted diabetic rats. The rats were divided into eight groups each consisting of five rats as follows:

The group no.1 administrated 2 g of glucose kg^{-1} of rat weight, the group no. 2. administrated 2 g of glucose kg^{-1} with 100 mg kg^{-1} of metformin, the third group administrated 2 g glucose kg^{-1} with 50 mg kg^{-1} of MCE. The fourth group adminisrated glibenclamide (600 μg kg^{-1}), followed by administration of 2 g of glucose kg^{-1} after 30 min of glibenclamide administration . The fifth group administrated 50 mg kg^{-1} of MCE, followed by administration of 2 g of glucose kg^{-1} after 30 min of MCE administration. Sixth group administrated glibenclamide (600 μg kg^{-1}, followed by 2 g of glucose kg^{-1} and metformin (100 mg kg^{-1}) was administrated after 30 min of glibenclamide administration. The seventh group administrated glibenclamide (600 μg kg^{-1}) followed by 2 g of glucose kg^{-1} and 50 mg kg^{-1} of MCE was administrated after 30 min of glibenclamide administration. The last one administrated 50 mg kg^{-1} of MCE, followed by 2 g of glucose kg^{-1} and metformin (100 mg kg^{-1}) was administrated after 30 min of Glibenclamide administration.

Blood samples of each group were withdrawn from the tail vein at 0, 30, 60 and 120 min. Glucose levels were measured immediately by Accu check glucometer (Accu check, Roche, USA).

***In vivo* assay:** For evaluation the hypoglycemic effects of aqueous ethanolic extract of MCE, glibenclamide and combination of them on diabetic states of STZ induced diabetic rats, the current biological experiment was conducted. The rats were divided into five groups of six rats in each group: group 1 (NR): Normal rats, group 2 (DR): Diabetic rats, group 3 (DR administrated MCE at dose of 50 mg kg^{-1}), group 4 (DR administrated at 600 μg kg^{-1} glibenclamide) and lastly group 5 (DR administrated MCE at dose of 50 mg kg^{-1} plus glibenclamide at dose of 600 μg kg^{-1}).

The daily oral treatment was administered in between 08.00 to 09.00 AM for 4 weeks.

Blood sampling: Samples of blood were collected from the tail vein and the blood sugar concentration was analyzed using Accu check glucometer (Accu check, Roche, USA). Samples of venous blood were taken at the beginning of the biological experiment and at 1, 2, 3 and 4 weeks from the start of the experiment.

Serum analysis: At the end of experimental period (4 weeks), the rats were fasted overnight and sacrificed by cervical decapitation. The blood of rats were centrifuged at 4000 rpm for 10 min to obtain the sera and kept in the deep freezer (-18°C) until analysis. Alanine aminotransferase, aspartate aminotransferase activities were assayed according to the procedures described by El-Anany and Ali[24]. Urea and creatinine were assayed according to the procedures described by El-Anany and Ali[24] and Qusti *et al.*[25], respectively.

Statistical analysis: Results are expressed as Mean ± Standard Deviation (SD). Data were analyzed according to the procedures described by Gomez and Gomez[26] by using Excel (Microsoft Office, 2007) and SPSS Version 18.0 (SPSS Inc., Chicago, IL, USA). Probability $p \leq 0.05$ indicated significance.

RESULTS

Phenolics of aqueous ethanolic extract of *Momordica charantia* L.: The total phenolic content of aqueous ethanolic extract of *Momordica charantia* L. (*Cucurbitaceaevar Guti*) fruit was 673.9±29.8 mg GAE/100 g dry extract.The amount of phenolic constitutes of aqueous ethanolic extract of *Momordica charantia* L. (*Cucurbitaceaevar guti*) fruit were tabulated in Table 1. The predominant phenolics of aqueous ethanolic extract of *Momordica charantia* L. (*Cucurbitaceaevar guti*) fruit were gallic acid, pyrogallol, catechin, Protocatechuic gentisic acid, chlorogenic acid, rutin, caffeic acid, vanillic acid, syringic acid and coumaric acid.

