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Phytochemical Screening, Gas Chromatography-mass Spectrometry Analysis, and Antidiabetic Effects of *Corchorus olitorius* Leaves in Rats

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Abstract

BACKGROUND: Therapies for diabetes mellitus are still meeting failure in most cases, especially in the developed stages of the disease due to incredible associating complications. Hence, there is a need for continuous development of curative therapies for that stubborn disease.

AIM: We aimed to investigate the antidiabetic effects of one of the most popular plants cultivated in Egypt, *C. olitorius*.

METHODS: Phytochemical screening of total alcoholic extract of *Corchorus olitorius* leaves and its aqueous and chloroform fractions revealed the presence of flavonoids, saponins, carbohydrates, tannins, coumarins, and alkaloids.

RESULTS: The gas chromatography-mass spectrometry analysis showed the presence of 12 and nine chemical compounds in aqueous and chloroform extracts, respectively. *C. olitorius* decreased serum glucose level and α -amylase activity. This effect was more pronounced in the total alcoholic extract and its chloroform fraction than the aqueous one. The extracts also adjusted the lipid profile, reduced liver injury parameters, and caused remarkable improvement and increase number, size, and density of functioning β -cells.

CONCLUSION: The findings suggest the antihyperglycemic and antioxidant effects of *C. olitorius* besides its beneficial effect on diabetic complications such as hyperlipidemia and liver injury. The presence of some phytochemicals such as theophylline, trans-2, 3-dimethoxycinnamic acid, 7-hydroxy-4-methyl coumarin, apigenin 7-glucoside, and glycitein may contribute to such pharmacological effects.

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Introduction

Type 1 diabetes is a chronic disease that is associated with several long-term complications, including microvascular and macrovascular events such as neuropathy, hyperlipidemia, nephropathy, and retinopathy. Nowadays, the life expectancy of type 1 diabetic patients is still about 12 years less than that of the general population [1]. The burden of long-term care and increased morbidity necessitate more studies to prevent, treat and even achieve a true cure for this complicated disease. In fact, the improvements achieved in insulin delivery systems provided better control of blood glucose; however, there is a continuing demand for newer therapies to cope with the devastating complications that occur alongside [2].

Corchorus olitorius L. belongs to family Tiliaceae and known as “Jute,” which is characterized by the presence of high ratios of amino acids; hence,

it is an essential source of dietary protein in Egypt and many other countries. Leaves of *C. olitorius* are edible and its ethnic soup is well-known in Egypt under the name of “Molukhyia.” The leaves were found to be rich sources of ω 3-octadecatriene fatty acid which is a higher concentration than any other vegetable [3]. The plant has been reported to possess anti-inflammatory [4], antibacterial, demulcent, bitter tonic, laxative, carminative, diuretic, useful in chronic cystitis, gonorrhoea, and cardiotoxic [5], [6]. In previous recent studies, it was shown that the ethanolic seed extract of *C. olitorius* was effective as an antidiabetic remedy [7] and leaf extract showed antihyperglycemic effects in type 2 diabetic animal model [8].

The present study aims to investigate the antidiabetic effects of *C. olitorius* different leaf extracts using Type 1 diabetic animal model that will be induced by streptozotocin (STZ). A comparative pharmacological study will be performed for the first time between the three extracts; the total alcoholic and its two fractions

(chloroform and aqueous) to find if the antidiabetic effect of the *C. olitorius* is ascribed to nonpolar (dissolved in chloroform fraction), polar compounds (dissolved in aqueous fraction) alone or due to total extract. Gas chromatography-mass spectrometry (GC-MS) analysis was used also to identify the potential bioactive phytochemicals in those two fractions.

Materials and Methods

Chemicals

Gliclazide (standard antidiabetic drug) was purchased from SERVIER subsidiary SERDIA Pharmaceuticals (Canada) in tablet form, grounded using a mortar, and dissolved in distilled water. Chloroform and methanol (purity: 95–99%) were purchased from El-Gomhouria Co. (Egypt). STZ was purchased from Sigma-Aldrich Chemie GmbH Co. (Germany).

Collection and identification of the plant material

C. olitorius leaves that used in the study were supplied by Haraz flour Milling, Cairo, Egypt. *C. olitorius* leaves were cleaned and stored in a cool and dry place before use. A voucher specimen (Reg. No.: Co, 2013-80) was deposited in the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University and it was air-dried and kept in a tightly closed container.

Preliminary phytochemical investigation

Phytochemical screening was performed using standard procedures based on colorimetric tests [9], [10]. *C. olitorius* leaves were screened for the presence of carbohydrates, saponins and/or glycosides, alkaloids, anthraquinones, unsaturated sterols, triterpenes, coumarins, tannins, cardiac glycosides, and flavonoids. The phytochemical screening revealed the presence of all the previous phytochemicals except anthraquinones and volatile compounds.

Preparation of extract

The powdered plant materials (1.5 kg) were extracted with ethanol (70%) (1 L/time). The extraction was done by a hot continuous percolation method in the Soxhlet apparatus for 24 h [11]. The extract was concentrated using a rotary evaporator till dry powder was obtained (~150 g). The total extract was dissolved at least amount water, extracted by chloroform (3 × 1 L). Using separating funnel, the organic layer and the aqueous layer were separated, and then two fractions

were concentrated using a rotary evaporator till dry powder was obtained (40 g of chloroform and 95 g of aqueous fractions). The final residues thus obtained were then subjected to GC-MS analysis [12].

GC-MS analysis

The chloroform and aqueous fractions of *C. olitorius* leaves were analyzed through GC-MS for the identification of different compounds. The GC-MS analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass selective detector (MSD, Agilent 7 000) equipped with a polar Agilent HP-5ms (5%-phenyl methyl polysiloxane) capillary column (30 m × 0.25 mm i.d. and 0.25 μm film thickness). The oven temperature was programmed initially at 40°C (for 3 min) to 280°C final at an increasing rate of 5°C/min (for 5 min). The carrier gas was helium with a linear velocity of 1 mL/min. The electron ionization system with ionization energy of 70 eV was used.

The identification of components

Interpretation of the mass spectrum of GC-MS was based on using the database of the National Institute Standard and Techniques (NIST Version, 2008), which have more patterns. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown compound was compared with the spectrum of the compound stored in the NIST data library (NIST08s), WILEY8, and Adams. The name, molecular weight, molecular formula, and structure of the components of the test material were obtained.

