

Original Article

Antioxidant and Antidiabetic Activities of *Cucumis Melo* Var. *Flexuosus* Leaf Extract

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Abstract

Cucumis melo var. *flexuosus* (*C. melo* var. *flexuosus*) is one of the ancient horticultural crops in many parts of the world. However, there has been no study reported its effects on diseases. Therefore, the aim of present study was to investigate antioxidant and antidiabetic activities of *C. melo* var. *flexuosus* leaf. First, the antioxidant activity of snake melon leaf extract was determined. Then the biochemical parameters for diabetic rats treated with snake melon (30, 60 and 120 mg/kg b.w) were investigated. The leaf extract showed a high antioxidant activity. Further, diabetic rats treated with snake melon leaf extract showed a significant decrease in the levels of blood glucose, total cholesterol, triglycerides and low-density lipoprotein cholesterol, lactate dehydrogenase, liver enzymes, and malondialdehyde and a significant increase in insulin, body weight, high-density lipoprotein cholesterol, total protein, catalase and superoxide dismutase levels. This study demonstrated the antioxidant and antidiabetic activities of leaf extract of snake melon.

Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia and hypoinsulinemia or insulin resistance (1) leading to a lot of complications (2). It is supposed that oxidative stress plays an essential role in the development of diabetic complications (3). But, antioxidants stand as a protective shield against the risk of oxidative stress (4, 5). The plants are famous for containing a variety of bioactive compounds, such as antioxidants (6)

and their low side effects (7).

Cucumis melo L. (*melon*) is one of the most popular horticultural crops worldwide which belongs to the *Cucurbitaceae* family (8). Many studies were done on different plants of this family due to their nutritional, medicinal, ethnoveterinary and ethnomedicinal values (9). *Cucumis melo* var. *flexuosus* is a member of this famous family that was known from ancient Egyptians and Romans (10). *C. melo* var. *flexuosus* has several names such as snake melon, Armenian cucumber, agoor, snake cucumber, and furious (11, 12). Its fruit is usually slender and is almost always bent and twisted (13). Immature fruits are harvested and consumed as a raw vegetable for salad and for medicinal use (8). Despite the fame of this plant as a vegetable used by many peoples, but its effect on our bodies is still

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(Received on July 1, 2018)

unknown. Therefore, the present study was aimed to evaluate the possible antioxidant and antidiabetic activities of *C. melo* var. *flexuosus* leaf extract.

Materials and Methods

Plant material and preparation of extract

Leaves of snake melon were collected from Benha, Egypt, in Augustus 2017 and authenticated by Dr. Mahran El Nagar (Department of Horticulture, Faculty of Agriculture, Benha University, Benha, Egypt). The fresh leaves (3.5 kg) were dried in the shadow, crushed to get 300 g of homogeneous fine powder and then extracted with 70% ethanol using a Soxhlet apparatus. The aqueous ethanolic extract was concentrated at 35-40°C using a rotary evaporator to give 30 g of solid extract. After that, the extract was stored at -10°C for experimental use.

Determination of total phenolic content (TPC)

Total phenolic content in the extract was determined by Folin-Ciocalteu reagent (14). Leave extract (200 µg/ml) was mixed with the Folin Ciocalteu reagent (400 µl) and 1.5 ml of 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml of distilled water. After 2 h, the absorbance at 765 nm was determined. The concentration of total phenolic content was determined as mg of gallic acid equivalent (GAE) per g of dry weight of plant powders. The whole experiment was repeated three times.

Determination of total flavonoid content (TFC)

Total flavonoid content was determined according to the method described by Kumaran (15). 1 ml of plant extract in ethanol (200 µg/ml) was mixed with 1 ml aluminum trichloride in ethanol (20 mg/ml) and a drop of acetic acid and then diluted with ethanol to 25 ml. The absorption at 415 nm was read after 40 min. Blank samples were prepared from 1 ml of plant extract and a drop of acetic acid and then diluted to 25 ml with ethanol. The total flavonoid content was determined using a standard curve of quercetin and

expressed as mg of quercetin equivalent (QE/g of extract dry materials). The whole experiment was repeated three times.

