

## Antidiabetic activity of phenolic compounds from Pecan bark in streptozotocin-induced diabetic rats

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### ABSTRACT

The *n*-butanol fraction (BF) of bark of Pecan tree, *Carya illinoensis* (Wangenh) K. Koch (Juglandaceae) afforded two new flavonol methyl ether: caryatin-3' sulfate (**6**) and caryatin-3' methyl ether-7-O- $\beta$ -D-glucoside (**7**) while five known phenolics (**1**–**5**) were isolated from its ethyl acetate fraction (EAF). The structures of isolated compounds were established based on 1D and 2D NMR spectroscopy. The isolated compounds were investigated for their hypoglycaemic, antioxidant as well as the aldose reductase (AR) inhibitory effect in lenses of streptozotocin diabetic rats. All the isolated compounds showed significant hypoglycaemic and antioxidant activities, except **5** and **6**. A marked AR-inhibitory effect was identified for compounds **2**, **3** and **7**.

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### 1. Introduction

Diabetes is a chronic and possibly life-threatening widespread endocrine disease, if left untreated. Globally, its prevalence continues to increase, owing to ageing and socio-economic changes (King et al., 1998). According to the International Diabetes Federation (2007), Egypt records one of the highest diabetes rates in the Middle East and North Africa region (MENA) with 11% of its population suffering from the disease; this estimate is expected to reach 13.5% by 2025. The polyol pathway appears to play an important role in the development of degenerative chronic complications associated with diabetes, such as neuropathy (Young et al., 1983) nephropathy (Dunlop, 2000), retinopathy (Engerman, 1989), cataract (Robinson et al., 1983) and cardiovascular diseases (Fuente and Manzanaro, 2003). Aldose reductase (AR), as a key enzyme in the polyol pathway, catalyzes the reduction of excess glucose, in various tissues (nerves, retina, lens and kidney), into sorbitol. The latter does not readily diffuse across cell membranes, and its intracellular accumulation has been implicated in the chronic complications of diabetes. Thus, aldose reductase inhibitors (ARIs) may offer the possibility of preventing the progression of these complications (Lee, 2002). In the context of the worldwide spread of diabetes, an intensive search for new

lead compounds with antidiabetic potential is necessary. Flavonoids as phenolic compounds not only inhibit the polyol pathway (by acting as ARIs), but are also involved in prevention of overproduction of superoxide and in metal ion chelation processes (Fuente and Manzanaro, 2003). Thus, plants rich in flavonoids could be promising candidates for herbal management of diabetes.

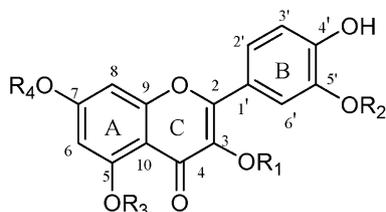
*Carya illinoensis* (Wangenh) K. Koch (Juglandaceae, Walnut Family) (Bailey, 1953), is among those plants reported to be rich in phenolics notably, flavonoid glycosides and aglycones, galloylated glycosides and condensed tannins building units (Nahla et al., 2007; Moheb et al., 1980; Cuong et al., 1996; Sasaki, 1964; Sasaki and Mikami, 1963). Pecan nuts were found to exert significant antioxidant and antihyperlipidemic effects (Abdelrahman et al., 2008; Villarreal-Lozoya et al., 2007), whereas leaves showed promising hepatoprotective effect (Nahla et al., 2007). Based on these reports, an evaluation of the antidiabetic potential of phenolics isolated there from pecan bark seemed of interest. Assessment of the isolated compounds was achieved through investigation of their hypoglycemic, antioxidant as well as aldose reductase inhibitory effects in streptozotocin-induced diabetic rats.

### 2. Results and discussion

A total of seven phenolic metabolites were isolated from the polar fractions of *C. illinoensis* bark. Their structures (Fig. 1) were identified as protocatechuic acid (**1**), quercetin (**2**), caryatin-3' methyl ether (**3**), azaleatin (**4**) and caryatin (**5**), caryatin-3' sulfate (**6**) and caryatin-3' methyl ether-7-O- $\beta$ -D-glucoside (**7**). The

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Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
6	CH <sub>3</sub>	SO <sub>3</sub> H	CH <sub>3</sub>	H
7	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	Glucose

Fig. 1. Chemical structure of the compounds isolated from *Carya illinoensis* bark.

known isolates (1–5) were readily identified by comparison of their chemical and spectroscopic data (UV, EI-MS, <sup>1</sup>H and <sup>13</sup>C NMR) with those corresponding in the literature.