Assessment of antidiabetic activity

Animals

Healthy male Wistar rats, weighing 200–220 g, were obtained from the animal house of the National Research Centre. Before initiating the experiments, the rats were allowed to acclimatize for a few days under standard environmental conditions (12 h dark/12 h light cycle; temperature 20–22°C; relative humidity 40–60%). The animals were cared for in accordance with the Guide to the Care and Use of Experimental Animals available from the Canadian Council on Animal Care. The ethical standards of the experiment are also in accordance with the guidelines provided by the CPCSEA and World Medical Association Declaration of Helsinki on Ethical Principles for Medical Research. The animal use was approved and conducted according to regulations of the ethics committee of the National Research Centre which gave its consent in accordance with the National Regulations on Animal Welfare and Institutional Animal Ethical Committee (Approval No: 17-062).

Induction of diabetes and animal grouping

Type 1 diabetes mellitus was induced by a single i.p. injection of a freshly prepared solution of STZ (52.5 mg/kg body weight) in 0.1 M citrate buffer (pH 4.3) after a fasting period of 24 h [13]. On the 3rd day of STZ injection, blood was collected from tail veins of rats and glucose level was measured. Rats with a serum glucose level of 180 mg/dL or greater were considered diabetic and selected in this study. A group of six rats was kept as a negative control group and received 1 mL saline orally (Group 1). After induction of diabetes, rats were divided into other eight equal groups (six rats per group). Group 2 (positive control) received STZ only, as mentioned above. Group 3 received oral administration of the reference drug (gliclazide, 10 mg/kg). Groups 4–9 rats were administered *C. olitorius* at two different oral doses; 50 and 100 mg/kg as reported in literature [14] as follows: Groups 4 and 5 received STZ and *C. olitorius* total alcoholic extract at two dose levels: 50 and 100 mg/kg bw, respectively. Groups 6 and 7 received *C. olitorius* aqueous extract at 50 and 100 mg/kg bw, respectively. Groups 8 and 9 received *C. olitorius* chloroform extract at 50 and 100 mg/kg bw, respectively.

Treatment with *C. olitorius* extracts was started 3 days after STZ injection and lasted for 14 days. At the end of the experiment, blood samples were drawn from the tail veins of rats [15] for recording fasting serum glucose in each group on days 3 (beginning of diabetes induction) and 17 (last day of the experiment, after treatment) of the study. Serum glucose was measured using glucose kit (Stanbio Laboratory, USA), according to Trinder [16]. Serum samples were obtained by centrifugation at 3 000 rpm for 10 min using the cooling centrifuge (Sigma and Laborzentrifugen, 2 k15, Germany) and used for biochemical measurements.

Biochemical assay

The collected sera were used for the determination of α -amylase, total antioxidant capacity (TAC), total cholesterol, triglycerides level, LDL, HDL, AST, and ALT using specific kits (Chronolab, Spain). The procedures were done according to the manufacturer's instructions. Percent of change of each indicator from positive control (STZ diabetic group) was calculated according to the following formula:

$$\% \text{ change} = \frac{[\text{Conc. in STZ group} - \text{Conc. in treated group}]}{\text{Conc. in STZ group}} \times 100$$

Histopathological examination

After sacrifice, animal organs (pancreas or liver) were dissected, extracted, and immediately fixed in buffered formalin. Tissues were further dehydrated, embedded into paraffin blocks, and serially sectioned at 4 microns thickness. Paraffin sections were stained with hematoxylin and eosin for routine histopathological study [17].

Further paraffin sections were stained immunohistochemically with insulin mouse Monoclonal Antibody INS05 (2D11-H5)-Thermo-Fisher.

Stained sections were examined using the light microscope OLYMPUS CX41 and photomicrographs were taken using digital camera OLYMPUS DP12.

Statistical analysis

All studied data were statistically analyzed using GraphPad Software Computer Program; version 6 hypothesis testing methods included one-way analysis of variance (ANOVA) using the Tukey test as *post hoc* test. Data were represented as mean \pm SEM. $p \leq 0.05$ was considered to be statistically significant.

Results

GC-MS analysis

GC-MS chromatogram of the aqueous extract showed 12 peaks indicating the presence of 12 phytochemical compounds, and the chromatogram of the chloroform extract showed nine phytochemical compounds. The mass spectra of identified compounds were matched with those found in NIST/NBS spectral database given in Tables 1 and 2.

Table 1: Phytochemical compound identified in aqueous extract of leaves of *Corchorus olitorius* L.

RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
3.49	Levogluconone	C ₆ H ₈ O ₃	126	5.0
3.76	6-Acetyl- β -D-mannose	C ₈ H ₁₄ O ₇	222	14.0
9.78	Ascaridole epoxide	C ₁₀ H ₁₆ O ₃	184	1.8
9.96	2-(2-Methyl-propenyl)-cyclohexanone	C ₁₀ H ₁₆ O	152	1.3
10.98	D-lyxo-D-manno-nononic-1,4-lactone	C ₉ H ₁₆ O ₃	268	0.5
11.77	N-acetyl-D-galactosaminitol	C ₈ H ₁₇ NO ₅	223	0.9
13.52	Digitoxin	C ₄₁ H ₆₄ O ₁₃	764	4.2
14.07	1-Deoxy-1-(methylamino) hexitol	C ₆ H ₁₃ NO ₅	195	5.4
15.05	Theophylline	C ₇ H ₈ N ₂ O ₂	180	7.2
17.58	Trans-2,3-dimethoxycinnamic acid	C ₁₁ H ₁₂ O ₄	208	2.0
20.08	Isoelemicin	C ₁₂ H ₁₆ O ₃	208	1.7
21.45	Nabilone	C ₂₄ H ₃₆ O ₃	372	6.2

The identification of the phytochemical compounds was confirmed based on the peak area, retention time, and molecular formula. The individual fragmentation patterns of necessary compounds were illustrated in Figure 1.

Table 2: Phytochemical compound identified in chloroform extract of leaves of *Corchorus olitorius* L.

