Antidiabetic and biochemical effects of new flavonols extracted from *Justicia ghiesbreghtiana* stem

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Background and objective
The main aim of this study was to examine the biochemical effects of two new natural flavonoid compounds isolated from *Justicia ghiesbreghtiana* stem extract on diabetes mellitus type II.

Materials and methods
One kilogram air-dried stems of *J. ghiesbreghtiana* were exhaustively extracted with 75% aqueous ethanol several times by heat treatment. The resulting extract was fractionated on a Sephadex LH-20 column using water and water/ethanol mixtures as eluents to yield six fractions, from which two new pure natural compounds 1 and 2 were isolated. The antidiabetic effect of compounds 1 and 2 was investigated in this study using 40 adult male rats.

Results and conclusion
Phytochemical investigation of the ethanolic extract of *J. ghiesbreghtiana* stem has led to the isolation of nine pure flavonoid compounds of quercetin type; two of them are new and have been identified as quercetin 3-*O*-α-L-arabinofuranoside-7-*O*-α-L-rhamnopyranoside-4′-*O*-β-D-galactopyranoside (1) and quercetin 3-*O*-α-L-arabinofuranoside-7-*O*-β-D-glucopyranoside-4′-*O*-β-D-galactopyranoside (2); in addition, two quercetin diglycosides, four quercetin monoglycosides, and aglycone quercetin were obtained. Their chemical structures have been established by conventional methods of chemical and physical analysis and confirmed by nuclear magnetic resonance spectroscopy. The biochemical study of compounds 1 and 2 on diabetic rats indicated improvement in kidney functions shown by reductions in both urea and creatinine levels. Moreover, glutathione peroxidase and superoxide dismutase concentrations were increased. Also, albumin and total protein levels were reduced, with an increase in liver functions, aspartate aminotransferase, and alanine aminotransferase levels, with a mild decrease in both cholesterol and triglyceride concentrations.

Keywords:
Acanthaceae, antidiabetic biochemical effects, *Justicia ghiesbreghtiana*, new natural quercetin glycosides, nuclear magnetic resonance spectroscopy

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Introduction

There has been growing interest in the therapeutic use of natural products, especially those derived from plants, for the treatment of diabetes. The family Acanthaceae (mostly herbs or shrubs) comprises about 250 genera and 2500 species including twining forms [1]. The chemistry of Acanthaceae shows a huge diversity of different compounds such as alkaloids, iridoids, lignans, flavonoids, terpenoids, and phenylpropanoid glycosides [2–6]. From *Justicia pectoralis* [7] *O*-methylated C-glycosylflavones were isolated, whereas the triterpenoid saponin and the 4-aryl-2,3-naphthalide lignans were obtained from the whole plant of *Justicia simplex* [8,9]. The acylated flavones, apigenin 7-(3″-acetyl-6″-E-P-coumaroyl glucoside) (the flavanone), naringenin 7-(3″-acetyl-6″-E-P-coumaroyl glucoside), and 9′-decarboxy rosmarinic acid-4′-*O*-(1→4)-galactosyl rhamnoside, were isolated from the ethanolic extract of aerial parts of *Blepharis ciliaris* [10,11]. The roots of *Andrographis paniculata* were found to contain methylated flavone glucosides, flavanone glucosides, and a steroid [12]. From the aerial parts of *Brillantaisia palisatii*, dirhamnosyl flavonoid, sitosterol, stigmasterol, phenylpropanoid glycosides, and triterpenes were isolated and identified; the dirhamnosyl flavonoid showed hypotensor activity [13]. Some plants belonging to this family have many biological and pharmacological activities such as antidiabetic, anti-HIV, antioxidant, antiviral, and antisyphilitic activities, and are especially useful for the treatment of gastrointestinal ailments [13]. Crude extracts from various plants of Acanthaceae have been investigated for their antimicrobial activities, whereby many of them showed growth-inhibitory activity against many microbial species [14]. The hexane extract of the roots of *Rhinacanthus nasutus kurz*, known as 'Thong phun chang', as well as their isolated compounds were found to exert a significant cytotoxic effect on P388 lymphocytic leukemia *in vitro* [15]. From our previous studies on Acanthaceae plants, we found that the twigs of *Justicia adhatoda* contain 10 flavonoid compounds; two of them are new, which were identified as 6-OH-genistein-6-*O*-

α-L-rhamnopyranoside-4′-*O*-β-D-glucopyranoside and 6-*O*-α-L-rhamnopyranoside-6,8-dihydroxy apigenin [16], whereas apigenin 6-C-β-D-galactopyranoside-4′-*O*-α-L-rhamnopyranoside and genkwanin 8-C-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranoside were isolated from its leaves [17]. Also from *Anisacanthus virgularis*' aerial parts 12 flavonoid compounds were isolated including two new ones, which were identified as 6,8-dihydroxy-apigenin-6-*O*-α-L-rhamnopyranoside-8-*O*-β-D-galactopyranoside and 6-OH-8,3′-dimethylether luteolin-6-*O*-α-L-rhamnopyranoside, and the pharmacological activities of these compounds as antioxidants and antimicrobials were studied [18].