Compound **6** was obtained as a yellowish amorphous powder. Its negative HRFAB-MS showed a molecular ion peak at *m/z* 409.0233 [M–H]<sup>–</sup> (calcd. 409.0229), corresponding to a molecular formula of C<sub>17</sub>H<sub>13</sub>O<sub>10</sub>S<sup>–</sup>. In positive HRFAB-MS mode, **6** showed a molecular ion peak at *m/z* 433.0221 [M+H+Na]<sup>+</sup> (calcd. 433.0205) corresponding to a molecular formula of C<sub>17</sub>H<sub>14</sub>O<sub>10</sub>SNa<sup>+</sup>. <sup>1</sup>H NMR spectrum of **6** (Table 1) indicated the presence of a disubstituted B-ring by an ABX-spin coupling system at δ<sub>H</sub> 7.94 (d, *J* = 2.1 Hz), 7.65 (dd, *J* = 8.7, 2.1 Hz), and 6.95 (d, *J* = 8.7 Hz) for H-2', H-6' and H-5', respectively. Additionally, an AM spin coupling system of two meta coupled proton signals at (δ<sub>H</sub> 6.46 and 6.35, each d, *J* = 1.8) were characteristic for H-8 and H-6, respectively in a 5,7 disubstituted A ring. The presence of two methyl ether groups was evidenced by two singlet signals at δ<sub>H</sub> 3.74 and 3.83, each integrated for three protons. The absence of 5-hydroxyl signal at 12 ppm confirmed presence of methyl ether at this position. These data were compatible with a structure of 3,5-dimethyl ether-3',4' disubstituted B-ring flavonol (Mabry et al., 1970). In addition, typically 17

carbon signals were similar to those of Caryatin (**5**) except for the resonance of ring B carbons that showed a typical shift due to the presence of a sulfate group (Barron et al., 1988): ca. 4.5 ppm upfield for the ipso carbon (C-3') and ca. 7.0, 3.5, 1.5 and 5.0 ppm downfield for C-2', C-4', C-5' and C-6', respectively. The effect of etherification at C-3 position was confirmed by the downfield location of C-3 (~+3 ppm) relative to that of free 3-OH and the presence of an intrinsic downfield methyl ether signal at δ<sub>C</sub> 59.7 (Agrawal, 1989). This was further confirmed by the observation of the three-bond correlation peak between the methyl ether protons signal at δ<sub>H</sub> 3.74 and the C-3 signal at δ<sub>C</sub> 139.9. Similarly, the connectivity of the methyl ether group to C-5 was further proved by the correlation peak of methyl ether protons signal at δ<sub>H</sub> 3.83 and C-5 at δ<sub>C</sub> 160.7. On the basis of the previous data together with UV data (Mabry et al., 1970), compound **6** could be identified as caryatin-3' sulfate.

