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Assessment Of Biological Activities Of *Cissus Rotundifolia* (Forssk.) Vahl. Growing In Yemen.

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ABSTRACT

The present work was undertaken to evaluate the beneficial effects of the ethanolic extract and its fractions (methylene chloride and n-butanol) prepared from the dried leaves and stems of C. rotundifolia, on antihyperglycemic, antihyperlipidemic, antioxidant and protection against glucocorticoid-induced secondary osteoporosis. All the tested samples showed significant in vitro α-glucosidase inhibitory activity with methylene chloride fraction showed the highest activity and exhibited antioxidant DPPH radical scavenging activity. In in vivo study, all the tested samples exhibited significant antidiabetic activity; with n-butanol fraction (300 mg/kg, p.o.) showed the most potent activity. A significant decrease in serum cholesterol and triglyceride levels were also observed. In addition, a significant reduction of malondialdehyde and increase of glutathione contents in the liver tissue in STZ diabetic rats. The ethanolic extract and n-butanol fraction (300 mg/kg, p.o.) showed a significant increase in serum calcium and phosphorus levels in vivo study in protection against glucocorticoid-induced secondary osteoporosis in rats. The high content of total phenolic and flavonoids contents supported the above results. In conclusion, the present study justified and proved the traditional use of C. rotundifolia in Yemen for the treatment of type II diabetes.

Keywords: Cissus rotundifolia; antihyperglycemic; antihyperlipidemic; antioxidant; osteoporosis

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INTRODUCTION

Diabetes mellitus (DM), dyslipidemia, and osteoporosis are most common metabolic disorders which increase with age. The most common pathophysiologies of these disorders are oxidative stress [1-3]. Although, there are several classes of drugs are currently used for management of these diseases, but the high-cost limitations and adverse effects such as hypoglycemia, weight gain, gastrointestinal problems, and liver toxicity are major concerns to looking for alternative options or medicine to treat or control these diseases. The natural products are a good clinical practice and they are showing a bright future in the therapy of metabolic disorders [4].

Cissus rotundifolia (Vitaceae) is a perennial tendril climber shrub, native to Africa and Arabian Peninsula. It is an edible plant used traditionally in Yemen for the treatment of gastrointestinal troubles, in loss of appetite, skin diseases, burns and fever and used as antidiabetic [5, 6]. Many studies showed effective of C. rotundifolia as antimicrobial, analgesic, anti-inflammatory, hepatoprotective, ant malarial and antidiabetic [5-8]. The acute toxicity of the Methanolic extract (70%) at 0.25, 0.5, and 1 g/kg b.wt. of the aerial parts of C. rotundifolia previously reported by Said, Aboutabl7 who did not observe any mortality in rats up to 24 h.

Many Cissus species used to treat different ailments such as antidiabetic, antimicrobial, antiviral, inhibit angiotensin converting enzyme, inhibit neutral endopeptidase, inhibit amino peptidase, rheumatism, arthritis, gastrointestinal tract, antitumor, anti-cell proliferation, fracture healing, increases bone strength, increase proliferation of Sertoli cells, protects bone from postmenopausal bone loss, ant dysentery, anti-diarrhea, diuretic, anti-inflammatory, anticonvulsant, anxiolytic, anti-parasitic, hypocholesterolemic and anti-snake venom [9].

This study was aimed to carry out the phytochemical screening and estimated the total phenolic and flavonoids contents of C. rotundifolia, as well as, the evaluation of the bioactivities of its ethanolic extract (96%) and fractions (methylene chloride and n-butanol) based on its traditional uses in Yemen and reported studies of the genus.

MATERIALS AND METHODS

Plant material and extraction

The aerial parts (leaves and stems) of C. rotundifolia were collected from Bani Habash Mountains, Almahweet, Yemen, in August 2014 and were dried in shade. Voucher samples (# 23.8.16.1) were kept at the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University. The powdered plant material (3 kg) was extracted with ethanol (96%) by cold maceration till exhaustion to give 300 g of green residue. The dried ethanolic extract (200g) was partitioned successively with methylene chloride and n-butanol then kept at 4 oC. Ethanolic extract and its fractions were dissolved in 1% tween 80 to prepare a solution of a concentration of 150 and 300 mg/ml and used for pharmacological studies.