The present study focuses on the chemistry of naturally occurring polyphenolic compounds together with their glycosides isolated from the aqueous ethanolic extract of *Justicia ghiesbreghtiana* stems and the examination of the biochemical effects of the isolated new compounds on diabetic rats. This plant is used in traditional medicine to treat various infections and inflammatory conditions [19]. Its methanolic extract is characterized by the presence of compounds of polyphenolic nature and has been shown to inhibit the reproduction of the influenza A virus [20]. An amide of threo-γ-hydroxyglutamic acid, justiciamide, was isolated from *J. ghiesbreghtiana*, which is the first amide of an uncommon amino acid found in this genus [21]. Thus, it was of interest to study its phytochemical constituents, especially polyphenolic components, which yielded nine pure flavonoid compounds of quercetin type; two of them were isolated for the first time in nature, namely, quercetin 3-*O*-α-L-arabinofuranoside-7-*O*-α-L-rhamnopyranoside-4′-*O*-β-D-galactopyranoside (**1**) and quercetin 3-*O*-α-L-arabinofuranoside-7-*O*-β-D-glucopyranoside-4′-*O*-β-D-galactopyranoside (**2**); in addition, the following known compounds were obtained: quercetin 3-*O*-α-L-arabinofuranoside-7-*O*-α-L-rhamnopyranoside and quercetin 3-*O*-α-L-arabinofuranoside-7-*O*-β-D-glucopyranoside, quercetin 3-*O*-α-L-arabinofuranoside, quercetin 7-*O*-β-D-glucopyranoside, quercetin 7-*O*-α-L-rhamnopyranoside, quercetin 4′-*O*-β-D-galactopyranoside, and the aglycone quercetin. Their chemical structures have been established by conventional methods of chemical and physical analysis and confirmed by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. The main aim of this prospective study is to examine the biochemical effects of the two newly isolated compounds extracted from *J. Ghiesbreghtiana* stem extract, which showed high activity in diabetic rats.

Materials and methods

Plant material

J. ghiesbreghtiana stems were collected from the Orman Botanical Garden (Egypt) and identified by Dr El-Gebaly. A voucher specimen (569JG) has been deposited in the National Research Centre Herbarium (CAIRC).

General methods

^{1}H (200 MHz) and ^{13}C (50 MHz) NMR (Varian Gemini 200 Spectrometer, England): chemical shifts were recorded in DMSO-d_6 and are given in values ppm. Ultraviolet (UV) spectra were measured on a Shimadzu spectrophotometer model UV-240 (England). Column chromatography was performed using Sephadex LH-20 (England). Paper chromatography (PC) was carried out on Whatman No. 1 using solvent systems (i) BAW (n-BuOH: AcOH: H_2O, 6: 1: 2); (ii) 15% AcOH (AcOH: H_2O, 15: 85); and (iii) H_2O, whereas for preparative PC, Whatman 3MM (England) was used. For enzymatic hydrolysis [0.5 ml of 0.05% of the enzyme (β-glucosidase, β-galactosidase) (BDH Chemicals Ltd, Poole, UK)], α-rhamnosidase (pectinase; Sigma-Aldrich) in 0.05 acetate buffer (pH 5) was added to 10 mg of flavonoid glycoside in 10 ml water. The mixture was incubated at 37–40°C for 24 h and finally the hydrolysate was examined by two-dimensional PC.

Extraction and isolation

One kilogram of air-dried stems of *J. ghiesbreghtiana* were exhaustively extracted with 75% aqueous ethanol several times (five extractions each with 3 l) under heat treatment. The combined extracts were concentrated under reduced pressure to yield a dry extract (376 g), which, by preliminary two-dimensional PC screening, was found to contain a type of flavonol mixture. The resulting extract was fractionated on a Sephadex LH-20 column (700 g, 40 × 1000 mm) using water and water/ethanol mixtures as eluents to yield six fractions (I–VI). These fractions were further fractionated using either preparative PC or a Sephadex LH-20 column to isolate and purify all the compounds obtained. The new natural compound 1 (265 mg) was eluted in a pure form from fraction II (10 g, 20% ethanol) by an elution technique using Whatman 3MM paper and the solvent system 15% AcOH for irrigation, whereas compound 2 (384 mg) was isolated in a pure form from the third fraction (40% EtOH, 15 g) using a Sephadex LH-20 column eluted with *n*-butanol.

Experimental animals

Forty adult Sprague–Dawely male rats, of the same age (4 months) and weight (120–150 g), obtained from

the animal house colony of the National Research Center, were used in this study. The animals were kept in stainless-steel cages under the same hygienic conditions under a 12 h light/dark cycle. They were fed on a well-balanced diet and had free access to tap water.

The animals were divided into the following groups:

Group I: 10 normal healthy adult male rats served as a control group.