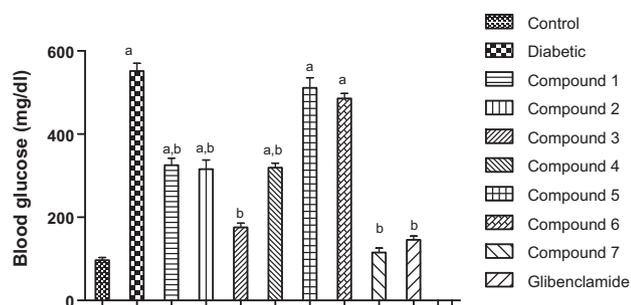
Compound **7** was obtained as a yellowish amorphous powder. Acid hydrolysis and GC were used to identify the sugar constituent of the compound. Compound **7** was acid hydrolyzed and the resulting sugar moieties were extracted, derivatized, and analyzed using GC along side with authentic sugars. The absolute configuration of the sugar was determined according to the method described by Hara et al. (1987). Based on the retention time of the authentic sugars, D-glucose was identified as the only sugar moiety in compound **7** (Hara et al., 1987). NMR data indicated that the sulfate group in **6** was replaced by a methyl ether group in **7**. This was further supported by the observation of the three-bond correlation peak between this methyl protons signal at δ<sub>H</sub> 3.85 and the C-3' signal at δ<sub>C</sub> 147.4 in the HMBC spectrum. The 1D and 2D NMR spectroscopic data, confirmed the aglycone moiety as quercetin 3,5,3'-tri methyl ether. One anomeric proton signal was assigned at δ<sub>H</sub> 5.07 (d, *J* = 7.2 Hz, β-glucosyl). The connectivity of glucose moiety to C-7 was deduced from the downfield shift of both signals of H-8 at δ<sub>H</sub> 6.89 and H-6 at δ<sub>H</sub> 6.58 compared to compound **6**. This was further confirmed by the long-range three-bond HMBC correlation between H-1'' at δ<sub>H</sub> 5.07 and C-7 at δ<sub>C</sub> 161.4 of the aglycone. The sugar moiety was deduced to have a β-<sup>4</sup>C<sub>1</sub>-pyranose stereo structure based on *J*-values of the anomeric

Table 1  
NMR spectroscopic data (400 MHz, solvent) for compounds (**6**) and (**7**).

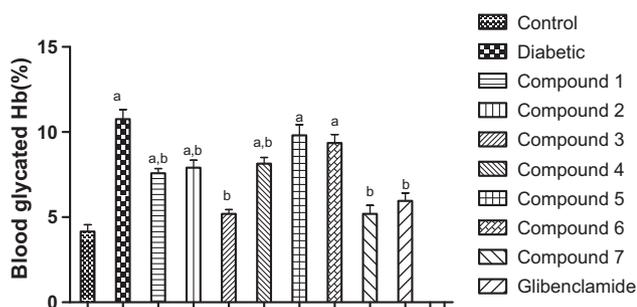
Compound ( <b>6</b> )				Compound ( <b>7</b> )			
Position	δ <sub>H</sub> ( <i>J</i> in Hz)	δ <sub>C</sub>	HMBC <sup>a</sup>	Position	δ <sub>H</sub> ( <i>J</i> in Hz)	δ <sub>C</sub>	HMBC <sup>a</sup>
2	-	151.3, C		2	-	152.1, C	
3	-	139.9, C		3	-	139.9, C	
4	-	172.1, C		4	-	172.1, C	
5	-	160.7, C		5	-	160.2, C	
6	6.35, d (1.8)	96.1, CH	5,7,8,10	6	6.58, d (2.1)	96.7, CH	7,8,10
7	-	162.4, C		7	-	161.4, C	
8	6.46, d (1.8)	94.7, CH	6,7,9,10	8	6.89, d (2.1)	95.9, CH	6,7,9,10
9	-	157.9, C		9	-	157.6, C	
10	-	107.3, C		10	-	109.0, C	
1'	-	121.3, C		1'	-	120.9, C	
2'	7.94, d (2.1)	122.3, CH	2,3',4',6'	2'	7.62, d (1.8)	111.7, CH	4',2,6'
3'	-	140.9, C		3'	-	147.4, C	
4'	-	151.1, C		4'	-	149.1, C	
5'	6.95, d (8.7)	117.0, CH	1',3',4'	5'	6.93, d (8.4)	115.5, CH	3',6'
6'	7.65, dd (8.7, 2.1)	124.6, CH	2,2',4'	6'	7.53, dd (8.7, 2.1)	121.6, CH	4',2,2'
5-OMe	3.83, s	55.8	5	1''	5.07, d (7.2)	99.9, CH	7
3-OMe	3.74, s	59.2	3	2''	3.25, t	73.1, CH-OH	
				3''	3.25, t	76.6, CH-OH	
				4''	3.11	69.8, CH-OH	
				5''	-	77.4, CH	
				6''	3.42, d	60.7, CH <sub>2</sub> -OH	
				5-OMe	3.85, s	55.7 <sup>b</sup>	5
				3-OMe	3.77, s	59.2	3
				3'-OMe	3.85, s	56.1 <sup>b</sup>	3'

<sup>a</sup> HMBC correlations are from carbon(s) stated to the indicated proton.