RT	Name of the compound	Molecular formula	Molecular weight	Peak area (%)
9.75	7-Hydroxy-4-methyl coumarin	C ₁₀ H ₈ O ₃	176	0.32
10.19	3,4-Dimethoxycinnamic acid	C ₁₁ H ₁₂ O ₄	208	0.34
10.84	D-saccharic acid 1,4-lactone	C ₆ H ₈ O ₇ H ₂ O	210	0.41
12.60	Camphoric acid	C ₁₀ H ₁₆ O ₄	200	3.80
13.84	Apigenin 7-glucoside	C ₂₁ H ₃₀ O ₁₀	432	4.36
13.90	β -tocopherol	C ₂₈ H ₄₈ O ₂	416	0.99
15.03	3-Oxo-7,8-dihydro- α -ionol	C ₁₃ H ₂₀ O ₂	210	11.56
15.46	4-Hydroxy-2-methoxybenzaldehyde	C ₈ H ₈ O ₃	152	30.95
15.58	Glycitein	C ₁₆ H ₁₂ O ₅	284	1.33

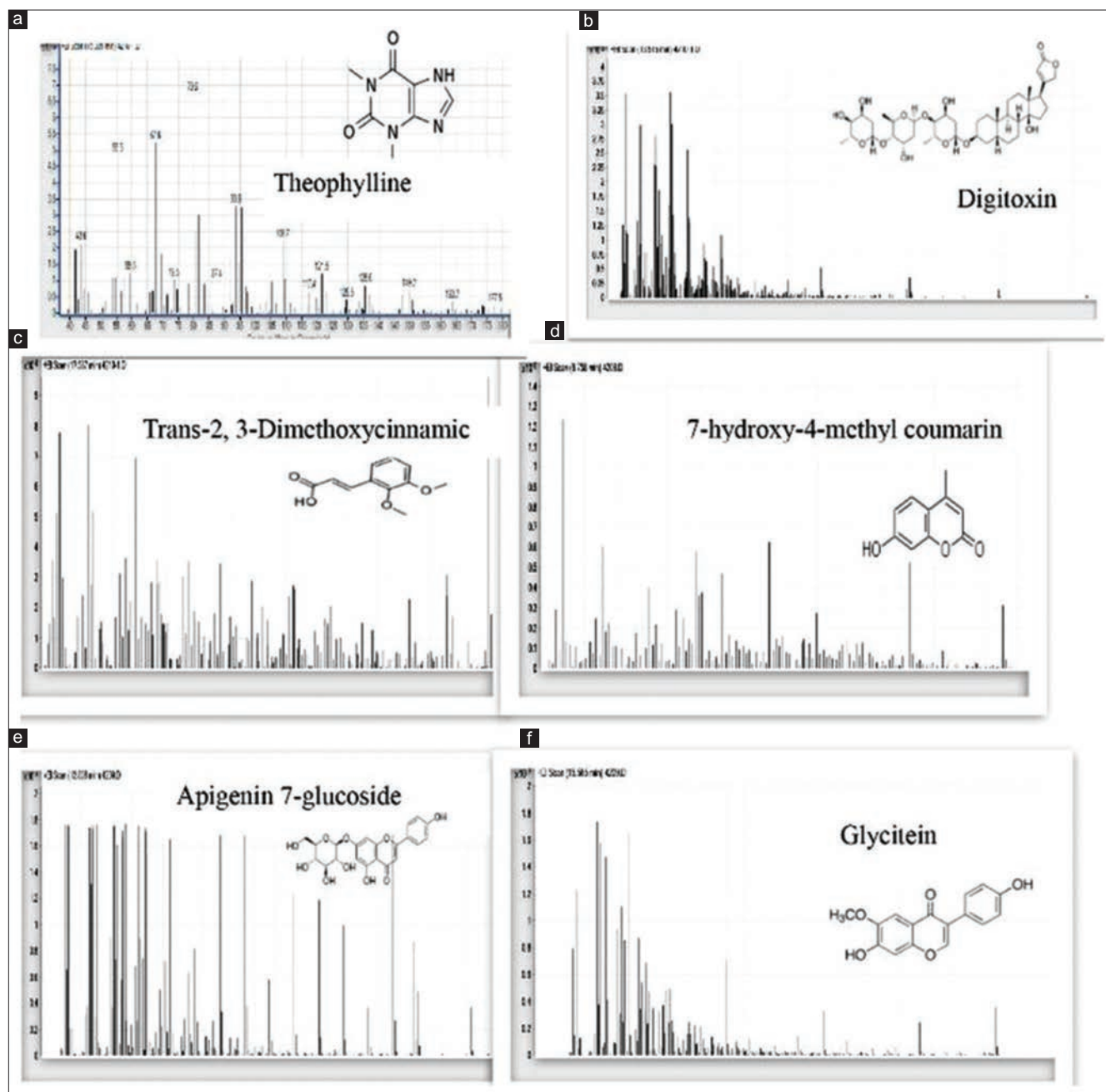


Figure 1: The individual fragmentation pattern of the important compounds in gas chromatography analysis. (a) Theophylline, (b) Trans-2,3-dimethoxycinnamic acid, (c) 7-Hydroxy-4-methyl coumarin, (d) Digitoxin, (e) Apigenin 7-glucoside, (f) Glycitein

Biochemical assay

Effect on fasting glucose level

As shown in Table 3, initial serum glucose was significantly increased ($p < 0.05$) in all STZ-treated groups as compared to the negative control one (confirming diabetes). At the end of the experiment, glucose level was significantly increased ($p < 0.05$) in the diabetic group exhibiting 24% increase from the initial value. After *C. olitorius* administration, glucose level was significantly decreased ($p < 0.05$) in gliclazide (standard drug), total alcoholic *C. olitorius* extract (100 mg/kg), aqueous (100 mg/kg), and chloroform *C. olitorius* fractions (50 and 100 mg/kg) by 66%, 65%, 38%, 66%, and 50%, respectively, compared

to STZ-diabetic group. It is worthy to note that total alcoholic and aqueous extracts decreased glucose level in a dose-dependent manner.