Group II: 30 adult male rats were rendered diabetic by the administration of an intraperitoneal injection of a freshly prepared alloxan monohydrate solution at a dose of 120 mg/kg body weight [22].

Group III: 10 alloxan-diabetic rats from group II were orally administered a solution of compound 1 (20 mg/kg body weight, lethal dose) using an orogastric tube daily for a period of 30 days.

Group IV: 10 alloxan-diabetic rats from group II were orally administered a solution of compound 2 (20 mg/kg body weight, lethal dose) daily for a period of 30 days.

Blood samples were collected using ocular vein puncture from the fasting (8–12 h) control group, the alloxan-diabetic group, and the two groups treated with compounds 1 and 2, respectively. Small portions of blood samples were placed in heparinized plastic tubes and assayed on the day of collection to prevent the conversion of glutathione into its reduced form to determine glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities as well as lipid peroxidation. Other portions of the blood samples were left to clot, and then centrifuged at 5000 rpm under cooling for 10 min to separate the sera for other biochemical analyses.

Biochemical studies

Determination of serum glucose concentration

Serum glucose concentration was determined enzymatically according to the method described by Trinder [23].

Determination of serum aminotransferase enzyme activities

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using kits provided by Pointe Scientific Inc. (Canton, USA), according to Tietz [24].

Determination of serum protein concentration

Quantitative determination of the total protein concentration in serum was carried out using kits provided by Pointe Scientific Inc., according to the method described by Weichselbaum [25].

Determination of serum albumin concentration

Serum albumin concentration was determined using kits supplied by Pointe Scientific Inc., according to Doumas *et al*. [26].

Determination of serum cholesterol and triglycerides

Quantitative determination of total cholesterol in serum was carried out according to the method described by Richmond [27]. Triglyceride concentration in the serum was determined using the enzymatic colorimetric method of Bucolo and David [28].

Determination of lipid peroxidation

The product of lipid peroxidation was determined as a thiobarbituric acid-reactive substance (TBARS) according to the method of Mihara and Uchiyamo [29].

Determination of glutathione peroxidase activity

Erythrocyte GPX activity was determined using the Ransel kit from Randox Laboratories (UK) according to the Paglia and Valentine's [30] method. The activity of GPX was expressed as units per gram of hemoglobin (Hb). The Hb concentration was determined using the cyanmet-Hb method according to Mahoney *et al*. [31].

Determination of superoxide dismutase activity

The activity of SOD was determined using the Ransel kit from Randox Laboratories. This method uses xanthine and xanthine oxidase to generate superoxide radicals that react with 2-(4-iodophexyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride to form a red formazan dye. The SOD activity is measured by the degree of inhibition of this reaction [32].

Determination of glutathione content in liver tissue

The glutathione content in liver tissue homogenate was estimated using the method of Beutler [33].

Determination of serum urea concentration

Enzymatic determination of serum urea was carried out according to the method of Fawcett and Scott [34] using kit provided by BioMerieux (Lyon, Rhône, France).

Determination of serum creatinine concentration

Serum creatinine concentration was determined according to the method described by Bartles *et al.* [35] using the kit provided by Pointe Scientific Inc.

Determination of serum testosterone concentration

Enzyme immunoassay kit for the quantitative measurement of testosterone in serum was provided by Biosource Company (London, UK), according to the method described by Hill *et al.* [36].

Determination of serum acid phosphatase activity

Serum acid phosphatase activity was determined according to a colorimetric method described by Moss [37] using the kit provided by Quimica Clinica Aplicada S.A. (Tarragona, Spain).

Statistical analysis

The data are expressed as mean ± SE. Statistical comparisons were performed by one-way analysis of variance, followed by Student's *t*-test. The results were considered statistically significant if the *P* values were 0.05 or less.

Spectral data of the two new compounds

Quercetin 3-O-α-L-arabinofuranoside-7-O-α-L-rhamnopyranoside-4′-O-β-D-galactopyranoside (1)