The dried powdered aerial parts (0.50 g) of C. rotundifolia were extracted twice, each for 15 min. with 10 ml acetone 80% containing 1% HCl by sonication. The extract was centrifuged at 3000 rpm for 10 min. and the supernatant was filtered. Then, the filtrate was transferred to a 25 ml volumetric flask and the volume was completed with distilled water. The extracts were filtered before spectrophotometric determination of the total phenolic and flavonoid contents [10].

Chemicals

Streptozotocin, gliclazide, and methylprednisolone were purchased from Sigma Chemical Co. (USA), whereas ascorbic acid, 4-Nitrophenyl-α-D-glucopyranoside (pNPG) and α-glucosidase enzyme were obtained from Sigma-Aldrich, Germany. Reagent Kits for glucose, TC, TG, MDA, GSH, calcium and phosphorus were purchased from Biodiagnostic, Egypt. Moreover, kits for insulin and acarbose were purchased from ALPCO Diagnostics (USA) and Alfa Aesar (Germany), respectively. Quercetin and gallic acid were obtained from E-Merck (Darmstadt, Germany) as authentic for colorimetric study.

Phytochemical screening

The ethanolic extract of the aerial parts of C. rotundifolia was screened for carbohydrates and/or glycosides, flavonoids, tannins, saponins, sterols and/ or triterpenes and alkaloids through applying various chemical tests [11, 12].

Determination of total phenolic content

The phenolic content was determined following Folin–Ciocalteu method.[13] An aliquot (0.2 ml) of the appropriately diluted extract was transferred into 10 ml volumetric flask. Then, diluted Folin–Ciocalteu reagent (0.5 ml) was added. After 3 min., 1 ml Na2CO3 solution (5g/L) was added. The volume was completed to 10 ml with distilled water. After 1 hr, the absorbance was measured at 725 nm against distilled water as the blank. The total phenolic content was measured as gallic acid equivalent after preparation of a standard curve.

Determination of total flavonoids content

Total flavonoid content was determined based on colorimetric assay using aluminum chloride method [14]. In brief, the prepared extract (1 ml) was transferred to a 10 ml volumetric flask containing 4 ml of distilled water, followed by the addition of 0.3 ml of solution of NaNO2 (0.5 g/L). After 5 min., 0.3 ml of AlCl3 solution (1 g/L) was added and 6 min. later, 2 ml of NaOH (1 mol/L) was added to the mixture. The total volume was made up to 10 ml with distilled water, the solution was mixed and the absorbance was measured at 510 nm against water as blank. Quercetin was used as standard and the equivalents (w/w) were determined from a standard calibration curve.

In vitro assessment of antioxidant activity using DPPH

The antioxidant activity of the ethanolic extract and its fractions (methylene chloride and n-butanol) was assessed in vitro by measuring their ability to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals compared to ascorbic acid as a standard according to the method reported by Brand-Williams[15]. The samples were dissolved in methanol (50 %) at concentrations of (10, 20, 40, 60, 80, 100 µg /ml), then mixed with 2 ml of 6x10-5 mol/L methanolic solution of DPPH. The mixtures were well shaken in a vortex (2500 rpm) for 1 min., then placed in a dark place at room temperature. The reduction of DPPH free radicals was measured by reading the absorbance of each sample against methanol at 517 nm. The experiment was performed in triplicate.