Effect on α amylase activity

Table 3 obviously showed that the activity of α amylase in the STZ-diabetic group was increased significantly ($p < 0.05$) compared to the negative control. The activity of α amylase was decreased in animal groups treated with gliclazide, total *C. olitorius* extract (100 mg/kg), aqueous *C. olitorius* fraction (100 mg/kg), and chloroform *C. olitorius* fractions (50 and 100 mg/kg) by 12%, 9%, 9%, 12%, and 8%,

Table 3: Effect of *Corchorus olitorius* L. on serum glucose, α amylase, and TAC

Treatment	Glucose (initial) (mg/dL)	Glucose (final) (mg/dL)	α -amylase activity (U/L)	TAC (mMol/L)
Control negative (normal)	101.80 \pm 4.70 ^a	106.0 \pm 3.8 ^a	1162.0 \pm 6.1 ^a	2.64 \pm 0.02 ^a
Control positive (STZ, 52.5 mg/kg)	424.40 \pm 18.10*	558.4 \pm 26.1*	1284.0 \pm 4.9*	2.33 \pm 0.04*
Gliclazide (10 mg/kg)	410.60 \pm 20.10*	187.5 \pm 10.1 ^a	1130.0 \pm 8.2 ^a	2.61 \pm 0.05 ^a
<i>C. olitorius</i> tot alc ext (50 mg/kg)	428.20 \pm 38.90*	582.7 \pm 35.6*	1269.0 \pm 38.5 ^c	2.30 \pm 0.02 ^c
<i>C. olitorius</i> tot alc ext (100 mg/kg)	325.30 \pm 24.80*	190.5 \pm 5.4 ^{ab}	1170.0 \pm 18.6 ^{ab}	2.60 \pm 0.05 ^{ab}
<i>C. olitorius</i> aqueous ext (50 mg/kg)	378.60 \pm 27.20*	490.6 \pm 22.1*	1266.0 \pm 16.2*	2.45 \pm 0.02*
<i>C. olitorius</i> aqueous ext (100 mg/kg)	366.50 \pm 34.00*	346.7 \pm 27.7 ^{abd}	1169.0 \pm 11.8 ^{bd}	2.35 \pm 0.04*
<i>C. olitorius</i> chloroform ext (50 mg/kg)	379.10 \pm 37.70*	191.6 \pm 6.2 ^b	1135.0 \pm 7.6	2.64 \pm 0.03 ^b
<i>C. olitorius</i> chloroform ext (100 mg/kg)	332.50 \pm 13.56*	279.2 \pm 22.5 ^{ab}	1183.0 \pm 3.2 ^b	2.65 \pm 0.03 ^a

Each value represents the mean \pm SEM (n=6). *Significantly different from control negative (saline) at p<0.05, ^aSignificantly different from control positive (STZ) at p<0.05, ^bSignificantly different from tot alc ext (50 mg/kg), ^cSignificantly different from tot alc ext (100 mg/kg), ^dSignificantly different from aqueous ext (50 mg/kg). Statistical analysis was carried out using one-way ANOVA test followed by Tukey *post hoc* test. TAC: Total antioxidant capacity, STZ: Streptozotocin, *C. olitorius*: *Corchorus olitorius*.

respectively, compared to STZ-diabetic group and returned to normal value. The effects of two doses (50 and 100 mg/kg) were significantly different in the groups treated with total alcoholic and aqueous extracts. From the data represented in the table, an inhibition in α amylase activity was more pronounced in the group treated with chloroform *C. olitorius* fraction (50 mg/kg) and gliclazide.

Effect on TAC

TAC was significantly decreased (p < 0.05) in the positive control group compared to the negative control group. Treatment with gliclazide, total alcoholic *C. olitorius* extract (100 mg/kg), and chloroform *C. olitorius* fractions (50 and 100 mg/kg) normalized TAC as shown in Table 3. The groups treated with total alc extract at different doses were significantly different from each other. The TAC increased by 12%, 11%, 13%, and 14% in the previous groups, respectively, compared to the STZ-diabetic group.

Effect on lipid profile

Lipid profile is presented in Table 4. Cholesterol and triglycerides levels increased significantly (p < 0.05) in diabetic groups as compared to the negative control group. Levels of cholesterol and triglycerides decreased significantly (p < 0.05) in animals treated with *C. olitorius* total alcoholic extract and its fractions except for triglycerides level in the group treated with the total alcoholic extract (100 mg/kg) and gliclazide comparing to diabetic group. Levels approached that of the negative control group in animals treated with aqueous and chloroform fractions of *C. olitorius* at both dose levels (except for the total cholesterol value

Table 4: Effect of *Corchorus olitorius* on serum lipid profile (mg/dL)

Treatment	Total cholesterol	Triglycerides	LDL	HDL
Control negative (normal)	89.17 \pm 1.00 ^a	48.79 \pm 1.50 ^a	37.60 \pm 0.30 ^a	39.80 \pm 3.51 ^a
Control positive (STZ, 52.5 mg/kg)	199.20 \pm 12.30*	146.60 \pm 6.30*	44.50 \pm 1.50*	22.89 \pm 1.46*
Gliclazide (10 mg/kg)	110.50 \pm 5.20 ^a	85.30 \pm 3.20 ^{ab}	37.90 \pm 0.60 ^a	37.10 \pm 0.72 ^a
<i>C. olitorius</i> tot alc ext (50 mg/kg)	132.70 \pm 2.60 ^{ab}	65.20 \pm 4.80 ^a	38.10 \pm 1.90 ^a	50.78 \pm 3.18 ^a
<i>C. olitorius</i> tot alc ext (100 mg/kg)	129.00 \pm 1.30 ^{ab}	73.67 \pm 5.10*	37.80 \pm 0.60 ^a	47.09 \pm 4.34 ^a
<i>C. olitorius</i> aqueous ext (50 mg/kg)	114.90 \pm 5.40 ^{ab}	60.64 \pm 4.20 ^a	41.60 \pm 1.10	58.75 \pm 4.15 ^{ab}
<i>C. olitorius</i> aqueous ext (100 mg/kg)	71.33 \pm 2.30 ^{ad}	55.87 \pm 3.10 ^{ad}	38.20 \pm 1.60 ^a	46.42 \pm 2.23 ^a
<i>C. olitorius</i> chloroform ext (50 mg/kg)	108.00 \pm 6.50 ^a	66.50 \pm 1.70 ^a	35.20 \pm 1.00 ^a	52.25 \pm 1.61 ^a
<i>C. olitorius</i> chloroform ext (100 mg/kg)	100.30 \pm 2.80 ^a	57.37 \pm 5.30 ^a	35.40 \pm 1.00 ^a	50.98 \pm 2.96 ^a

Each value represents the mean \pm SEM (n = 6). *Significantly different from control negative (saline) at p<0.05, ^aSignificantly different from control positive (STZ) at p<0.05, ^bSignificantly different from tot alc ext (50 mg/kg), ^cSignificantly different from tot alc ext (100 mg/kg), ^dSignificantly different from aqueous ext (50 mg/kg). Statistical analysis was carried out using one-way ANOVA test followed by Tukey *post hoc* test. *C. olitorius*: *Corchorus olitorius*, LDL: Low-density lipoprotein, HDL: High-density lipoprotein.