R_f values × 100: 25(1), 72(2), 84(3). UV spectral data (λ_{max}, nm) MeOH: 255, 349; +NaOMe: 269, 208sh, 377; +NaOAc: 262, 350; +NaOAc/H_3BO_3: 255, 266, 350; +$AlCl_3$: 277, 298sh, 355, 400; +$AlCl_3$/HCl: 265sh, 278, 297sh, 348, 398 (sh, shoulder). ^{1}H NMR spectral data: aglycone moiety: δ (ppm) 7.3 (d, *J* = 2.0 Hz, H-2′), 7.26 (dd, *J* = 2.0 and 8.0 Hz, H-6′), 6.84 (d, *J* = 8.0 Hz, H-5′), 6.9 (d, *J* = 8.0 Hz, H-8), 6.3 (d, *J* = 8.0 Hz, H-6); sugar moiety: δ (ppm) 5.45 (d, *J* = 1 Hz, H-1 of α-L-arabinofuranoside), 5.25 (d, *J* = 2 Hz, H-1 of α-L-rhamnopyranoside), 5.0 (d, *J* = 7 Hz, H-1 of β-D-galactopyranoside), 4.21 (dd, *J* = 2 and 5 Hz, H-2 of α-L-rhamnopyranoside) and 0.84 (d, *J* = 6 Hz, CH_3 of rhamnose), 3.08–3.88 (m, rest of sugar protons). ^{13}C NMR spectral data: aglycone moiety: δ (ppm) 156.5 (C-2), 133.6 (C-3), 177.6 (C-4), 161.3 (C-5), 98.8 (C-6), 162.5 (C-7), 94.4 (C-8), 165.0 (C-9), 104.6 (C-10), 125.9 (C-1′), 117.1 (C-2′), 146.9 (C-3′), 146.6 (C-4′), 115.6 (C-5′), 121.5 (C-6′); 3-*O*-α-L-arabinofuranoside: δ (ppm) 108.0 (C-1″), 82 (C-2″), 77.2 (C-3″), 86.3 (C-4″), 61.1 (C-5″); 7-*O*-α-L-rhamnopyranoside: δ (ppm) 99.1 (C-1‴), 70.1 (C-2‴), 70.2 (C-3‴), 71.5 (C-4‴), 70.5 (C-5‴), 17.5 (C-6‴); 4′-*O*-β-D-galactopyranoside: δ (ppm) 102.3 (C-1⁗), 72.1 (C-2⁗), 75.5 (C-3⁗), 68.1 (C-4⁗), 75.1 (C-5⁗), 60.1 (C-6⁗).

Quercetin 3-O-α-L-arabinofuranoside-7-O-β-D-glucopyranoside-4′-O-β-D-galactopyranoside (2)

R_f values×100: 22(1), 83(2). UV spectral data (λ_{max}, nm) MeOH: 256, 348; +NaOMe: 269, 209sh, 376; +NaOAc: 262, 349; +H_3BO_3: 255, 267, 350; +$AlCl_3$: 279, 297sh, 356, 400; +HCl: 266sh, 279, 295sh, 349, 397. ^{1}H NMR spectral data: aglycone moiety: δ (ppm) 7.29 (d, *J* = 2.0 Hz, H-2′), 7.25 (dd, *J* = 2.0 and 8.0 Hz, H-6′), 6.83 (d, *J* = 8.0 Hz, H-H-5′), 6.89 (d, *J* = 8.0 Hz, H-8), 6.28 (d, *J* = 8.0 Hz, H-6); sugar moiety: δ (ppm) 4.90 (d, *J* = 7.5 Hz of β-D-glucopyranoside), 5.25 (d, *J* = 1 Hz, H-1 of α-L-arabinofuranoside), 5.0 (d, *J* = 7 Hz, H-1 of β-D-galactopyranoside), 3.08–3.88 (m, rest of sugar protons). ^{13}C NMR spectral data: aglycone moiety: δ (ppm) 156.5 (C-2), 133.6 (C-3), 177.6 (C-4), 161.3 (C-5), 98.8 (C-6), 162.5 (C-7), 94.4 (C-8), 165.0 (C-9), 104.6 (C-10), 125.9 (C-1′), 117.1 (C-2′), 146.9 (C-3′), 146.6 (C-4′), 115.6 (C-5′), 121.5 (C-6′); 3-*O*-α-L-arabinofuranoside: δ (ppm) 108.0 (C-1″), 82 (C-2″), 77.2 (C-3″), 86.3 (C-4″), 61.1 (C-5″); 7-*O*-β-D-glucopyranoside: δ (ppm) 100.4 (C-1‴), 73.2 (C-2‴), 77.2 (C-3‴), 69.9 (C-4‴), 76.5 (C-5‴), 60.9 (C-6‴); 4′-*O*-β-D-galactopyranoside: δ (ppm) 102.3 (C-1⁗), 72.1 (C-2⁗), 75.5 (C-3⁗), 68.1 (C-4⁗), 75.1 (C-5⁗), 60.1 (C-6⁗).

Results and discussion

One of the most significant results of the present study is the improvement in the kidney functions in response to the two isolated new compounds (**1** and **2**) extracted from *J. ghiesbreghtiana* stem extract, with a parallel reduction in the concentrations of both urea and creatinine level in serums, which decreased highly significantly. Also, both GPX and SOD levels were increased; in addition, the levels of albumin and total protein were reduced. A significant result is the marked improvement in the different biochemical symptoms of the serum glucose level; liver functions markedly improved in terms of both AST and ALT levels, with a mild decrease in both cholesterol and triglyceride levels.

Recently, many traditionally used medicinal plants have been tested for their antidiabetic potential by various investigators in experimental animals. Working along the same line, we carried out a study on the two new compounds **1** and **2** isolated from *J. ghiesbreghtiana* stem for their antidiabetic biochemical effects.