DPPH radical scavenging activity

The method of Chew *et al.* (16) has been used for the scavenging ability of DPPH (2, 2-diphenyl-1-picrylhydrazyl) antioxidant test with slight modification. 1 ml of different concentrations of diluted extracts of the plant parts in ethanol was added to 1 ml of DPPH (0.15 mM in ethanol). 1 ml DPPH with 1 ml ethanol was prepared as a control. The reaction mixtures were mixed very well by hand and then incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm with a UV-Visible spectrophotometer. The ascorbic acid was used as a positive control and the ethanol was used as a blank. The DPPH scavenging ability of plant extracts was calculated using the following equation :

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

The Abs control is the absorbance of DPPH + ethanol; Abs sample is the absorbance of DPPH + sample. All measurements were done in triplicate.

Acute toxicity study

The mean lethal dose (LD50) of the aqueous ethanolic extract of *C. melo* var. *flexuosus* leaf was determined in rats using the method described by Lorke (17).

Experimental animals

Male Wistar albino rats weighing 123–124 g were purchased from Helwan Farm of Egyptian Organization for Vaccine and Biological Preparations, Egypt. The rats were fed a standard chow diet and allowed to drink water ad libitum during the experimental period (30 days). The rats were housed in animals cages under standard environmental conditions (22±1°C, 12 h light/12 h dark cycle). Animals were acclimatized for a period of 7 days in our laboratory condition prior to the experiment.

Induction of diabetes

The overnight fasted rats were injected with a single dose of streptozotocin or STZ (60 mg/kg body weight in 0.1 M citrate buffer, pH 4.5). After 72 h of STZ administration, the tail vein blood was collected to determine fasting blood glucose level and only rats with blood glucose higher than 200 mg/dl were considered diabetic and included in the experiment

Experimental design

The animals were divided into five groups of six rats in each group as follows: Group 1: Normal control; Group 2: diabetic control; and Groups 3–5: diabetic rats treated orally with *C. melo* var. *flexuosus* leaf extract at doses of 30, 60, and 120 mg/kg bw, respectively. At 5th day of the experiment, different doses of *C. melo* var. *flexuosus* leaf extract were administered orally using an intragastric tube daily to the above groups for 30 days. On the 31th day post-treatment, the overnight fasting animals were ether-anesthetized. The blood samples were collected from a post caval vein and directly transported to tubes containing EDTA. The blood samples were separated by centrifugation at 1500 xg for 15 min. Then, the supernatants were stored as plasma at -20°C until assayed.

Biochemical analysis

Determination of plasma insulin

Plasma insulin level was measured by using rat insulin ELISA kit purchased from BioVendor – Research and Diagnostic Products (Japan).

Determination of lipid profile and lactate dehydrogenase

Serum concentrations of triglyceride (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were determined using commercially available kits supplied by Reactivos GPL (Spain). Low-density lipoprotein cholesterol (LDL-C) was calculated according to Friedewald's formula (18):

$$\text{LDL} = [(\text{TC} - \text{HDL}) - \text{TG}/5]$$

Lactate dehydrogenase (LDH) activity in plasma sample was measured with an assay kit (Sigma-Aldrich Chemical, USA).

Determination of liver enzymes and total protein

Plasma aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total protein (TP) were carried out using Randox Laboratories Diagnostics kits (UK).

Determination of oxidative stress markers

Plasma malondialdehyde (MDA) content and catalase (CAT) activity were determined by colorimetric methods using kits purchased from BioVision (USA). Plasma superoxide dismutase activity (SOD) activity was determined by an enzymatic colorimetric method using kits purchased from BioVision (USA).

Statistical analysis

Data were expressed as Mean±SD for six readings. The data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (19). Statistical analysis was performed using the Statistical Package for Social Science (SPSS) computer program, Version 20.00 produced by IBM Software, Inc. Chicago, USA. Differences were considered significant at P<0.05.

Results

Total phenolic and flavonoid contents of *C. melo* var. *flexuosus* leaf extract

Fig. 1 shows the total phenolic content (112 mg GAE/g dry weight) and total flavonoid content (32.13 mg QE/g dry weight) of *C. melo* var. *flexuosus* leaf extract.