in the group treated with aqueous extract; 50 mg/kg) which exerted 42%, 64%, 46%, and 50% decrease in total cholesterol level and 59%, 62%, 55%, and 61% decrease in triglycerides level, respectively, compared to STZ-diabetic group. Whereas, gliclazide treatment decreased cholesterol and triglycerides level by 44% and 42%, respectively, compared to the STZ-diabetic group. For cholesterol, aqueous extract (50 mg/kg) treatment showed a significant difference from the group treated with the same extract at 100 mg/kg. HDL-cholesterol level significantly (p < 0.05) decreased in the STZ-treated group as compared to the negative control. HDL-cholesterol showed a remarkable increase in all extract treatment groups (p < 0.05) compared to the diabetic group and its level was higher than the normal value. Whereas, LDL-cholesterol level increased significantly (p < 0.05) in the diabetic group compared to the negative control group. After *C. olitorius* administration, LDL-cholesterol levels were decreased in all treated groups (p < 0.05) compared to the diabetic group except for the aqueous extract (50 mg/kg) treated group. The highest decrease was observed in the chloroform fraction treated groups (50 and 100 mg/kg) which were 21% and 20% when compared to the STZ-diabetic group. There was no significant difference in LDL-cholesterol levels in all treatment groups as compared to the negative control group.

Effect on liver enzymes

As illustrated in Table 5, ALT and AST revealed a significant increase (p < 0.05) in the positive control group compared to the negative control. Administration of the different extracts of *C. olitorius* showed a significant decrease (p < 0.05) in ALT and AST activities compared to the positive control group. ALT activity was decreased by 23%, 35%, 40%, 52%, 40%, and 50% after administration of total alcoholic extract (50 and 100 mg/kg), aqueous extract (50 and 100 mg/kg), and chloroform fraction (50 and 100 mg/kg), respectively. The effect on AST activity was more pronounced in the

Table 5: Effect of *Corchorus olitorius* L. on serum liver enzymes (U/L)

Treatment	ALT	AST
Control negative (normal)	114.5 ± 5.3 ^a	155.5 ± 1.9 ^a
Control positive (STZ, 52.5 mg/kg)	221.2 ± 16.3 [*]	253.5 ± 12.3 [*]
Gliclazide (10 mg/kg)	175.3 ± 6.2 ^{ab}	220.1 ± 19.1 ^{ab}
<i>C. olitorius</i> Tot alc ext (50 mg/kg)	169.7 ± 7.4 ^{ab}	182.5 ± 2.4 ^a
<i>C. olitorius</i> Tot alc ext (100 mg/kg)	143.3 ± 7.6 ^a	212.8 ± 2.6 ^{ab}
<i>C. olitorius</i> aqueous ext (50 mg/kg)	135.2 ± 9.6 ^a	213.1 ± 8.6 ^{ab}
<i>C. olitorius</i> aqueous ext (100 mg/kg)	105.2 ± 3.2 ^a	210.1 ± 6.4 ^a
<i>C. olitorius</i> Chloroform ext (50 mg/kg)	133.3 ± 3.2 ^a	217.6 ± 7.0 ^{ab}
<i>C. olitorius</i> Chloroform ext (100 mg/kg)	109.3 ± 5.4 ^a	218.5 ± 4.4 ^{ab}

Each value represents the mean±SEM (n=6). ^{*}Significantly different from control negative (saline) at p<0.05, ^{ab}Significantly different from control positive (STZ) at p<0.05. Statistical analysis was carried out using one-way ANOVA test followed by Tukey post hoc test. *C. olitorius*: *Corchorus olitorius*, STZ: Streptozotocin.

group treated with the total alcoholic extract (50 mg/kg). The improvement in biochemical parameters achieved by *C. olitorius* extracts was comparable to the standard antidiabetic drug gliclazide.

Histopathological findings

The histologic appearance of the pancreatic tissues of the negative control rats showed that normal islets of Langerhans are surrounded by ordinary

pancreatic acini. Islets were regular with well-defined boundaries. Cells within islets showed oval to rounded nuclei, disposed in organized architecture. Surrounding pancreatic acini were lined by pyramidal cells showing ample apical cytoplasm and rounded, bland, basophilic basoluminal nuclei (Figure 2a). The pancreas of diabetic untreated rats showed disruption of the architecture and microstructure within the islets of Langerhans. Prominent vacuolar degeneration was observed with focal necrosis. Karyolysis was seen with foci of pyknosis and scattered eosinophilic bodies. Changes were most prominent at the center of the islets. Islets also showed irregularity in their shape and appeared small and shrunken (Figure 2b). Scattered islets showed dense inflammatory cellular infiltrate (insulitis). Outside the islets of Langerhans, the pancreatic ductal and acinar epithelium appeared normal. Treatment with reference drug (gliclazide) showed improvement in size, cellularity, and architectural arrangement within islets, approximating negative control (Figure 2c). Treatment with total alcoholic extract (100 mg/kg) caused remarkable improvement in the pancreas (Figure 2e);