<sup>b</sup> Results may be interchanged.



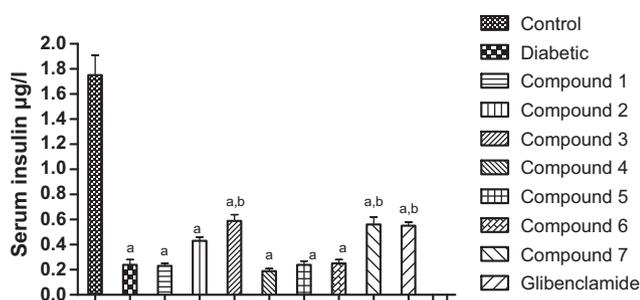
**Fig. 2.** Effect of oral administration of the isolated compounds (1–7) on blood glucose levels in STZ-induced diabetic rats. All values are expressed as means SEM of 6 observations. (a) Significant difference from normal control group at  $P < 0.005$ . (b) Significant difference from control diabetic group at  $P < 0.005$  m normal control group at  $P < 0.005$ .



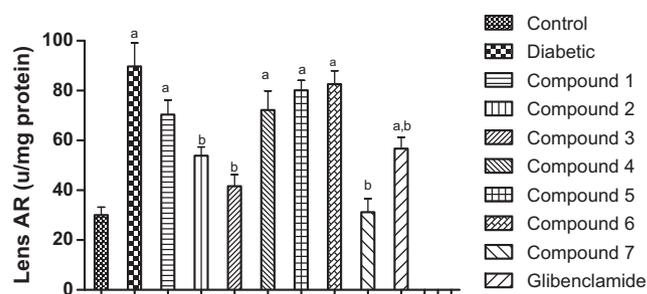
**Fig. 3.** Effect of oral administration of the isolated compounds (1–7) on blood glycated Hb in STZ-induced diabetic rats. All values are expressed as means SEM of 6 observations. (a) Significant difference from normal control group at  $P < 0.005$ . (b) Significant difference from control diabetic group at  $P < 0.005$  m normal control group at  $P < 0.005$ .

proton and  $\delta$ -values of its  $^1\text{H}$  and  $^{13}\text{C}$ -resonances (Table 1). On the basis of the previous data, compound 7 could be identified as caryatin-3'-methyl ether-7-O- $\beta$ -D-glucoside. This is the first report for isolating compounds 6 and 7.

Sterptozotocin is well known for its selective pancreatic islets  $\beta$ -cell cytotoxicity. It produces oxygen free radicals in the body, which causes pancreatic injury and could be responsible for increased blood glucose level. In case of diabetic rats under study, the blood glucose and HbA1c levels were increased with significant decrease in serum insulin compared to normal rats (Figs. 2–4). However, the oral administration of all isolated compounds (except compounds 5 and 6) led to a statistically significant hypoglycemic effect relative to glibenclamide (Fig. 2), where compounds 3 and 7 were the most potent. Moreover, treatment with the isolated compounds (except compounds 5 and 6) resulted in a significant decrease in HbA1c level



**Fig. 4.** Effect of oral administration of the isolated compounds (1–7) on blood insulin levels in STZ-induced diabetic rats. All values are expressed as means SEM of 6 observations. (a) Significant difference from normal control group at  $P < 0.005$ . (b) Significant difference from control diabetic group at  $P < 0.005$  m normal control group at  $P < 0.005$ .



**Fig. 5.** Effect of oral administration of the isolated compounds (1–7) on lens AR levels in STZ-induced diabetic rats. All values are expressed as means SEM of 6 observations. (a) Significant difference from normal control group at  $P < 0.005$ . (b) Significant difference from control diabetic group at  $P < 0.005$  m normal control group at  $P < 0.005$ .