In vitro α-glucosidase inhibitory activity

The α-glucosidase inhibitory activity of the ethanolic extract and its fractions were assayed according to the procedure described by Li, Wen[16]. The ethanolic extract and its fractions were weighed and serial dilutions of 0.5, 1, 2, 3, 5 mg/ml were made up of equal volumes of dimethylsulfoxide. The effect of ethanolic extract and its fractions on the activity of α-glucosidase was assessed in 96-well flat bottom microplate. Each well contains 20 µl (100 mM) potassium phosphate buffer (pH 6.8), 20 µl of (2.5 mM) p-nitrophenyl-α-D-glucopyranoside (PNG, substrate) dissolved in (0.1mM) potassium phosphate buffer (pH 6.8) and 20 µl sample. The enzymatic reaction was initiated by addition of 20 µl of the enzyme solution (0.2 U/ml) and the plate was incubated at 37 oC for 15 min. Finally, the absorbance was measured spectrophotometrically at 405 nm and acarbose was used as a positive control with various concentrations (0.05, 0.1, 0.25, 0.5 and 0.6 mg/ml).

Experimental animals

The study was conducted on adult male albino Wistar rats, weighing 150 - 200 g each. They were purchased from locally bred strains, allocated in groups and were allowed to accommodate for one week in the animal house of National Research Centre, Cairo, Egypt before subjecting them to experimentation. They were provided with a standard pellet diet and were given water ad libitum. All the experimental works with the animals were carried out after obtaining approval from Institutional Animal Ethical Committee of the National Research Center.

Evaluation of antidiabetic, antihyperlipidemic and antioxidant activities in STZ-induced diabetic rats

Induction of diabetes mellitus and experimental design

Diabetes mellitus was induced by a single intraperitoneal injection of streptozotocin (STZ) solution (55 mg/kg) dissolved in citrate buffer (0.1 M, pH 4.5) [17]. Because of hypoglycemia induced after injection of STZ, the animals were allowed to drink 5% glucose solution during night[18]. After 72 hour blood samples were withdrawn from the retro-orbital venous plexus under light ether anesthesia and the serum was separated by centrifugation for the determination of glucose level. Only rats with serum glucose levels more than 250 mg/dl were selected as diabetic animals and randomly divided into 9 groups (groups II-VIII) six rats each.
Groups I: Rats received 1 ml of saline and served as a normal control.

Groups II: Diabetic rats serving as diabetic control (STZ).
Groups III: Diabetic rats received gliclazide (10 mg/kg, p.o.) and serving positive control group.
Groups IV - V: Diabetic rats received ethanolic extract (150 and 300 mg/kg, p.o.).
Groups VI-VII: Diabetic rats received methylene chloride fraction (150 and 300 mg/kg, p.o.).
Groups VIII-IX: Diabetic rats received n-butanol fraction (150 and 300 mg/kg, p.o.).

After 14 days of the treatment, the animals were kept for an overnight fasting and the blood samples were collected from retro-orbital plexus and allowed to clot for 30 min. at room temperature. The blood samples were centrifuged at 5000 rpm for 20 min. and serum was separated and stored at -80°C for determination of glucose, insulin, cholesterol and triglycerides levels. After the collection of blood samples, all rats were sacrificed by cervical dislocation. The liver was rapidly removed from each animal and washed in ice-cold 0.9 % saline. Specimens of livers were stored at -80° for the estimation of glutathione (GSH) and malondialdehyde (MDA) contents.

Estimation of serum biochemical parameters

Determination of serum glucose and insulin levels

Serum glucose was estimated by glucose oxidase-peroxidase method according to the method of Trinder [19]. The absorbance was measured at 510 nm and the results were expressed as mg/dl. Whereas insulin was estimated by a radioimmunoassay technique using the ALPCO Insulin (Rat) ELISA kit according to the method of Judzewitsch[20].

Determination of cholesterol and triglycerides levels

Triglycerides were estimated by the enzymatic method by using diagnostic kit according to the method of Fossati and Prencipe[21]. The absorbance was measured at 510 nm and the results were expressed as mg/dl. Also, total cholesterol was estimated by the enzymatic method by using diagnostic kit according to the method of Allain[22].

Determination of lipid peroxidation product (MDA) and glutathione (GSH) content in liver tissue

The products of lipid peroxidation (mainly, MDA) were determined as thiobarbituric acid-reactive substances according to the method of Uchiyama and Mihara[23]. The resulting pink colored chromogen was extracted with n-butanol and was measured at 532 nm. The liver reduced glutathione (GSH) content was determined according to the method of Beutler and Kelly[24], based on the fact that both protein and non-protein thiol (SH-) groups (mainly, GSH) react with Ellman's reagent [5,5`-dithiobis (2-nitrobenzoic acid)] to form a stable yellow color of 5-mercapto-2-nitrobenzoic acid, which can be measured colorimetrically at 412 nm.