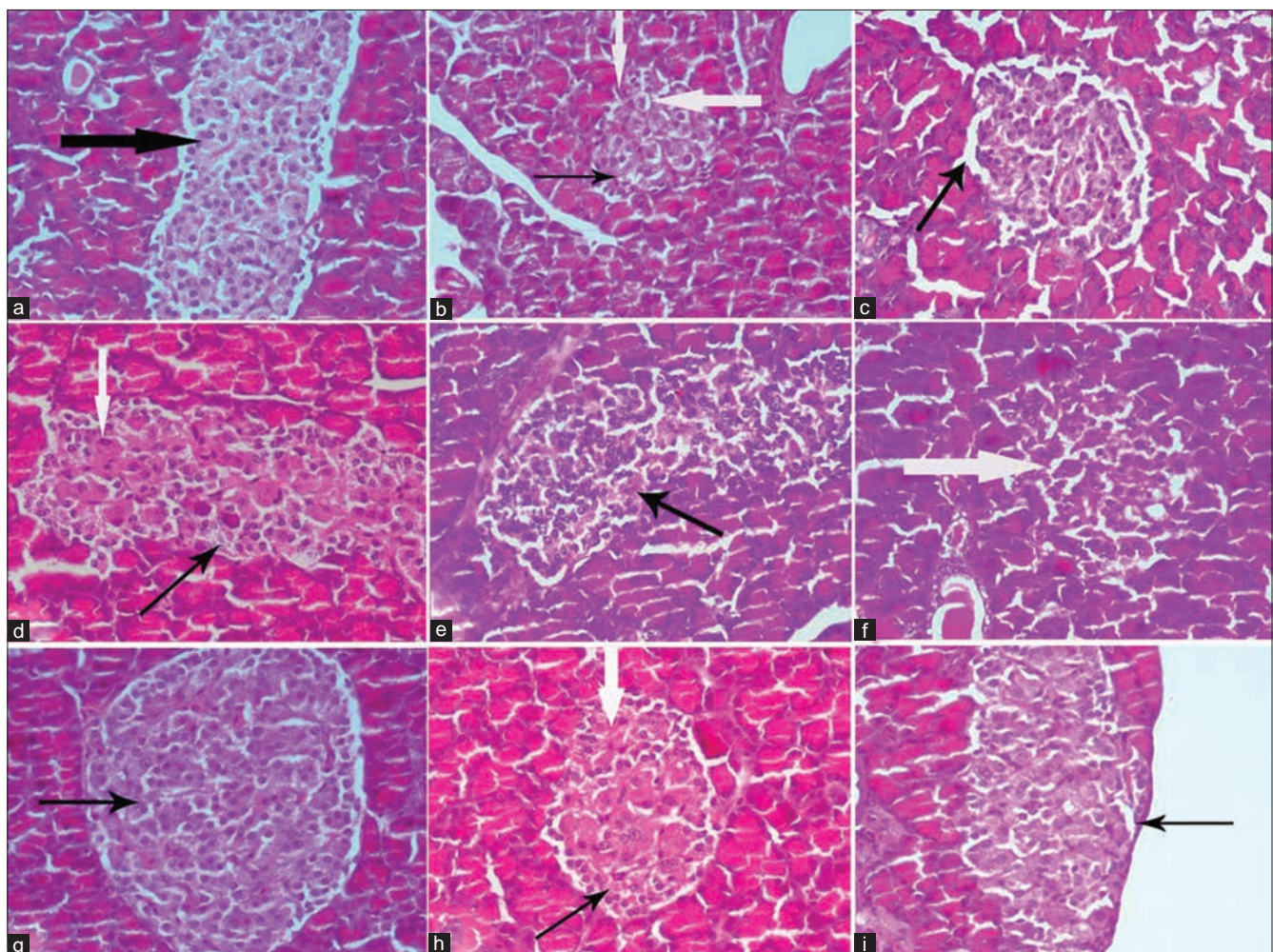


Figure 2: A photomicrograph of pancreatic tissue using hematoxylin and eosin (H and E): (a) -ve control: Large islet of Langerhans (arrow). (b) +ve control: Shrunken, small islet (arrow), degenerated cells (white). (c) Gliclazide improved islet size (arrow) and cellularity. (d) Total alc (50 mg): Vacuolated, pyknotic cells, and eosinophilic bodies (white). (e) Total alc (100 mg): Improved islet (arrow). (f) Aq. ext (50 mg): Small islet and residual degenerated cells (arrow). (g) Aq. ext (100 mg): Improved islet size and cellularity (arrow). (h) Chloroform (50 mg): Residual microvesicular degeneration (black) and scattered hyperchromatic nuclei (white) (i) Chloroform (100 mg): Improved islet (arrow) (H and E, ×400)

in the form of increase and restoration of number, size, as well as increase within the density of cells inside the islets. Vacuolar degeneration was diminished and necrosis was not seen. Inflammation also decreased. Improvement was most marked using total alcoholic extract, high dose (Figure 2e), and chloroform extract, high dose, approximating normal (Figure 2i), then followed by total alcoholic extract, low dose (Figure 2d). Improvement was less in the case of aqueous, high dose (Figure 2g). No improvement could be noticed on treatment with each of chloroform extract; low dose (Figure 2h) and aqueous; low dose (Figure 2f).

As shown in Figure 3, the liver tissue of the control rat showed preserved architecture. Ordinary, polyhedral hepatocytes were seen with ample eosinophilic cytoplasm and central, rounded vesicular, bland nuclei were seen. Hepatocytes were seen disposed in cords, one cell thick each, radiating from a central vein, and separated by sinusoids (Figure 3a). Liver tissue of positive control rats showed marked dilatation and congestion of central veins. Arteries showed thick walls. Hepatocytes showed hydropic and microvesicular degeneration (Figure 3b). Aggregates of inflammatory cells were seen, especially at the periphery of dilated vessels. Congestion was seen within sinusoids as well. Pyknotic and hyperchromatic nuclei were noticed within scattered hepatocytes. Liver tissue of diabetic rat treated with reference drug (gliclazide) showed remarkable improvement; picture approximating negative control (Figure 3c).

Liver tissue of diabetic rat treated with total alcoholic extract; high dose showed considerable improvement, still mild congestion was seen within the central vein and sinusoids (Figure 3e). The liver tissue of diabetic rats treated with chloroform extract; high dose showed neither congestion nor dilatation of central vein and sinusoids; however, scattered hepatocytes showed hyperchromatic nuclei with minimal residual microvesicular degeneration (Figure 3i).

Immunohistochemical findings

Islets of Langerhans of diabetic rats showed a marked reduction in number, size, and decreased density of functioning β -cells as demonstrated by an anti-insulin monoclonal antibody (Figure 4b) as compared to normal islets (Figure 4a). However, diabetic rats treated with *C. olitorius* chloroform extract high dose showed prominent improvement in β -cells number, size, and density in the islets of Langerhans (Figure 4i). It was better than other extracts (Figure 4d, e, f, g, h) and gliclazide (Figure 4c).