Radical scavenging activity of *C. melo* var. *flexuosus* leaf extract

C. melo var. *flexuosus* leaf extract antioxidant activity was evaluated over a range of concentrations and the results of DPPH scavenging effect were plotted in Fig. 2. *C. melo* var. *flexuosus* leaf extract showed

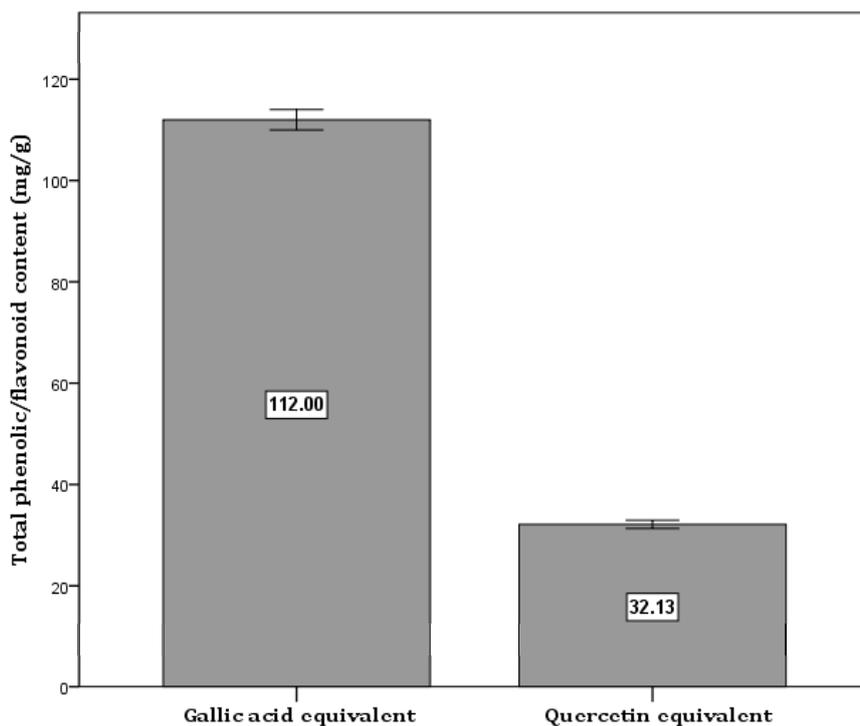


Fig. 1 : Total phenolic and flavonoid content of *C. melo* var. *flexuosus* leaf extract represented as equivalent of gallic acid and quercetin respectively.