Protective effect of C. rotundifolia against glucocorticoid-induced secondary osteoporosis in rats

Induction of osteoporosis and Experimental design

Osteoporosis was induced by three times a week of subcutaneous injection of methylprednisolone (MP; 10 mg/kg) for six weeks. The procedure was performed according to a method of Derakhshanian[25]. Forty-eight rats were randomly divided into eight groups (6 rats per group), and treated for 6 weeks. Groups I: Rats received 1 ml of saline and serving as normal control; Groups II: Rats that received methyl prednisolone (MP; 10 mg/kg, sc) three times a week serving as MP control; Groups III- IV: Rats received MP (10 mg/kg, 3 times/week, s.c.) + orally ethanolic extract (150 and 300 mg/kg, b.wt., dailly); Groups V - VI: Rats received MP (10 mg/kg, 3 times/week, s.c.) + orally methylene chloride fraction (150 and 300 mg/kg, b.wt., dailly); Groups VII - VIII: Rats received MP (10 mg/kg, 3 times/week, s.c.) + orally n-butanol fraction (150 and 300 mg/kg, b.wt., dailly).

Estimation of biochemical parameters

At the end of the study, animals were killed under general anesthesia with chloral hydrate (400 mg/kg i.p.) and blood was collected by cardiac puncture. Blood was centrifuged at 3000 rpm for ten min for the separation of serum. The separated serum stored immediately at -20 oC. finally, calcium was estimated by OCPC method [26] and phosphorus was estimated by molybdate UV method [27].

Statistical analysis

Data were presented as means ± S.E. Statistical analysis of the data was performed using one-way analysis of variance (ANOVA) followed by Tukey Kramer multiple comparisons test for post-hoc analysis. Statistical significance was acceptable to a level of $P < 0.05$. Analysis of data was carried out by software program GraphPad Prism (version 6).

RESULTS AND DISCUSSION

Phytochemical screening

The preliminary phytochemical screening revealed that the dried powdered aerial parts of C. rotundifolia contain; Carbohydrates and/or glycosides, sterols and/or triterpenes, tannins, ± coumarins, flavonoidal aglycones and/or glycosides, while the alkaloids were absent.

Total phenolic and flavonoids contents

Phytochemical study of the aerial parts of C. rotundifolia revealed the presence of high amount of flavonoids (2.93 mg QE/g DW), and phenolic (5.60 mg GAE/g DW). Several studies reported that the phenolic compounds showed direct action on pancreatic β-cells, stimulating insulin secretion through the activation of specific cellular targets and protecting these cells from damages mediated by oxidative stress and inflammation, both typically elevated in diabetes 28. Therefore, the antihyperglycaemic effect of C. rotundifolia could be attributed to its phenolic and/or flavonoid contents.

In vitro assessment of α-glycosidase inhibitory activity

All the tested samples showed significant α-glucosidase inhibitory activity, with methylene chloride fraction showed the highest activity (IC50 1.23 ± 0.02 mg/ml) compared to acarbose (IC50 0.79 ± 0.01 mg/ml) (Table 1). Similarly, another study was done on C. arnottiana, by Sama30, who found that the ethanolic extract (10 mg/ml) of the fruits of C. arnottiana showed appreciable α-glucosidase inhibitory activity with IC50 values 2.81 mg/ml.