Discussion

This study investigates the antidiabetic effects of one of the most popular plants cultivated in Egypt,

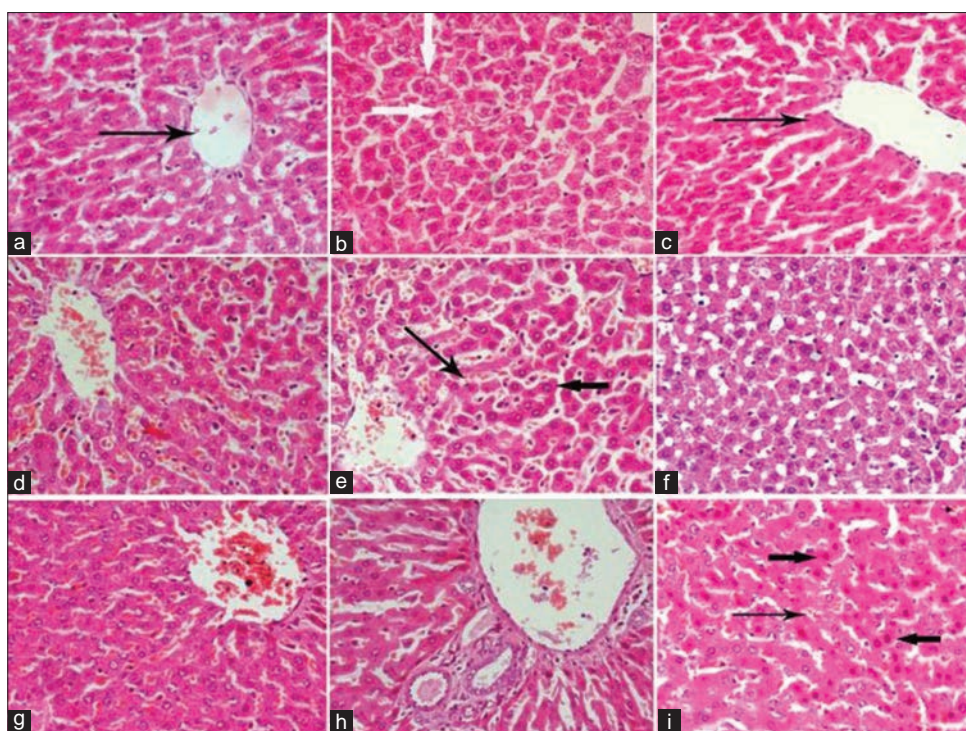


Figure 3: A photomicrograph of liver tissue using hematoxylin and eosin (H and E). (a) -ve control: Preserved architecture. (b) +ve control: Hydropic degeneration (arrow). (c) Gliclazide: Preserved architecture (arrow). (d) Total alc (50 mg): Mildly congested central vein, scattered inflammatory cells. (e) Total alc (100 mg): Mildly congested central vein (thin arrow). (f) Aq. ext. (50 mg): Normal hepatocyte. (g) Aq. Ext. (100 mg): Mild congested central vein. (h) Chloroform (50 mg): Mild congestion of dilated central vein, proliferating bile ducts, and normal hepatocytes. (i) Chloroform (100 mg): Mildly congested blood sinusoids (thin arrow) and pyknotic hepatocytes (thick arrow) (H and E, $\times 400$)

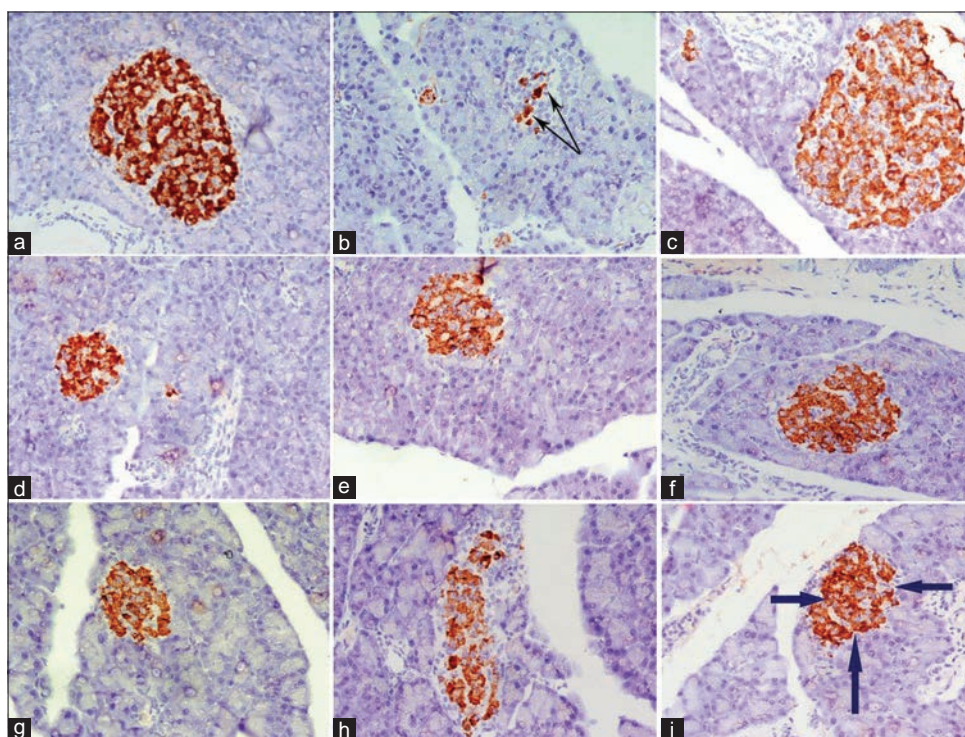


Figure 4: Immunohistochemical detection of insulin in pancreatic tissue: (a) -ve control rat: Large islets and plenty of β -cells with strong intensity and insulin antibody. (b) +ve control: Marked reduction in number, size, and density of β -cells (black arrow) (c) Gliclazide: Marked improvement of β -cells. (d) Total alc (50 mg): markedly improved β -cells. (e) Total alc (100 mg): Improved β -cells. (f) Aq. ext. (50 mg): Markedly improved of β -cells. (g) Aq. Ext. (100 mg): Increased β -cells. (h) Chloroform (50 mg): Markedly improved β -cells. (i) Chloroform (100 mg): Prominently improved β -cells number, size, and density (white arrows) (immunoperoxidase – $\times 400$)

C. olitorius. The individual fragmentation pattern of *C. olitorius* aqueous and chloroform extracts showed the presence of components from different chemical classes. These phytochemicals were reported to have pharmacological activities with a potential antidiabetic effect such as cinnamic acid and its derivatives, coumarins, apigenin, and glycitein. In a recent reported study, cinnamic acid and its derivatives were found to be associated with protective effects against diabetes and its complications [18]. Similarly, coumarins were found to possess antidiabetic effects [19]. Apigenin is a member of flavones family and showed a hypoglycemic effect in STZ-induced diabetic rats through enhancing GLUT4 translocation and improving the pancreatic architecture, suggesting glucose lowering potential of this flavone as well as β -cell preserving efficacy [20]. Glycitein is a naturally isoflavone with multiple health benefits attributed to different biological functions. It has been demonstrated that glycitein has an antihyperglycemic effect [21], [22]. Theophylline is a potent bronchodilator that acts as an adenosine receptor antagonist. Adenosine was found to be linked to glucose homeostasis. Adenosine 1 receptor antagonism improved glucose tolerance in Zucker rats through enhancing glucose uptake in skeletal muscle [23]. Derivatives of theophylline were accordingly synthesized and showed a hypoglycemic effect [24].