DPPH radical scavenging activity of aqueous ethanolic extract of *Momordica charantia* L.: Antioxidant activities of aqueous ethanolic extract of *Momordica charantia* L. (*Cucurbitaceaevar guti*) fruit, as determined by the DPPH

Table 1: HPLC analysis of phenolic compounds (mg/100 g DW) of aqueous ethanolic extract of *Momordica charantia* L.

Phenolic compounds (mg/100 g)	Aqueous ethanolic extract of *Momordica charantia* L.
Gallic	151.90
Pyrogallol	13.80
Catechin	12.70
Protocatechuic	8.90
Gentisic acid	6.80
Chlorogenic acid	35.80
Rutin	90.70
Caffeic	0.17
Vanillic	1.90
Syringic acid	8.90
Coumaric acid	16.80

Table 2: DPPH scavenging activity of aqueous ethanolic extract of *Momordica charantia* L.

Concentration (μg mL^{-1})	100	200	300	400	500
Inhibition (%)					
Extract	23.50^{j}	36.2^{h}	42.8^{f}	61.3^{d}	78.0^{b}
BHT	29.10^{i}	38.4^{g}	46.1^{e}	63.2^{c}	86.1^{a}
LSD at 0.05%*	0.41				

Values are Means±Standard Deviation (SD) of three determinations, values followed by the same letter are not significantly different ($p<0.05$), *Least significant difference at $p\leq0.05$ according to Duncan's multiple-range test

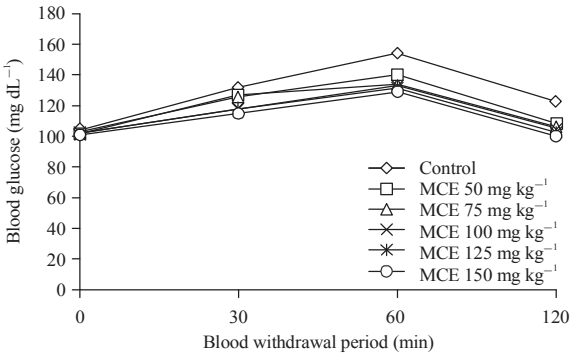

Fig. 1: Effect of graded doses of aqueous ethanolic extract of *Momordica charantia* L. fruit (MCE) on blood glucose levels during (OGTT) in normal rats (n = 5)

radical scavenging method, are shown in Table 2. DPPH inhibition percentage values were dose dependent, these inhibitions were gradually and significantly ($p\leq0.05$) increased with increasing the concentrations of both extract and Butylatedhydroxytoluene (BHT). The aqueous ethanolic extract of *Momordica charantia* L. exhibited 78% DPPH radical-scavenging activity at 500 μg mL^{-1} which was comparable to that of BHT as positive standard at a concentration of 500 μg mL^{-1} (86.1%) (Table 2).

Effect of graded doses of aqueous ethanolic extract of *Momordica charantia* L. fruit on blood glucose levels during (OGTT) in normal rats: Figure 1 shows the effect of graded doses of aqueous ethanolic extract of *Momordica charantia* L. fruit on blood glucose levels (OGTT) in normal rats. The results showed that there were no significant differences in the initial fasting blood sugar levels of all groups under investigation. After oral administration of glucose in normal rats, blood glucose levels were significantly ($p\leq0.05$) higher at 60 min. Significant reductions ($p\leq0.05$) were recorded after 120 min of oral administration of glucose, these reduction ranged from ~14-24%. Aqueous ethanolic extracts of *Momordica charantia* L. fruit produced significantly the higher reductions ($p\leq0.05$) in blood glucose at 120 min ranged from~ 21-24%. The highest hypoglycemic activity (~24%) was observed after 120 min for those rats administrated the aqueous ethanolic extract of *Momordica charantia* L. fruit at dose of 50 mg kg^{-1} b.wt. According to these findings the *Momordica charantia* L. fruit extract at level 50 mg kg^{-1} was selected for further study.