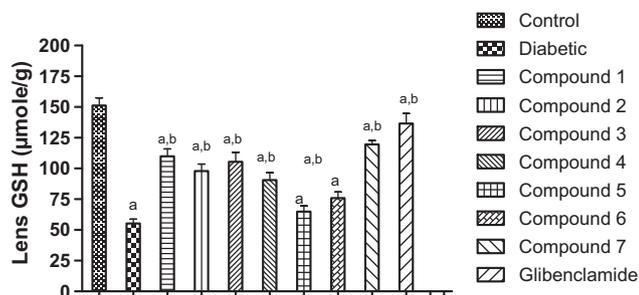
(Fig. 3). This might attribute to the improvement of glycemic status. On the other hand, treatment with isolated compounds revealed that only compounds 3 and 7 showed significant increase in serum insulin level, while the rest of tested compounds did not alter its level (Fig. 4). During hyperglycemic event, the elevated glucose level enhances the activity of AR, which catalyzes the reduction of glucose into sorbitol. In this study, AR activity in diabetic rat lens was significantly increased as compared to normal rats. Compounds 2, 3 and 7 exhibited a marked AR inhibitory effect in lenses (Fig. 5). The catechol moiety on the B ring of flavonols plays an important role against AR enzyme (Matsuda et al., 2002) although it is not sufficient by itself, and this confirms the inactivity of compounds 4–6 as ARIs. Furthermore, the methyl ether groups at 3, 5, 3' may be responsible for potentiation of the activity as in (3) which showed higher activity than its demethylated derivative (2). In contrast, an OH group at C-4' was important for AR inhibitory property. Finally, the presence of neighboring O-methyl group in phenolics generally inhibited the AR activity (Chethan et al., 2008). The increased activity of AR in the diabetic rats under study resulted in the decrease of NADPH/NADP<sup>+</sup> ratio, which has an impact on other NADPH-dependent enzymes, such as glutathione reductase (GSH). This decrease in GSH may be also due to an impaired ability of the lens to concentrate amino acids required for GSH biosynthesis coupled to faster GSH efflux under hyper osmotic condition. Furthermore, elevation of lens MDA level (Fig. 7) suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals (Amresh et al., 2007). The isolated compounds as ARIs have beneficial effects on lens GSH level in experimental models of diabetes and galactosemia, by elimination of osmotic stress (prevention of lens polyol accumulation), which is essentially required for normalization of GSH level (Obrosova and Stevens, 1999). The isolated compounds (except for 5 and 6) exerted an antioxidant activity through increasing GSH and decreasing MDA (Figs. 6–7).

In conclusion, the possible mechanism by which the isolated compounds exert their hypoglycemic effect could be by: induction of pancreatic insulin secretion from  $\beta$ -cell of islets of Langerhans, their antioxidant effect and AR inhibitor effect. Moreover, compounds 3 and 7 isolated from pecan bark could be a good source for medical foodstuffs and lead compound as alternatives for ARIs currently used. Furthermore, this is the first report of the isolation and biological activity of compounds 6 and 7.

### 3. Experimental

#### 3.1. General

Pure samples were separately measured as MeOH solutions with different diagnostic shift reagents (Mabry et al., 1970) on a UV



**Fig. 6.** Effect of oral administration of the isolated compounds (1–7) on lens GSH levels in STZ-induced diabetic rats. All values are expressed as means  $\pm$  SEM of 6 observations. (a) Significant difference from normal control group at  $P < 0.005$ . (b) Significant difference from control diabetic group at  $P < 0.005$  m normal control group at  $P < 0.005$ .

IKON 940 spectrophotometer. HR-FABMS was measured in the JOEL JMX-AX 505, HAD mass spectrophotometer at an ionization voltage of 70 eV. ESI-MS were measured in Agilent 6320, Ion Trap MS. NMR analyses were run on JEOL GLM 300 and JEOL JNM ECA 500 MHz spectrometers relative to TMS in DMSO.

An HP 6890 GC (Hewlett-Packard, USA) equipped with a 60 m DB-1- (J&W Scientific, 0.25 mm ID, 0.25  $\mu$ m film thickness). Samples were injected in the splitless mode, an inlet and transfer line temperature of 250  $^{\circ}$ C, and a constant He flow of 1.0 ml min $^{-1}$ . Separation was achieved with a temperature program with an initial temperature of 80  $^{\circ}$ C and initial time of 2 min, which was then ramped to 315  $^{\circ}$ C at 5  $^{\circ}$ C min $^{-1}$  and held at 315  $^{\circ}$ C for 12 min.