STZ is a commonly used agent in the induction of diabetes in experimental animals [25]. When used at a large dose (50–75 mg/kg b.w.), STZ produces the destruction

of insulin-secreting β -cells through the generation of free radicals with methylation of DNA. Structural and biochemical alterations developed in this model resemble the consequences of Type 1 diabetes mellitus [26]. In the present study, upon the treatment of diabetic rats with the three extracts of *C. olitorius*, serum glucose level was successfully decreased. Histopathological investigation revealed that *C. olitorius* treatment caused remarkable improvement in the pancreatic tissue as reflected by an increased number, size and density of functioning β -cells. Taken together, it may be concluded that this plant act as an antihyperglycemic agent through stimulation of β cells regeneration or functionality. In previous studies, it was shown that the ethanolic seed extract of *C. olitorius* was effective as an antidiabetic remedy [7] and leaf extract showed antihyperglycemic effects in type 2 diabetic animal model [8].

Alpha-amylase is an enzyme involved in carbohydrates digestion. It enhances the hydrolysis of glycosidic linkage in some oligosaccharides and starch. It breaks down complex polysaccharides into oligo- and disaccharides which are further hydrolyzed into monosaccharides by α -glucosidase enzyme. These simpler forms can be then readily absorbed into the portal vein and increase serum glucose levels [27]. The activity of the α -amylase enzyme increased in the present study by injection of STZ and inhibited by the administration of *C. olitorius* extracts. Inhibition of α -amylase keeps carbohydrate digestion and monosaccharide absorption controlled. Their digestion

is delayed and, accordingly, postprandial hyperglycemia is alleviated. Therefore, amylase inhibitors are of potential therapeutic importance in the treatment of diabetes [28]. Hence, the antihyperglycemic capability of *C. olitorius* can be explained, in part, by regulating carbohydrate kinetics *in vivo* through inhibition of the α -amylase degrading enzyme. This agrees with previous studies that reported the effect of Jute leaves on α -amylase but in type 2 diabetes [8]. In coincidence with current histopathological findings, the antihyperglycemic effect was more pronounced in groups treated with chloroform extract than that of aqueous fraction, suggesting that it contains more active phytochemicals against diabetes.

Treatment with *C. olitorius* showed antioxidant potential, as evidenced by increased TAC. Oxidative stress is one of the main mechanisms that mediate the deleterious effects of diabetes and eventually aggravates diabetic complications. Reactive hydrogen peroxide radicals are formed due to glucose oxidation and dismutation with subsequent generation of superoxide anion radicals [29]. Thus, the ability of *C. olitorius* to restore the altered antioxidant status reveals its free radical scavenging potential. The presence of high contents of flavonoids, saponins, carbohydrates, tannins, and triterpenes further explains the antioxidant effect of the extracts. These phytochemicals act as reducing agents because of their strong redox properties, hydrogen donating, and singlet oxygen quenching activities [30]. It can be deduced that the antioxidant property of *C. olitorius* extract mediates, in part, its antihyperglycemic activity and consequently prevention of diabetic complications. The antioxidant effect was noticed in both the total alcoholic and its chloroform fraction.

Hyperlipidemia is one of the important complications of diabetes and plays a major role in disease prognosis. At normal conditions, insulin prevents the degradation of triglycerides and mobilization of fatty acids from fat stores. However, insulin shortage enhances lipoprotein lipase inactivation which stimulates the assembling of free fatty acids into phospholipids and cholesterol, resulting in raising serum lipids. Treatment with compounds with lipoprotein lipase enhancing effect could suppress elevated serum glucose. The proposed mechanism was controlling lipid metabolism and improving insulin resistance [31]. In parallel, the present lipid profile testing showed an elevation in serum levels of total cholesterol, triglycerides, and LDL-C, whereas HDL-C decreased in STZ-diabetic rats [8]. Treatment with *C. olitorius* extracts not only improved serum phospholipids abnormalities but also normalized their values. The aqueous and chloroform fraction were equally effective against hyperlipidemia. The presence of flavonoids has shown to be associated with decreasing the risk of atherosclerosis due to their ability to lower cholesterol and triglycerides levels and prevention of LDL oxidation [32].

In the present study, the significant increase in serum ALT and AST levels that were observed in STZ-induced diabetic rats represents liver damage compared to negative control rats. Liver necrosis in STZ-induced diabetic rats leads to increased plasma activities of transaminases due to leakage of these enzymes from liver cytosol into the bloodstream [33]. Oral administration of *C. olitorius* showed a protective effect on liver tissue by reducing the elevated levels of ALT and AST. This is in parallel with a recent investigation which showed the hepatoprotective effect of *C. olitorius* and suggested the ability of the plant to maintain the integrity of the hepatocellular membrane [34].

Conclusion

The present study explores that suppression of α -amylase and antioxidant activity is two proposed mechanisms that could mediate the antihyperglycemic effect of *C. olitorius* leaves. This effect varied with the different extracts of *C. olitorius*. The chloroform fraction exerted its antidiabetic activity in a dose-dependent manner and was superior in these effects to the aqueous one, indicating the higher efficacy of the nonpolar constituents.

C. olitorius total alcoholic extract (at a dose of 100 mg/kg) and its aqueous (at a dose of 100 mg/kg), and chloroform fractions (at both dose levels; 50 and 100 mg/kg) also protected against diabetic complications such as hyperlipidemia and determinant effects on the liver tissue. These antidiabetic effects can be ascribed to the presence of some bioactive phytochemical constituents such as theophylline, *trans*-2, 3-dimethoxycinnamic acid, 7-hydroxy-4-methyl coumarin, apigenin 7-glucoside, and glycitein. The results support the traditional use of this edible plant with the additive effect as an antidiabetic remedy.

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