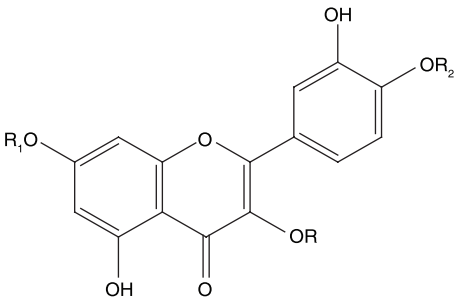
The aqueous ethanolic extract of *J. ghiesbreghtiana* stem led to the isolation and identification of nine natural flavonoid compounds of quercetin type. The seven known compounds were identified as quercetin 3-*O*-α-L-arabinofuranoside-7-*O*-α-L-rhamnopyranoside, 3-*O*-α-L-arabinofuranoside-7-*O*-β-D-glucopyranoside, 3-*O*-α-L-arabinofuranoside, 7-*O*-β-D-glucopyranoside, 7-*O*-α-L-rhamnopyranoside,

4′-*O*-β-D-galactopyranoside, and the aglycone quercetin. The two new compounds were identified as follows:

Compound **1** was obtained as a yellow amorphous powder and appeared as a dark brown spot on PC changing to brown when exposed to ammonia vapors. Its UV spectral data in MeOH and with the addition of diagnostic reagents showed that its flavonol type substituted at 3, 7, and 4′ positions, which yielded a spectrum with absorption maxima at 255 nm (band II) and 349 nm (band I) characteristic of a flavanol skeleton. The 28 nm bathochromic shift in band I produced on the addition of NaOMe with a decrease in its intensity proved that 4′ substituted with the absence of any shift in the same band on the addition of NaOAc/H_3BO_3, indicating the absence of a 3′,4′-*O*-dihydroxyl group in ring B and a substituted one at 4′, whereas the absence of a shoulder at 328 nm in the NaOMe curve and the shift (1 nm) in band II upon the addition of NaOAc suggested that position 7 was occupied. Complete acid hydrolysis of compound **1** yielded quercetin as the aglycone and the three sugars rhamnose, arabinose, and galactose, which were identified by comparative paper chromatography (Co-Pc) using authentic markers. The intermediate, quercetin 3-*O*-α-L-arabinofuranoside-7-*O*-α-L-rhamnopyranoside, was obtained on enzymatic hydrolysis using β-galactosidase and identified by UV spectral data, R_f value, that is, galactose is in position 4′ of compound 1. Its partial acid hydrolysis yielded the intermediate quercetin 7-*O*-α-L-rhamnopyranoside-4′-*O*-β-D-galactopyranoside, which was identified as before, that is, arabinose was attached to position 3 and rhamnose was attached at position 7. Further confirmation of compound **1** was performed with ^{1}H NMR spectral data, which showed signals of the quercetin skeleton besides three signals of three anomeric sugar protons at δ ppm 5.45 (d, J = 1 Hz, H-1 of α-L-arabinofuranoside), 5.25 (d, J = 2 Hz, H-1 of α-L-rhamnopyranoside), 5.0 (d, J = 7 Hz, H-1 of β-D-galactopyranoside), 4.21 (dd, J = 2 and 5 Hz, H-2 of α-L-rhamnopyranoside), and 0.84 (d, J = 6 Hz, CH_3 of rhamnose) [38]. Its structure was confirmed by ^{13}C NMR, which yielded the three signals of the three anomeric sugar carbons at δ ppm (108.0, 99.1, and 102.3 of α-L-arabinofuranoside, α-L-rhamnopyranoside, and β-D-galactopyranoside, respectively) together with the upfield shift of C-3, 7, and 4′ signals, which appeared at δ ppm 133.6, 162.5, and 146.6, respectively, whereas the unsubstituted ones appeared at 135.9, 164.2, and 148.5, respectively [39]. Thus, its structure is the new natural isolated compound quercetin 3-*O*-α-L-arabinofuranoside-7-*O*-α-L-rhamnopyranoside-4-*O*-β-D-galactopyranoside.