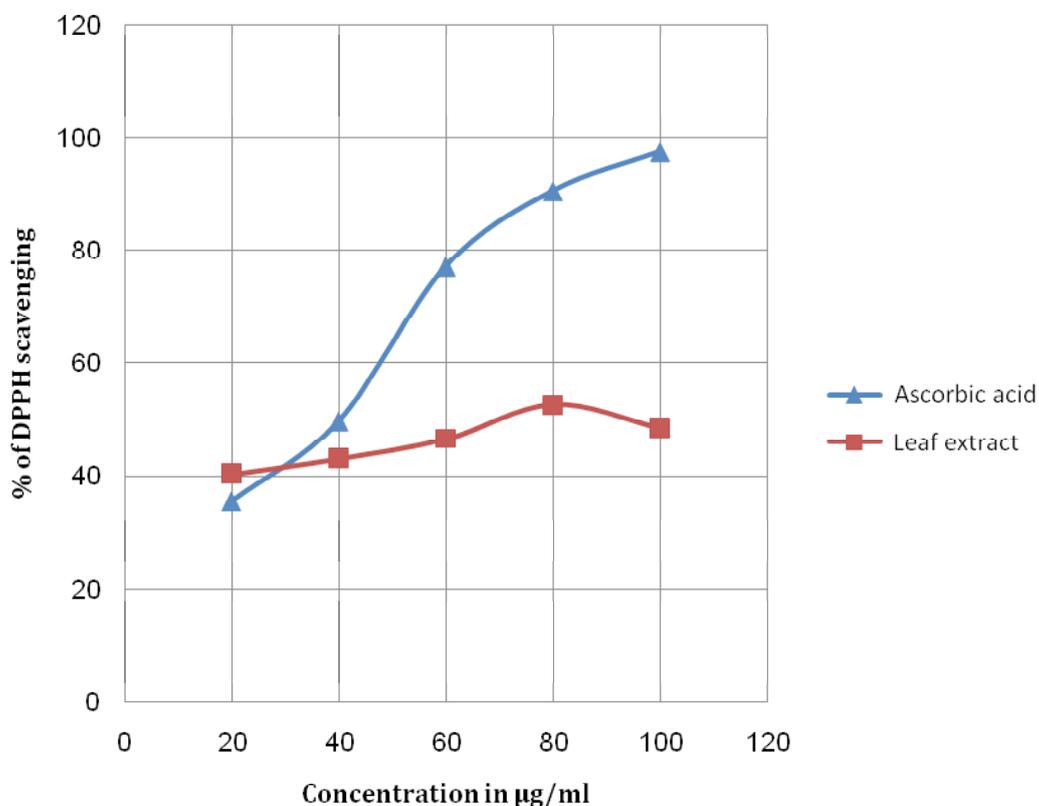


Fig. 2 : Antioxidant capacity of *C. melo* var. *flexuosus* leaf extract (DPPH assay).

maximum inhibition (52.72%) of DPPH free radical at 80 µg/ml. It was observed that the free radical was scavenged in a concentration dependant manner up to 80 µg/ml.

Acute toxicity study

Intragastric administration of *C. melo* var. *flexuosus* leaf extract at different doses 30, 60, 120, 240, and 480 mg/kg body weight did not produce any sign of morbidity and mortality in the five rat groups (Four rats each) during the 14 days period of an experiment for acute toxicity. This result indicates that the LD50 was above 480 mg/kg body weight for the aqueous ethanolic leaf extracts of *C. melo* var. *flexuosus*.

Effect of *C. melo* var. *flexuosus* leaf extract on body weight and plasma insulin

As shown in Table I, diabetic rats exhibited significant decrease (p<0.05) in the body weight and plasma insulin level as compared with normal rats. Oral administration of *C. melo* var. *flexuosus* leaf extract at the doses of 30, 60 and 120 mg/kg significantly elevated the body weight and plasma

insulin levels in diabetic rats in a dose-dependent manner.

Effect of *C. melo* var. *flexuosus* leaf extract on lipid profile and lactate dehydrogenase

Diabetic rats showed significant elevation (p<0.05) of TG, TC, LDL-C, and LDH and decrease (p<0.05) in plasma level of HDL-C in comparison with the normal group. However, treatment of diabetic rats with *C. melo* var. *flexuosus* leaf extract decreased TG, TC, LDL-C, and LDH levels and increased plasma level of HDL-C in a dose-dependent manner (Table II).

Effect of *C. melo* var. *flexuosus* leaf extract on liver enzymes and total protein

The data for plasma liver enzymes and total protein are presented in Table III. Plasma activities of AST, ALT, and ALP (liver enzymes) were significantly elevated (p<0.05) in diabetic rats when compared to normal rats. Treatment of diabetic rats with *C. melo* var. *flexuosus* leaf extract significantly reduced the activities of liver enzymes with respect to diabetic control rats. On the contrary, plasma level of TP

TABLE I: Effect of *C. melo* var. *flexuosus* leaf extract on the body weight and plasma insulin level in STZ-diabetic rats.

Parameters	Normal group	Diabetic group	Diabetic groups treated with		
			30 mg/kg	60 mg/kg	120 mg/kg
Initial body weight (g)	123.63±0.48	123.00±0.82	124.03±0.31	123.95±0.31	123.75±0.29
Final body weight (g)	227.43±0.74 ^a	99.48±0.43 ^e	117.43±0.71 ^d	124.23±0.59 ^c	180.73±0.64 ^b
Insulin (ng/ml)	3.49±0.01 ^a	1.52±0.01 ^e	1.83±0.02 ^d	2.08±0.03 ^c	2.21±0.01 ^b

Data are expressed as Means±SD (n=6). Values within a row not sharing a common letter differ significantly at P<0.05.

TABLE II: Effect of *C. melo* var. *flexuosus* leaf extract on lipid profile and lactate dehydrogenase in STZ-diabetic rats.