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Table 1: α-Glucosidase inhibitory activity of the ethanolic extract (96%) and its fractions (methylene chloride and n-butanol) of C. rotundifolia

	IC_{50} mg/ml ± SD
Standard	
Acarbose	0.79 ± 0.01
Extract/Fractions	
Ethanolic extract	2.09 ± 0.01
Methylene chloride fraction	1.23 ± 0.02
n-Butanol fraction	1.89 ± 0.02

SD; standard deviation

In vitro assessment of antioxidant activity using DPPH

As seen in the table (2) n-butanol fraction showed the highest activity to scavenge DPPH radical with IC50 25.04 ± 0.76 µg/ml while, the ethanolic extract showed the lowest activity with IC50 90.72 ± 8.68 µg/ml, compared to ascorbic acid (IC50 38.55 ± 0.83 µg/ml). These results were in agreement with the study reported by Vijayalakshmi29, on the ethanolic extract of C. quadrangular and the isolated flavonoids fraction, which showed good dose-dependent free radical scavenging activity with IC50 values of 125 and 12 µg/ml for ethanolic extract and flavonoids fraction, respectively.

Table 2: Free radicle scavenging activity (DPPH assay) of the ethanolic extract (96%) and its fractions (methylene chloride and n-butanol) of C. rotundifolia.

	IC_{50} µg/ml ± SD
Standard	
Ascorbic acid	38.55 ± 0.83
Extract/Fractions	
Ethanolic extract	90.72 ± 8.68
Methylene chloride fraction	70.82 ± 6.54
n-Butanol fraction	25.04 ± 0.76

SD; standard deviation

Evaluation of antidiabetic activity in STZ-induced diabetic rats

Rats injected with STZ showed a significant increase in blood glucose and decrease in serum insulin levels as compared with normal control (Table 3). Diabetic rats which treated by gliclazide (10 mg/kg, p.o.) for two weeks showed a significant decrease on glucose level and significant increase on insulin level as compared with diabetic control group, as well as administration of ethanolic extract and its fractions (methylene chloride and n-butanol) at two doses (150 and 300 mg/kg, p.o.) exhibited the same significant effect on serum glucose and insulin level. Administration of n-butanol fraction (300 mg/kg, p.o.) to diabetic rats showed the most potent activity as the level of serum glucose was decreased by 61.42 % and insulin level was increased by 3.71 folds as compared to diabetic control group as illustrated in table (3). Findings of the present study concerning the antihyperglycemic effect of C. rotundifolia are in accordance with those reported by Al-Mehdar and Al-Battah31, who reported that the water extract (100 mg/kg) of the leaves of C. rotundifolia showed a decrease in the level of serum glucose. In the present study, we reported that ethanolic extract and its fractions (methylene chloride and n-butanol) showed strong antidiabetic and antioxidant activity. This is in accordance with results reported by Lekshmi32**,** on C. quadrangular, who found that the ethyl acetate extract (50,100 and 200 mg/kg) of the stems extract had potential antidiabetic activity, and showed significant improvement of insulin sensitivity, reduced liver damage and oxidative changes.

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Table 3: Effects of gliclazide, ethanolic extract of C. rotundifolia & its fractions on serum glucose and insulin levels.

Groups	Glucose level (mg/dL)		Serum insulin level (µIU/ml)
	After 72 hours of treatment	After 14 days of treatment	
Normal control	87.09 ± 4.83	91.67 ± 11.01	8.52 ± 0.48
Diabetic control (STZ)	313.61 ± 24.05*	237.3 ± 30.47*	2.10 ± 0.46*
Gliclazide (10 mg/kg b.wt.)	256.03 ± 25.38	86.90 ± 20.26@	8.00 ± 0.49@
Ethanolic extract			
150 mg/kg, b.wt.	285.06 ± 20.89	114.76 ± 22.82@	5.64 ± 0.69@
300 mg/kg, b.wt.	255.43 ± 29.71	111.08 ± 14.39@	6.92 ± 0.56@
Methylene chloride fraction			
150 mg/kg, b.wt.	287.70 ± 26.46	117.30 ± 12.80@	4.62 ± 0.62@
300 mg/kg, b.wt.	277.74 ± 18.92	107.54 ± 19.28@	6.52 ± 0.53@
n-Butanol fraction			
150 mg/kg, b.wt.	310.01 ± 24.23	128.11 ± 11.65@	7.68 ± 0.44@
300 mg/kg, b.wt.	292.58 ± 38.41	91.56 ± 10.77@	7.80 ± 0.50@

Data represent the mean of 6 rats ± Standard error, *; Significant compared to the normal control, @; Significant compared to the diabetic control (STZ) at $p < 0.0001$.