Compound **2** (yellowish brown amorphous powder) was identified through its R_f values, color reactions, and UV spectral data as a flavonol substituted at 3, 7, and 4′ positions. Its complete acid hydrolysis yielded the aglycone quercetin and the three sugars, glucose, arabinose, and galactose, which were identified by Co-Pc using authentic markers. Enzymatic hydrolysis using β-galactosidase yielded the intermediate quercetin 3-*O*-α-L-arabinofuranoside-7-*O*-β-D-glucopyranoside, which was identified as above, that is, galactose is in position 4′ of compound 2. Its partial acid hydrolysis yielded the intermediate quercetin 7-*O*-β-D-glucopyranoside-4′-*O*-β-D-galactopyranoside, which was identified as before, that is, arabinose was attached to position 3 and glucose was attached to position 7. ^{1}H NMR spectrum of compound 2 confirmed its structure as quercetin 3-*O*-α-L-arabinofuranoside-7-*O*-β-D-glucopyranoside-4′-*O*-β-D-galactopyranoside through the presence of the signals of the quercetin skeleton besides three signals of three anomeric sugar protons at δ ppm 5.3 (d, J = 7.5 Hz of β-D-glucoside), 5.25 (d, J = 1 Hz, H-1 of α-L-arabinofuranoside), 5.0 (d, J = 7 Hz, H-1 of β-D-galactopyranoside), and 3.08–3.88 (m, rest of sugar protons) [38]. This proposed structure was confirmed by ^{13}C NMR, which showed the three anomeric sugar carbons signals at 108.0, 100.4, and 102.3 ppm assignable to C-1 of α-L-arabinofuranoside, β-D-glucopyranoside, and β-d-galactopyranoside, respectively, together with the upfield shift of C-3, 7, and 4′ signals, which appeared at δ ppm 133.6, 162.5, and 146.6, respectively (where the unsubstituted ones appeared at 135.9, 164.2, and 148.5, respectively) [39]. Thus, from the above data, compound 2 can be identified as the new natural compound: quercetin 3-*O*-α-L-arabinofuranoside-7-*O*-β-D-glucopyranoside-4′-*O*-β-D-galactopyranoside, which was isolated for the first time in nature (Fig. 1).

Figure 1



Structure of the isolated compounds **1** and **2**.
Compound 1: R = α-L-arabinofuranoside, R_1 = α-L-rhamnopyranoside, R_2 = β-D-galactopyranoside.
Compound **2**: R = α-L-arabinofuranoside, R_1 = β-D-glucopyranoside, R_2 = β-D-galactopyranoside.

Biological study

Table 1 shows the serum glucose level of control rats, alloxan-diabetic rats, and diabetic rats treated with compounds **1** and **2**. The results show a highly significant increase in the serum glucose level in alloxan-diabetic rats compared with the control rats. Diabetic rats treated with compounds **1** and **2** showed a highly significant decrease in serum glucose level compared with alloxan-diabetic rats. The production of glucose by gluconeogenesis is an energy-expensive process as the production of one mole of glucose from pyruvate will require 6 mol of ATP [40]. It is therefore likely that the necessary energy was peroxide by the increased rate of lipid oxidation. The reduction in serum glucose from 299 ± 4.2 to 109 ± 0.5 mg/dl after treatment of alloxan-diabetic rats with compound 1 indicates that it could induce blood glucose homeostasis through regeneration of endocrine pancreas and by increasing insulin secretion and stimulating the enzyme glycogen synthetase, which traps glucose moieties into pre-existing glycogen chains [41].

Liver function assessments for control rats, alloxan-diabetic rats, and diabetic rats treated with compounds 1 and 2 are shown in Table 2, from which the marked dearrangement in liver functions is clear. The serum levels of AST, ALT, triglycerides, and cholesterol increased significantly to 72.3 ± 5.8 m/l, 64.0 ± 3.4 m/l, 144.2 ± 3.2 mg/dl, and 123 ± 1.1 mg/dl, respectively, in alloxan-diabetic rats compared with the control rats, whereas serum albumin and total protein levels decreased markedly to 2.6 ± 0.5 and 5.3 ± 0.2 mg/dl, respectively. The high level of ALT is indicative of severe hepatocellular damage as it is more liver specific than AST. Triglycerides are synthesized in the liver from fatty acids and glycerol and as such they are transported as very low-density lipoproteins to adipose tissue store [42]. However, the significantly high level of serum triglycerides in alloxan-diabetic rats (Table 2) may be associated with the reduced triglyceride content of muscle, which indicates rapid disruption of the glucose fatty acid cycle [43].

A considerable improvement in liver function of diabetic male rats was observed after treatment with compounds 1 and 2. The serum level of AST was highly significantly reduced from 72.3 ± 5.8 to 51 ± 1.2 and 47 ± 1.4 μ/l for 1 and 2, respectively. The serum level of ALT was highly significantly reduced from 64.0 ± 3.4 to 44.2 ± 1.8 and 39 ± 4.5 μ/l for 1 and 2, respectively, whereas a mild decrease in the serum levels of cholesterol and triglycerides was observed in Table 2. The protein content remained unchanged or decreased in alloxan-diabetic rats, that is, in liner of diabetic animals but skeletal and cardiac muscles are most common to the major site of net protein less during diabetes [44,45]. Table 2 also shows how a reduction in the level of total protein in diabetic rats mechanisms for intracellular protein breakdown is cytosolic ATP-dependent. It has been reported that the plasma concentration of a number of the regulatory substances such as glucagone, glucocorticoids, and branched chain amino acids, which also affect protein metabolism, are altered during the insulin-deficient state. It is noteworthy that the in-vivo presence of other hormones, particularly alkysoid and corticosteroid hormone, can affect protein turnover either alone or in contact with insulin [45]. In fact, treatment of diabetic rats with compounds **1** and **2** led to an increase in the level of total protein (Table 2). There was a highly significant decrease in serum albumin level in diabetic rats as compared with the control group, whereas a mild increase in serum albumin level was observed after the treatment of diabetic rats with compounds **1** and **2**. These compounds have shown a protective effect against oxidation stress, which has been found to be mainly because of an increased production of free radicals because of a marked reduction in antioxidant defenses [46].