Effect of co-administration of 50 mg kg^{-1} of MCE and metformin or glibenclamide in on blood glucose of diabetic rats by OGTT: Blood sugar concentrations in diabetic rats varied from 223-233 mg dL^{-1}. Blood glucose levels reached peak after 60 min of oral administration of glucose. Control group of diabetic rats had significantly ($p\leq0.05$) the highest (315 mg dL^{-1}) concentration of blood sugar at 60 min after glucose administration (Fig. 2).

Glucose levels of rats treated with the extract or extract-drug combinations had significantly the lower values of blood glucose compared to control group. The levels of blood glucose significantly ($p\leq0.05$) declined after 120 min of glucose administration for all groups. However, glucose levels of control group remained the higher after 120 min. The highest ($p\leq0.05$) reductions were observed when rats were given combination of glucose and metformin as well as a combination of glucose and MCE after 30 min of glibenclamide administration. Moderate reductions at 120 min (19.66, 17.41 and 21.34%), respectively were observed when diabetic rats administered glibenclamide (600 μg kg^{-1}) 30 min prior to administration of 2 g kg^{-1} glucose; rats administered 50 mg kg^{-1} of MCE before 30 min of administration of 2 g kg^{-1} of glucose as well as those rats administered 50 mg kg^{-1} of MCE before 30 min of administration (Fig. 2) of 2 g kg^{-1} of glucose with 100 mg kg^{-1} of metformin.

Diabetic rats treated with the combination of aqueous ethanolic extract of *Momordica charantia* L. fruit (50 mg kg^{-1}) and metformin (100 mg kg^{-1}) achieved a more significant ($p<0.05$) decrease in blood glucose level compared with those rats administrated the monotherapies. The results also indicate that the blood glucose levels at 120 min in those rats treated with metformin (100 mg kg^{-1}) were similar to those treated with 50 mg kg^{-1} of *Momordica charantia* L. fruit extract (Fig. 2).

Table 3: Effect of aqueous ethanolic extract of *Momordica charantia* L. (50 mg kg^{-1}), glibenclamide (600 µg kg^{-1}) and combination of them on some liver and kidney functions in streptozotocin-induced diabetic rats

	Liver functions		Kidney functions	
Groups	Aspartate aminotransferase (AST) (IU L^{-1})	Alanine aminotransferase (ALT) (IU L^{-1})	Urea (mg dL^{-1})	Creatinine
1	34.0±2.4^{b}	25.3±3.1^{c}	29.4±3.0^{c}	0.56±0.07^{b}
2	49.9±6.1^{a}	46.3±1.4^{a}	49.0±1.8^{a}	0.79±0.08^{a}
3	41.8±4.1^{ab}	36.2±2.1^{b}	36.4±2.7^{b}	0.57±0.05^{b}
4	44.7±2.0^{a}	39.7±3.1^{b}	34.1±1.3^{bc}	0.58±0.06^{b}
5	40.1±4.1^{ab}	35.1±4.2^{b}	33.1±2.3^{bc}	0.56±0.11^{b}
LSD at 0.05%*	7.3	5.4	4.28	0.14

Data are expressed as Mean±SD, given values represent means of five determinations, values followed by the same letter are not significantly different ($p<0.05$), *Least significant difference at $p\leq0.05$ according to Duncan's multiple-range test

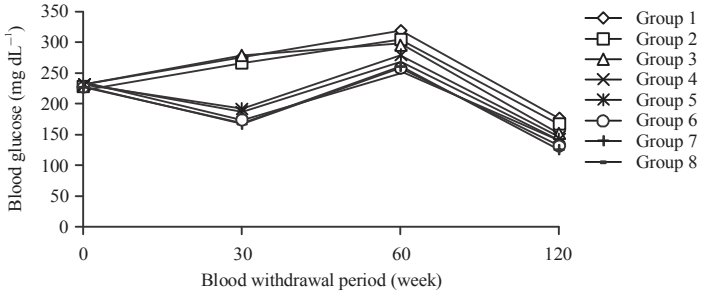

Fig. 2: Effect of aqueous ethanolic extract of *Momordica charantia* L. fruit (MCE), metformin, glibenclamide and co-administration of them on blood glucose levels during (OGTT) in diabetic rats (n = 5)