Evaluation of antihyperlipidemic activity in STZ-induced diabetic rats

Data of table (4) showed that the serum cholesterol and triglycerides levels were significantly increased in STZ-induced diabetic rats compared to normal control. Administration of gliclazide (10 mg/kg, p.o.) and the two doses (150 and 300 mg/kg, p.o.) of the ethanolic extract and its fractions (methylene chloride and n-butanol) to diabetic rats exerted a significant decrease on triglycerides level as compared to untreated diabetic rats. Also all treatment regimens showed a significant decrease in total cholesterol level when administrated to diabetic rats except ethanolic extract (150, 300 mg/kg, p.o.) groups as compared to diabetic control group. These results were in agreement with the reported results by Lino33 on the methanolic extract of the leaves of C. verticillata, which showed decrease plasma glucose, triglycerides and total cholesterol levels in the alloxan-induced diabetic rats and increase in the number of pancreatic β-cells.

Table 4: Effects of gliclazide, ethanolic extract of C. rotundifolia & its fractions on serum TC and TG levels after two weeks of treatment.

	Serum TC level (mg/dL)	Serum TG levels (mg/dL)
Normal Control	217.6 ± 4.53	195.3 ± 20.35
Diabetic control (STZ)	307.6 ± 4.67*	372.0 ± 39.16*
Gliclazide (10 mg/kg, b.wt.)	224.9 ± 2.73@	222.0 ± 25.89@
Ethanolic extract		
150 mg/kg, b.wt.	246.5 ± 9.86	213.9 ± 13.54@
300 mg/kg, b.wt.	246.4 ± 10.35	208.0 ± 9.06 @
Methylene chloride fraction		
150 mg/kg, b.wt.	228.7 ± 22.57@	215.6 ± 8.01@
300 mg/kg, b.wt.	201.1 ± 16.89@	211.8 ± 7.27@
n-Butanol fraction		
150 mg/kg, b.wt.	192.7 ± 16.82@	189.3 ± 14.98@
300 mg/kg, b.wt.	171.3 ± 29.06@	188.4 ± 16.85@

Data represent the mean of 6 rats ± Standard error, *; Significant compared to the normal control, @; Significant compared to the diabetic control (STZ) at $p < 0.0001$, TC; Total cholesterol, TG; Triglycerides.

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Evaluation of antioxidant activity in STZ-induced diabetic rats

As represented in table (5) significantly higher lipid peroxidation product MDA and decreased the concentration of major intracellular antioxidant GSH contents were found in diabetic rats compared to normal control group. Oral administrations of gliclazide (10 mg/kg, p.o.), ethanolic extract (300 mg/kg, p.o.) and its fractions (methylene chloride and n-butanol) at two doses (150 and 300 mg/kg, p.o.) showed a significant decreased in the content of MDA. On the other hand, gliclazide and all tested samples at dose (300 mg/kg, p.o.) were significantly normalized the liver GSH content of diabetic rats. Data of the present investigation showed that induction of diabetes by STZ induced oxidative stress as evidenced by a significant increased on lipid peroxidation products like MDA and significantly decreased on non-enzymatic antioxidant GSH content in the liver tissue of diabetes rats. This may lead to the activation of stress pathways, which ultimately leads to tissue damage. This hypothesis is in agreement of recently reports which considered the oxidative stress as a causative factor in the diabetes pathogenesis and play a major role on pancreatic β-cell damage 34. The previous results were further supported from the in vitro antioxidant study of C. rotundifolia, which revealed significant inhibition of DPPH radical in a dose-dependent manner. In addition, in vivo study further supported the antioxidant potential of tested samples, where it was found a significant decreased in MDA and a significant increased in GSH contents. The potent antioxidant effect of C. rotundifolia may be involved in decreasing serum glucose level in diabetic rats, this suggestion is in accordance with the result reported by Ceriello35, who found that the oxidative stress is a common pathogenic mechanism behind many diseases including diabetes and its complications.