Parameters	Normal group	Diabetic group	Diabetic groups treated with		
			30 mg/kg	60 mg/kg	120 mg/kg
TG (mg/dl)	87.65±0.31 ^e	141.05±0.12 ^a	137.17±0.49 ^b	129.05±0.22 ^c	116.60±0.26 ^d
TC (mg/dl)	72.93±0.20 ^e	94.76±0.24 ^a	89.04±0.31 ^b	81.43±0.30 ^c	76.83±0.14 ^d
LDL-C (mg/dl)	32.63±0.17 ^e	59.04±0.06 ^a	52.15±0.39 ^b	46.57±0.27 ^c	40.58±0.56 ^d
HDL-C (mg/dl)	28.31±0.05 ^a	15.63±0.11 ^e	19.74±0.02 ^d	21.14±0.04 ^c	24.28±0.01 ^b
LDH (U/L)	58.67±0.35 ^e	78.50±0.46 ^a	74.70±0.36 ^b	70.03±0.25 ^c	64.77±0.49 ^d

Data are expressed as Means±SD (n=6). Values within a row not sharing a common letter differ significantly at P<0.05.

TABLE III : Effect of *C. melo* var. *flexuosus* leaf extract on the activity of liver enzymes and total protein in STZ-diabetic rats.

Parameters	Normal group	Diabetic group	Diabetic groups treated with		
			30 mg/kg	60 mg/kg	120 mg/kg
AST (IU/L)	34.80±0.20 ^e	81.90±0.46 ^a	56.50±0.62 ^b	52.33±0.76 ^c	44.43±0.51 ^d
ALT (IU/L)	32.17±0.27 ^e	63.87±0.12 ^a	55.60±0.66 ^b	49.63±0.35 ^c	42.73±0.64 ^d
ALP (IU/L)	23.47±0.50 ^e	55.87±0.32 ^a	47.5±0.59 ^b	41.13±0.62 ^c	36.40±0.72 ^d
TP (g/dl)	5.56±0.04 ^a	3.28±0.02 ^e	3.74±0.05 ^d	4.03±0.08 ^c	4.35±0.04 ^b

Data are expressed as Means±SD (n=6).

Values within a row not sharing a common letter differ significantly at P<0.05.

TABLE IV : Effect of *C. melo* var. *flexuosus* leaf extract on oxidative stress markers in STZ- diabetic rats.

Parameters	Normal group	Diabetic group	Diabetic groups treated with		
			30 mg/kg	60 mg/kg	120 mg/kg
MDA (nmol/μl)	0.31±0.00 ^e	1.01±0.05 ^a	0.85±0.00 ^b	0.72±0.00 ^c	0.64±0.00 ^d
CAT (μmol/ml)	0.31±0.00 ^a	0.16±0.00 ^e	0.19±0.00 ^d	0.22±0.00 ^c	0.27±0.01 ^b
SOD (U/ml)	6.29±0.03 ^a	2.76±0.05 ^e	3.17±0.03 ^d	4.18±0.02 ^c	4.79±0.07 ^b

Data are expressed as Means±SD (n=6).

Values within a row not sharing a common letter differ significantly at P<0.05.

was significantly decreased (p<0.05) in diabetic group as compared to the normal group. Administration of *C. melo* var. *flexuosus* leaf extract for diabetic rats significantly increased TP level when compared to the normal level. *C. melo* var. *flexuosus* leaf extract showed a maximum effect at a dose of 120 mg/kg.

Effect of *C. melo* var. *flexuosus* leaf extract on oxidative stress markers

Table IV reveals significant decrease (p<0.05) in antioxidant enzyme activities (SOD and CAT) and increase (p<0.05) in MDA level in diabetic group when compared with normal group. Treatment of diabetic rats with *C. melo* var. *flexuosus* leaf extract significantly raised antioxidant enzyme activities and lowered MDA level as compared to the diabetic group in a dose-dependent manner.

Discussion

Many plant extracts and their products have been shown to have antioxidant activities, these antioxidants act as free radical scavengers by preventing and repairing damages caused by ROS associated with different diseases including diabetes

(20). The DPPH free radical scavenging method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis (21). DPPH scavenging activity for *C. melo* var. *flexuosus* leaf extract was 52.72% at a concentration of 80 μg/ml. The antioxidant activity of the extract may be attributed to the high content of total phenolic (112 mg GAE/g dry weight) and total flavonoid (32.13 mg QE/g dry weight).