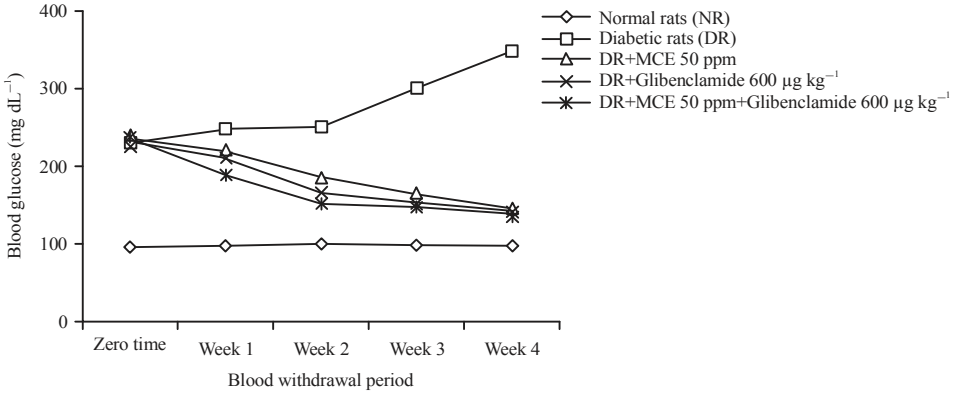

Fig. 3: Hypoglycemic effect of aqueous ethanolic extract of *Momordica charantia* L. fruit (MCE), glibenclamide and combination of them on fasting blood glucose levels in STZ induced diabetic rats (n = 6)

These findings indicate that the effectiveness of the aqueous ethanolic extract of *Momordica charantia* L. fruit (50 mg kg^{-1}) was similar to the metformin (100 mg kg^{-1}). Accordingly, the aqueous ethanolic extract of *Momordica charantia* L. fruit at dose of 50 mg kg^{-1} can be used instead of metformin, especially for renal or hepatic diseases.

Effect of aqueous ethanolic extract of MCE, glibenclamide and combination of them on diabetic states of STZ induced diabetic rats: After these preliminary experiments, the fundamental experiment was performed in order to determine the hypoglycemic activities of aqueous ethanolic extract of *Momordica charantia* L. fruit, glibenclamide and combination of them in STZ induced diabetic rats. Figure 3 shows the impact of aqueous ethanolic extract of *Momordica charantia* L. fruit; glibenclamide and combination of them on blood glucose levels in STZ induced diabetic rats for 4 weeks of continuous treatment. No significant changes in the level of blood glucose were shown for control group; however the administration of STZ caused significant ($p\leq0.05$) increase in

blood glucose levels starting from the beginning of the experiment and towards the end of the experiment. In STZ rats, the daily administration of aqueous ethanolic extract of *Momordica charantia* L. fruit, glibenclamide and combination of them caused a significant ($p<0.05$) progressive reduction in blood glucose levels compared to diabetic control rats (Fig. 3). The dose of 50 mg kg^{-1} of *Momordica charantia* L. fruit extract reduced the blood glucose levels by 7.18, 21.13, 30.26 and 38.71% on 7th, 15th, 22nd and 30th day of treatment respectively. Similar reductions of 7.69, 28.0, 33.78 and 39.0%, respectively were showed in blood glucose levels for diabetic rats treated daily with 600 µg kg^{-1} of glibenclamide on the same days. The highest reductions (20.68,35.54, 38.18 and 55.09%) were observed for those diabetic rats treated with a combination of aqueous ethanolic extract of *Momordica charantia* L. fruit (50 mg kg^{-1}) and 600 µg kg^{-1} of glibenclamide on 7th, 15th, 22nd and 30th day of treatment, respectively. These findings indicate that the hypoglycemic effectiveness of the aqueous ethanolic extract of *Momordica charantia* L. fruit at dose of 50 mg kg^{-1} was similar to the glibenclamide (600 µg kg^{-1}).