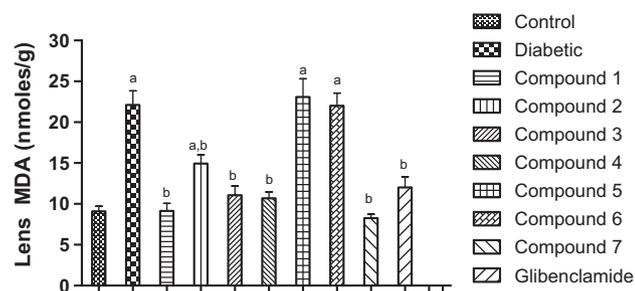
### 3.2. Material

#### 3.2.1. Plant material

Samples of the bark of *C. illinoensis* (Wangenh) K. Koch, were collected in April 2006 from the trees in the yellow mountain area, Giza, Egypt. The identity of the plant was authenticated by the agriculture engineer Therése Labib, Orman Garden, Cairo, Egypt. A voucher specimen (No. C-5) is kept in the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

#### 3.2.2. Reference materials

Reference sugar samples (D and L-glucose), L-cysteine methyl ester hydrochloride and TMS-HT kit (hexamethyldisilazane-trimethylchlorosilane (HMDS-TMCS) were commercially obtained from (Sigma-Aldrich Chemical Co., Steinheim, Germany).



**Fig. 7.** Effect of oral administration of the isolated compounds (1–7) on lens MDA levels in STZ-induced diabetic rats. All values are expressed as means  $\pm$  SEM of 6 observations. (a) Significant difference from normal control group at  $P < 0.005$ . (b) Significant difference from control diabetic group at  $P < 0.005$  m normal control group at  $P < 0.005$ .

### 3.3. Animals

Adult male albino rats (180–200 g) Wistar strain rats were purchased from laboratory animals' house of National Cancer Institute, Cairo-Egypt. They were housed in the central animal house of Faculty of Pharmacy, Cairo University, Cairo, Egypt. Rats were fed on a standard laboratory diet and water *ad libitum* and maintained at 22  $^{\circ}$ C temperature, 85% relative humidity, and a 12 h day and night cycle. Animal experiments were carried out following the nationally accepted ethical guidelines for the care of laboratory animals.

### 3.4. Extraction, isolation and identification

The air-dried powdered bark of *C. illinoensis* (2 kg) was extracted with 70% EtOH (6 l  $\times$  1 l) at 70  $^{\circ}$ C under reflux. The ethanolic extract was evaporated under reduced pressure to give 180 g of a dark brown residue. The residue was suspended in water (200 ml) and the soluble fraction was partitioned successively with hexane (4 ml  $\times$  500 ml), chloroform (4 ml  $\times$  500 ml), ethyl acetate (7 ml  $\times$  500 ml), and *n*-butanol (7 ml  $\times$  500 ml). EAF (23 g) was chromatographed over Silica gel 60H (Sigma) VLC ( $\emptyset$  10 cm  $\times$  20 cm, 150 g) with CH<sub>2</sub>Cl<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>-EtOAc mixtures, EtOAc and EtOAc-MeOH mixtures with gradient increase of polarity up to 100% methanol. Similar fractions were pooled to give seven main fractions. Fraction I (3 g) was subjected to repeated chromatographic fractionations; CC on Sephadex LH-20 with MeOH as an eluent, CC on Silica gel 100C<sub>18</sub>- reversed phase (70–230 mesh, Fluka using MeOH: water, 3:7 as an eluent) to afford compounds 1 (115 mg) and compound 2 (58 mg). Fraction II (2 g) was rechromatographed on a Silica gel 100C<sub>18</sub>- reversed phase (70–230 mesh, Fluka) column using MeOH:H<sub>2</sub>O, 2:8 as an eluent to afford compound 3 (40 mg). Fraction III (1 g) was subjected to purification twice on CC Sephadex LH-20 with *n*-BuOH-HOAc-H<sub>2</sub>O (4:1:5, top layer) and Sephadex LH-20 column using 80% MeOH respectively to yield compound 4 (17 mg). Fraction IV (1.2 g) was repeatedly fractionated on CC Sephadex LH-20 with 70% MeOH, to afford compound 5 (20 mg).