To assess the indices of oxidative stress that are associated with the development of complications in diabetes, TBARSs were measured as an index of malondialdehyde production and hence lipid peroxidation, compared with control, and diabetic liver and pancreas, showed a significant increase in the TBARS level during the experimental period [47]. The data of lipid peroxidation for TBARS, GPX, and SOD in diabetic rats and diabetic rats treated with compounds **1** and **2** are shown in Tables 3 and 4. A highly significant increase in lipid peroxidation was observed in alloxan-diabetic male rats compared with the corresponding control and data in Tables 3 and 4 show the levels of TBARS in whole blood (89.2 ± 5.3 nmol/g Hb) and in the liver (77.1 ± 1.2 nmol/g fresh tissue), whereas a highly significant decrease in lipid peroxidation was detected after treatment with compounds 1 and 2 (78.2 ± 1.1 and 74.1 ± 1.9 nmol/g Hb and 68.1 ± 1.2 and 70.3 ± 2.0 nmol/g fresh tissue, respectively). In contrast, a highly significant decrease in both GPX and SOD activities was detected in alloxan-diabetic rats compared with control rats, whereas the activity of GPX and SOD showed a highly significant increase after treatment with compounds **1** and **2** as shown in Tables 3 and 4.

Table 1 Serum glucose level of control rats, alloxan-diabetic rats, and diabetic rats treated with compounds 1 and 2 at the end of 30 days

Items	Serum glucose (mg/dl)
Control group (n = 10)	74.1 ± 0.8
Alloxan-diabetic rats (n = 10)	299 ± 4.2**
Treatment with 1 (n = 10)	109 ± 0.5**
Treatment with 2 (n = 10)	157 ± 1.4**

Values are presented as mean ± SE of 10 animals. **P < 0.01, highly significant change.

Table 2 Liver profile for control group, alloxan-diabetic rats, and diabetic rats treated with compounds 1 and 2 at the end of 30 days

Items	AST (μ/l)	ALT (μ/l)	Total protein (mg/dl)	Albumin (mg/dl)	HDL-cholesterol (mg/dl)	TG (mg/dl)
Control group (n = 10)	32.4 ± 1.0	33.0 ± 1.7	5.9 ± 0.4	3.6 ± 0.3	92.2 ± 4.8	97.1 ± 3.3
Alloxan-diabetic rats (n = 10)	72.3 ± 5.8**	64.0 ± 3.4**	5.3 ± 0.2*	2.6 ± 0.5**	123 ± 1.1**	144.2 ± 3.2
Treatment with 1 (n = 10)	51 ± 1.2**	44.2 ± 1.8**	6.1 ± 0.2 (NS)	3.7 ± 0.6*	129 ± 1.1 (NS)	134.1 ± 1.2 (NS)
Treatment with 2 (n = 10)	47 ± 1.4**	39 ± 4.5**	5.8 ± 0.3 (NS)	3.1 ± 0.1*	130 ± 1.3 (NS)	136.4 ± 1.0 (NS)

Values are mean ± SE of 10 animals. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high density lipoprotein; TG, triglyceride. *P < 0.05, significant change. **P < 0.01, highly significant change.

Table 3 Lipid peroxidation as thiobarbituric acid-reactive substance, glutathione peroxidase, and superoxide dismutase activities in whole blood of the control group, alloxan-diabetic rats, and diabetic rats after treatment with compounds 1 and 2 (20 mg/kg) at the end of 30 days

Items	Control group (n = 10)	Alloxan-diabetic rats (n = 10)	Treatment with 1 (n = 10)	Treatment with 2 (n = 10)
TBARS (nmol/g Hb)	45.4 ± 1.4	89.2 ± 5.3**	74.1 ± 1.1**	78.2 ± 1.9**
Glutathione peroxidase (μ/g Hb)	55.2 ± 1.1	43.1 ± 1.2**	45.2 ± 1.1**	44.1 ± 0.9**
Superoxide dismutase (μ/g Hb)	3.9 ± 0.14	2.3 ± 0.13**	2.91 ± 0.08**	2.5 ± 0.14**

Values are mean ± SE of 10 animals. Hb, hemoglobin; TBARS, thiobarbituric acid-reactive substance. **P < 0.01, highly significant change.

Table 4 Level of thiobarbituric acid-reactive substance, glutathione, and superoxide dismutase in the liver of control group, alloxan-diabetic rats, and diabetic rats treated with compounds 1 and 2 (20 mg/kg) at the end of 30 days

Items	Control group (n = 10)	Alloxan-diabetic rats (n = 10)	Treatment with 1 (n = 10)	Treatment with 2 (n = 10)
TBARS (nmol/g fresh tissue)	46.3 ± 2.1	77.1 ± 1.2**	68.1 ± 1.2*	70.3 ± 2.0*
Glutathione peroxidase (mg/g fresh tissue)	7.1 ± 0.1	4.8 ± 0.3**	5.1 ± 0.2*	4.9 ± 0.1 (NS)
Superoxide dismutase (μ/g fresh tissue)	5100 ± 140	2700 ± 120**	3400 ± 200**	2950 ± 190 (NS)

Values are mean ± SE of 10 animals. Hb, hemoglobin; TBARS, thiobarbituric acid-reactive substance. *P < 0.05, significant change. **P < 0.01, highly significant change.