Effect of aqueous ethanolic extract of MCE, glibenclamide and combination of them on some liver and kidney functions in diabetic rats: Table 3 shows the effect of aqueous ethanolic extract of *Momordica charantia* L. fruit (50 mg kg^{-1}), glibenclamide (600 µg kg^{-1}) and combination of them on some liver and kidney functions in streptozotocin-induced diabetic rats. Results indicated that the activities of AST and ALT enzymes of diabetic rats suffered significant ($p<0.05$) increases of up to 46.63 and 83.06%, respectively, when compared to normal controls. Treatment of diabetic rats with aqueous ethanolic extract of *Momordica charantia* L. fruit (50 mg kg^{-1}), glibenclamide (600 µg kg^{-1}) or combination of them caused marked reductions in the elevated activities of AST and ALT enzymes of diabetic rats. The treatment of diabetic rats with the combination of aqueous ethanolic extract of *Momordica charantia* L. fruit (50 mg kg^{-1}) and glibenclamide (600 µg kg^{-1}) showed significantly ($p<0.05$) higher reduction of ALT and AST levels compared to each one of them separately. The concentrations of creatinine and urea were significantly ($p<0.05$) higher in STZ-diabetic rats compared to normal control rats (Table 3). Diabetic rats administered with aqueous ethanolic extract of *Momordica charantia* L. fruit (50 mg kg^{-1}), glibenclamide (600 µg kg^{-1}) or combination of them showed significant ($p\leq0.05$) decreases in the level of creatinine and urea (Table 3). Interestingly, No significant differences in the concentrations of creatinine and urea were shown for those rats administrated combination of aqueous ethanolic extract of *Momordica charantia* L. fruit (50 mg kg^{-1}) with glibenclamide (600 µg kg^{-1}) and those of control group.

DISCUSSION

The results of the current investigation showed that the hypoglycemic activity of the aqueous ethanolic extract of *Momordica charantia* L. fruit at dose of 50 mg kg^{-1} was similar to the glibenclamide (600 µg kg^{-1}).

The medicinal properties of the plants are mainly attributed to their phytochemical constituents such as polyphenols, saponins, alkaloids etc.[27-30]. The total phenolic contents of aqueous ethanolic extract of *Momordica charantia* L. (*Cucurbitaceaevar Guti*)) fruit of the current study was higher than those of Choo *et al.*[31]. In the same time, is in good agreement with those of Ng *et al.*[32]. Phenolics are polar aromatic compounds that are readily soluble in polar solvents such as water, alcohols and water-alcohol mixtures[33]. The recovery of polyphenolic molecules in different plant samples is affected by the polarity of extracting solvents and the solubility of phenolics in extraction systems[34-36]. Determining of Phenolic components by using HPLC system has a feature over determination of total phenolic content by Folin-Ciocalteu method, as it provides more accurate quantitative about individual components[37]. *Momordica charantia* L. has high contents of phenolics such as gallic acid, gentisic acid, catechin and epicatechin[38]. Ibrahim *et al.*[39] studied the antioxidant and phenolic compounds of certain cucurbitaceous plants planted in Egypt, they showed that the presence of chlorogenic acid at level of 16.3 and 27.7 mg/100 g DW in *C. colocynthis* L. and *C. sativus* L., respectively and they reported also that gallic acid was the major compound of phenolics of *M. charantia* L. herb.

DPPH method has been used to examine antioxidant activity in complex biological systems because this assay is sensitive, requiring only a small quantity of samples and used for testing both lipophilic and hydrophobic substances[40]. The radical scavenging activity of poly-phenolics affected by the molecular structure and the substitution style of the hydroxyl groups and the possibility of stabilization of the resulting phenoxyl radicals through hydrogen donation or by expanded electron delocalization[41]. The hypoglycemic effect of aqueous ethanolic extract of *Momordica charantia* L. fruit may be due to the existence of triterpene, proteid, steroid, charantin, charantin, alkaloid and phenolic compounds which possesses hypoglycemic activity[42-43]. Streptozotocin (STZ) is a glucose analog, has a specific cytotoxic action on the pancreatic β-cells in the islets of Langerhans and induces diabetes

mellitus in experimental animals[44]. Streptozotocin (STZ) is an antibiotic which produced by *Streptomyces achromogenes*[45]. The action of STZ may be due to vitiate glucose oxidation and reduction of biosynthesis and secretion of insulin. The STZ produces reactive oxygen species (ROS) in the body, resulting in pancreatic damage, as well as could be responsible for raised the concentrations of blood glucose and peroxidation of lipids[46].