In our study, streptozotocin (STZ) was selected for induction diabetes (type 1) in rats. STZ selectively destroys the pancreatic insulin secreting β-cells, leaving less active cells and resulting in diabetes mellitus (22). The medicinal plant compounds may have mechanisms acting as an insulin-like effect, improving insulin sensitivity, augmenting glucose-dependent insulin secretion and stimulating the regeneration of islets of Langerhans in the pancreas of STZ-induced diabetic rats (23). From the result of the present experiment, it was observed that treatment with *C. melo* var. *flexuosus* leaf extract increased the plasma insulin in level in diabetic rats. We hypothesized that *C. melo* var. *flexuosus* leaf extract can stimulate insulin secretion from remnant pancreatic β-cells due to the presence of high content of total phenolic and flavonoid which was noticed in

the phytochemical analysis for the *C. melo* var. *flexuosus* leaf extract.

In the present study, reduction in body weight in diabetic rats was observed which may be due to the low level of plasma insulin. It was suggested that loss of body weight in diabetic rats could be due to loss of tissue proteins and unavailability of carbohydrates for utilization as an energy source caused by lack or deficiency of insulin (23). A significant increase in body weight was observed in diabetic rats treated with *C. melo* var. *flexuosus* leaf extract which may be due to the ability of the extract to increase plasma insulin and total protein levels as observed in our results.

In the present study, the high levels of TG, TC, LDL-C, LDH and low level of HDL were observed in diabetic rats. Nagar and Chauhan (24) reported that the abnormally high concentration of serum lipid in diabetic mainly due to increased mobilization of free fatty acids from peripheral fat depots. During diabetes, insulin deficiency or insulin resistance increases the level of circulating free fatty acids by enhancing the activity of hormone-sensitive lipase (25). In the same time, Giribabu *et al.* (26) suggested that the high activity of LDH in diabetes could be related to lower amount of insulin as insulin has been reported to affect the activity of LDH. It was observed that plasma lipid profile and LDH activity were reversed towards normal after administration of *C. melo* var. *flexuosus* leaf extract. Thus, the extract could be helpful in improving lipid metabolism which will, in turn, help to prevent diabetic complications such as coronary heart diseases and atherosclerosis. To our knowledge, this is the first study reporting the hypolipidemic activity of *C. melo* var. *flexuosus* leaf extract in STZ-induced diabetes.

In the present study, the high plasma levels of AST, ALT, and ALP in diabetic rats indicates possible damage to the liver. This result was supported by Giribabu *et al.* (26) who reported that plasma levels of AST and ALT were increased following hepatocyte

injury while ALP level was elevated in biliary tree obstruction. Hassan *et al.* (7) also reported that the high serum levels of AST, ALT, and ALP in diabetic rats are indicative of cellular leakage and loss of functional integrity of the hepatic cell membranes implying hepatocellular damage. However, diabetic groups treated with *C. melo* var. *flexuosus* leaf extract showed a significant reduction in the levels of these enzymes when compared to the diabetic untreated group. This means that *C. melo* var. *flexuosus* leaf extract has some hepatoprotective potentials in diabetic rats by decreasing plasma AST, ALT, and ALP levels.

The present study also revealed a significant decrease in the level of plasma TP in diabetic rats. This could be due to increased peroxidation. On the other hand, *C. melo* var. *flexuosus* leaf extract treated rats showed an increased level of TP, suggesting that *C. melo* var. *flexuosus* leaf extract has antioxidant capacity.

In this study, the activities of SOD and CAT significantly decreased coupling with a marked increase in the level of MDA in diabetic rats, which could be due to hyperglycemia. This finding is in correlation with the findings of Hassan *et al.* and Wang *et al.* (7, 27). Ibrahim and Abd El-Maksoud (28) also reported that hyperglycemia leads to overproduction of reactive oxygen species (ROS). However, administration of *C. melo* var. *flexuosus* leaf extract increased the activities of plasma SOD and CAT and decreased the level of MDA which could be a result of the high antioxidant activity of the extract as reported in our results.

Conclusion

It can be concluded that the aqueous ethanolic extract of *C. melo* var. *flexuosus* leaf has an antidiabetic effect and that may be due to the presence of secondary metabolites like phenols and flavonoids which are responsible for antioxidant activity in the extract.

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