Table 5: Effects of gliclazide, ethanolic extract of C. rotundifolia & its fractions on MDA and GSH contents in the liver tissue after two weeks of treatment.

	MDA content (nM/g)	GSH content (µM/g)
Normal Control	110.6 ± 7.45	14.38 ± 0.49
Diabetic control (STZ)	168.7 ± 3.97 *	10.37 ± 0.47*
Gliclazide (10 mg/kg, b.wt.)	110.5 ± 15.18 @	13.47 ± 0.43@
Ethanolic extract		
150 mg/kg, b.wt.	132.6 ± 5.60	11.81 ± 0.32
300 mg/kg, b.wt.	115.7 ± 12.47@	13.31 ± 0.30@
Methylene chloride fraction		
150 mg/kg, b.wt.	111.5 ± 14.66@	11.60 ± 0.49
300 mg/kg, b.wt.	94.74 ± 11.16@	13.09 ± 0.51@
n-Butanol fraction		
150 mg/kg, b.wt.	109.0 ± 6.32@	11.30 ± 0.59
300 mg/kg, b.wt.	104.6 ± 10.95@	13.57 ± 0.69@

Data represent the mean of 6 rats ± Standard error, *; Significant compared to the normal control, @; Significant compared to the diabetic control (STZ) at $p < 0.0001$.

Protective effect of C. rotundifolia against glucocorticoid-induced secondary osteoporosis in rats

Significant reduction of serum calcium and phosphorus levels were observed in MP rats compared to normal control. This may be because MP enhances urinary excretion of calcium and reduces its intestinal absorption 36. After oral administration of daily treatment during six weeks, only ethanolic and n-butanol extracts at dose 300 mg/kg b.wt., showed significant increased ($P < 0.0001$) in the serum calcium and phosphorus levels, while, the other groups showed improvement in serum calcium and phosphorus levels, as compared to untreated MP group table (6), these results were in agreement with another study reported by 37, on the ethanolic extract (75 and 100mg/kg) of the aerial parts of C. quadrangularis, which showed

significant increase in bone thickness, bone density, bone hardness, serum estradiol, serum vitamin D3 and serum calcium.

Table 6: Effects of ethanolic extract and its fractions (methylene chloride and n-butanol) of C. rotundifolia on serum calcium and phosphorus levels

	Serum calcium level (mg/dl)	Serum phosphorus level (mg/dl)
Normal Control	10.79±0.07	7.03±0.52
MP control (10 mg/kg, s.c).	9.52±0.28*	3.887±0.42*
Ethanolic extract 150 mg/kg, b.wt. 300 mg/kg, b.wt.	 9.71±0.18 10.79± 0.21@	 4.76±0.48 6.88±0.40@
Methylene chloride fraction 150 mg/kg, b.wt. 300 mg/kg, b.wt.	 9.69±0.20 9.98±0.11	 4.37±0.26 4.46±0.47
n-Butanol fraction 150 mg/kg, b.wt. 300 mg/kg, b.wt.	 9.66±0.17 10.50±0.15@	 5.05±0.29 7.46±0.58@

Data represent the mean of 6 rats ± Standard error, *; Significant compared to the normal control, @; Significant compared to the methyl prednisolone control (MP) at $p < 0.0001$. sc; subcutaneous route.

CONCLUSION

From this study, we can conclude that ethanolic extract and its fractions (methylene chloride and n-butanol) of C. rotundifolia, possess significant antihyperglycemic activity in STZ induced diabetic rats. The possible mechanism of this antidiabetic activity may be due to stimulation of the activity of the remnant pancreatic ß-cells due to its high content of phenolic compounds. Also, this plant exhibited antihyperlipidemic and antioxidant activities which provide more advantage on counteracting of diabetes complications. Statistically, there is no significant difference between of C. rotundifolia samples and gliclazide in the aspect of decreased STZ-induced hyperglycemia, hyperlipidemia and ameliorated oxidative stress. On other hand, the ethanolic extract and n-butanol fraction of C. rotundifolia showed the inhibitory effect of glucocorticoid on bone formation.

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