Glibenclamide is oral antidiabetic drug has been used as a hypoglycemic drug for type 2 DM patients from 1973 until now[47]. The main mechanism of action of this drug is stimulation of insulin secretion[48]. Glyburide, also called glibenclamide . Glyburide drug acts through the promotion of the secretion of insulin from the β-cells of the pancreas in people with normal and type 2 DM. Glibenclamide should be given at least 30 min and preferably 60 min before meals to include of the height of postprandial glucose. Therefore it is recommended to take this drug from 10 p.m. to 11 p.m. to be effective in lowering the fasting blood glucose levels in the morning[49]. Metformin is a hypoglycemic drug used to improve glucose tolerance in patients with type 2 diabetes, metformin drug decreases both basal and postprandial blood glucose. The mode of action of metformin is decreasing production of hepatic glucose, inhibiting the absorption of intestinal glucose as well as improving utilization of glucose[50]. The antihyperglycemic activity of aqueous ethanolic extract of *Momordica charantia* L. fruit (MCE may be attributed to its preventative and protective effect against STZ-mediated injury to the β-cells of the pancreas and probably because of the regeneration of damaged β-cells and also probably because of reduction of gluconeogenesis and glycogenesis[51]. Sulfonylureas i.e., glibenclamide, are the commonly used agent for the or treating type 2 diabetes DM, these drugs act by enhancing the secretion of insulin. This action increases the responses of the β-cells, which, which leads to increase insulin levels at all blood sugar concentrations[52]. On the other hand, metformin stimulate tissue absorption of glucose and increase insulin receptor bindings[53]. At the hepatocellular level, metformin is known to improve insulin-mediated glycogen synthesis and inhibit gluconeogenesis[54]. The hypoglycemic effect of aqueous ethanolic extract of *Momordica charantia* L. fruit may be due to the existence of hypoglycemic compounds such as glycosides terpenoid,and flavonoids[15,16].

Our results showedthat the effectiveness of the aqueous ethanolic extract of *Momordica charantia* L. fruit (50 mg kg^{-1}) is similar to the metformin (100 mg kg^{-1}). Most common undesirable influences of metformin are digestive disorders, abdominal pain and metallic taste. It is recommended that metformin should not be taken for patients who suffer from kidney disorders or hepatic problems or heart failure[55].

During the *in vivo* assay administration of STZ caused marked increases in blood glucose levels. STZ has potent alkylating property[56] and is specifically cytotoxic to the pancreatic β-cells in mammals. The pancreatic β-cell preferentially uptakes STZ leading to the formation of superoxide radicals. Furthermore, the liberation of no radical lead to the destruction of β-cells through necrosis process[57]. The extracts of *Momordica charantia* had immediate influence on the regeneration of the islets of pancreas and might enhancing the secretion of insulin from the pancreatic β-cells[58]. Daily administration of the ethanolic extract of *Momordica charantia* L. fruit to rats reduced the level of sugar in the blood by inhibiting gluconeogenesis process by suppressing the hepatic gluconeogenic enzymes and enhancing glucose oxidation pathways through activation of glucose-6-phosphate dehydrogenase[59]. The highest reductions were observed for those diabetic rats treated with a combination of aqueous ethanolic extract of *Momordica charantia* L. fruit and glibenclamide. Pharmacokinetic factors as alteration of absorption due to binding that has been reported with co-administration of some herbs[60]. When the medical drug is taken orally, it transmits through digestive system in mostly the same way as food and herbs taken. Therefore, when it is mixed with herb, each can change the others pharmacokinetic profile, such as absorption, distribution, metabolism and/or excretion. Some agents interfere with the body's ability to absorb herbs. Similarly, some herbs and food can lessen or elevate the effect of a drug[61,62]. Liver plays a central role in carbohydrate metabolism which function can be affected in diabetes[63]. Results indicated that the activities of AST and ALT enzymes of diabetic rats suffered significant increases ($p \leq 0.05$). These increases may be attributed to the fact that STZ mediated liver injuries which can cause leakage of liver enzymes into the blood[64], indicated the hepatotoxic effect of streptozotocin. High activities of serum aminotransferasesare a common marker of hepatic diseases and were detected more frequently among diabetics than normal population[65-67]. Treatment of diabetic rats with MCE caused decreases in activities of AST and ALT enzymes of diabetic rats. Most of the properties of *Momordica charantia* L. fruit extract are due to the polyphenolic component of *Momordica charantia* L. fruit. The inhibition of radical species could, therefore, be one of the mode of actions involved in the efficient hepatoprotective and curative properties of *Momordica charantia* L. fruit extract. Treatment of diabetic rats with polyphenolic extract showed potential hepatoprotective activity[68-69].