Table 5 shows the kidney function profile of control rats, alloxan-diabetic rats, and diabetic rats treated with compounds **1** and **2**. Highly significant increases in serum urea and creatinine concentration were observed in alloxan-diabetic rats compared with the control rats. Thus, urea measurements have been considered to provide a means of assessment of renal function as 50% or more of urea filtered to the glomerulus is passively reabsorbed through the tubules [48]. Table 5 shows a highly significant increase in serum urea level in alloxan-diabetic rats. Normal level of urea is less than 45 mg/ml and creatinine level is less than 0.1 mg/ml and as a result of the side effect of alloxan injection associated with increasing level of glucose which lead to consequent reduction in the glomerular filtration rate and retention of urea. Table 5 also shows a highly significant increase in serum creatinine in diabetic rats. Due to the increase of the kidney enzymes (Urea and Creatinine), is associated with decrease in the glomerular filtration rate [42]. After treatment with compounds **1** and **2**, highly significant decreases in serum urea and creatinine concentration were detected from 92.2 ± 1.4 to 61.7 ± 3.6 mg/dl and from 2.6 ± 0.1 to 1.3 ± 0.12 mg/dl, respectively, for compound **1** and from 92.2 ± 1.4 to 55.5 ± 2.6 mg/dl and from 2.6 ± 0.1 to 1.5 ± 0.15 mg/dl, respectively, for compound **2**.

Table 5 Kidney function profile for the control group, alloxan-diabetic rats, and diabetic rats treated with compounds 1 and 2 at the end of 30 days

Items	Urea (mg/dl)	Creatinine (mg/dl)
Control group (n = 10)	47.4 ± 2.2	0.8 ± 0.04
Alloxan-diabetic rats (n = 10)	92.2 ± 1.4**	2.6 ± 0.1**
Treatment with 1 (n = 10)	55.5 ± 2.6**	1.5 ± 0.15**
Treatment with 2 (n = 10)	60.1 ± 1.7**	1.3 ± 0.12**

Values are mean ± SE of 10 animals. **P < 0.01, highly significant change.

The levels of serum testosterone, total, and prostatic acid phosphates of control rats, alloxan-diabetic rats, and diabetic rats treated with compounds **1** and **2** are shown in (Table 6). A highly significant decrease in serum testosterone level is accompanied with the increase of total and prostatic acid phosphatase activities and that was observed in alloxan-diabetic rats compared with control rats. After treatment with compounds **1** and **2**, a highly significant increase in serum testosterone

Table 6 Testosterone, total, and prostatic acid phosphatase levels in the serum of the control group, alloxan-diabetic rats, and diabetic rats treated with compounds 1 and 2 at the end of 30 days

Items	Control group (n = 10)	Alloxan-diabetic rats (n = 10)	Treatment with 1 (n = 10)	Treatment with 2 (n = 10)
Testosterone (μg/100 ml)	720 ± 3.1	750 ± 3.4**	740 ± 2.1*	722 ± 1.1**
Total acid phosphatase (μ/l)	8.0 ± 0.34	15.9 ± 0.18**	13.7 ± 0.2**	14.1 ± 2.4**
Prostatic acid phosphatase (μ/l)	8.9 ± 0.3	9.3 ± 0.2**	5.4 ± 2.1**	6.1 ± 2.0**

Values are mean ± SE of 10 animals; *P < 0.05, significant change; **P < 0.01, highly significant change.

level accompanied by a highly significant decrease in total and prostatic acid phosphatase activities were observed. It has been reported by BalaSubramanian *et al*. [49] that diabetes mellitus is associated with a significant reduction in serum testosterone levels and weight of accessory sex gland. The sperm content of tepidity male regions also decreased. These results are in agreement with our data (Table 6), in which we found a highly significant decrease in serum testosterone level in alloxan-diabetic rats may reflect highly significant increase in total acid phosphatase and prostatic acid phosphatase activities.

In conclusion, in the current study, we observed an improvement in the kidney functions in response to the two new isolated compounds extracted from *J. ghiesbreghtiana* stem extract, with a parallel reduction in both urea and creatinine levels. Moreover, GPX and SOD concentrations were increased. Also, albumin and total protein levels were reduced. There was a marked improvement in the different biochemical symptoms in terms of the serum glucose level; liver functions developed in terms of both AST and ALT levels, with a mild decrease in both cholesterol and triglyceride concentrations.

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Conflicts of interest

There are no conflicts of interest.

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