The continuous hyperglycemia, haemodynamic changes within the kidney tissue and free radical generation mediated tension in diabetes produce renal disorder which results rising

of urea and creatinine concentrations in blood[51,70]. It has been showed that the metabolic abnormalities observed in uncontrolled diabetes lead to gluconeogenesis[71] and consequently urea production, which are substantially enhanced in diabetes[72]. This study results indicate that no significant differences in the concentrations of creatinine and urea were shown for those rats administrated combination of aqueous ethanolic extract of *Momordica charantia* L. fruit (50 mg kg^{-1}) with glibenclamide (600 μg kg^{-1}) and for control group. This finding could be attributed to the ability of these agents to regulate the concentration of blood sugar, since diabetic hyperglycaemia results in kidney disorders[73]. Chronic hyperglycemia and dyslipidemia are associated with a different of metabolic dysfunctions in diabetic patients[74], causing oxidative stress, exhaustion the activity of the antioxidative system and resulting in increased the amounts of Reactive Oxygen Species (ROS)[75]. Oxidative Stress may cause the injury's of liver and kidney tissues[76], which is noted in the elevated concentrations of AST, ALT activities as well as of urea and creatinine. Polyphenolic compounds may, therefore, prevent the injury and death of pancreatic β-cells, and/or enhance the regeneration of this type of cells in diabetic rats[77].

The findings of this study thus suggested that aqueous ethanolic extract of *Momordica charantia* L. fruit (MCE) is a safe and effective treatment for the diabetic patients.

CONCLUSION

Phenolics content of MCE was 673.9 mg GAE/100 g dry extract. The aqueous ethanolic extract of MC exhibited 78.08% DPPH radical-scavenging activity at 500 μg mL^{-1}. The highest hypoglycaemic activity (23.85%) during OGTT for normal rats was observed after 120 min for those rats administrated the aqueous ethanolic extract of *Momordica charantia* L. fruit at dose of 50 mg kg^{-1} b.wt. The hypoglycemic effectiveness of the aqueous ethanolic extract of *Momordica charantia* L. fruit at dose of 50 mg kg^{-1} was similar to the glibenclamide (600 μg kg^{-1}). The AST and ALT enzymes of diabetic rats suffered significant increases, when compared to normal controls. Treatment of diabetic rats with aqueous ethanolic extract of MEC, glibenclamide or combination of them caused marked reductions in the elevated activities of AST and ALT enzymes of diabetic rats. Adminstration of MCE attenuates the changes of the functions of liver and kidney of diabetic rats.

SIGNIFICANCE STATEMENTS

This study discovers the hypoglycemic effects of aqueous ethanolic extract of *Momordica charantia* L. fruit (MCE) and the interacting effects of co-administration of MCE on the antidiabetic activity of metformin and glibenclamide. This study showed that the aqueous ethanolic extract of *Momordica charantia* L. (*Cucurbitaceaevar Guti*) fruit at dose 50 mg kg^{-1} exhibited significant anti-hyperglycemic activity in diabetic rats. The findings of this study will help in advancing the search for natural compounds that could be used as hypoglycemic agents or incorporated into drugs for treatment of the diabetic Patients.

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