BF (6 g) was fractionated on a polyamide column (12 cm  $\times$  115 cm, 400 g), eluted with H<sub>2</sub>O followed by a gradient of H<sub>2</sub>O/MeOH mixtures up to 100% MeOH. Similar fractions were pooled together to yield five collective fractions (I–V). Fraction II (1.5 g) was chromatographed on a sephadex LH-20 column with MeOH, followed by silica gel 100C<sub>18</sub>- reversed phase (70–230 mesh, Fluka) for column chromatography using MeOH:H<sub>2</sub>O, 2:8 as an eluent to afford compounds 6 (23 mg) and compound 7 (28 mg).

Caryatin-3'-sulfate (6): yellow amorphous powder; (c,MeOH); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 340<sub>(2.7)</sub>, 260<sub>(2.76)</sub>, 210 (sh)<sub>(2.9)</sub> nm. For <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>), <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) and HMBC (DMSO-*d*<sub>6</sub>): see (Table 1). HR-FABMS (negative): *m/z* 409.0233 (calcd. for [M]<sup>-</sup>: 409.0229), HR-FABMS (positive): *m/z* 433.0221 (calcd. for [M+H+Na]<sup>+</sup>: 433.0205).

Caryatin-3'-methyl ether-7-O- $\beta$ -D-glucopyranoside (7): yellow amorphous powder; UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 350<sub>(2.74)</sub>, 330<sub>(2.88)</sub>, 260<sub>(2.98)</sub> nm. For <sup>1</sup>H (300 MHz, DMSO-*d*<sub>6</sub>), <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>) and HMBC (DMSO-*d*<sub>6</sub>): see (Table 1). ESI-MS (negative): *m/z* 506.14 (calcd. for [M]<sup>-</sup>: 506.46).

### 3.5. Assessment of diabetes

Diabetes was induced in overnight fasted by a single intraperitoneal injection of a freshly prepared solution of streptozocin (STZ) (Wohaieb and Godin, 1987). Blood samples were obtained from the tips of the rats' tails. Each rat with a blood glucose level above 250 mg/dl was considered diabetic. The

diabetic rats were allocated into 9 groups. Twelve rats in each group were taken, keeping mortality due to hyperglycemia into consideration. For analysis only six rats from each group were used. The 1st group served as a diabetic control. Groups from 2 to 8 were given the seven isolated compounds (10 mg/kg b.w. orally in 0.5% CMC). The 9th group was given glibenclamide (0.6 mg/kg daily, orally in 0.5% CMC) (Pari and Maheswari, 1999). A group of rats was given 0.5% CMC orally and served as normal control rats. Treatments were given daily and continued for six weeks. Blood glucose level was determined using a glucometer Accucheck sensor (Roche, USA) and then sacrificed by decapitation. Blood was collected into either plain, or EDTA containing tubes for the estimation of either serum insulin or glycated hemoglobin (HbA1c) respectively. The first set of blood samples was allowed to clot, centrifuged at  $1000 \times g$  at  $4^\circ C$  for 15 min and the separated sera were used for the estimation of insulin level using Rat Insulin ELISA kit, DRG international Inc. (USA), using ELISA reader, ASYS Expert plus, Hitech GmbH (Austria). Other blood samples, collected on EDTA, were used for determination of HbA1c% in blood by (HPLC) using the kit supplied from Bio Rad D-10 hemoglobin testing system (France). The two lenses of each rat were immediately dissected by the posterior approach, washed with normal saline, blotted dry and homogenized in 50 mM phosphate buffer pH 7.4 and homogenized using electrical homogenizer (Potter-Elvehjem glass homogenizer) to make 5% homogenate. The homogenate was then divided into three aliquots; Two portions were added to appropriate volume of 2.23% KCl or 7.5% sulfosalicylic acid, centrifuged at  $1000 \times g$  and the resulting supernatants were used for the assessment of lens (MDA) (Uchiyama and Mihara, 1978) and (GSH) (Beutler et al., 1963) levels, respectively. The last portion of the homogenate was mixed with ice cold medium (20 mM dithiothreitol and 1 mM EDTA) and ultracentrifuged at  $105,000 \times g$  at  $4^\circ C$  for 30 min using Dupont Sorvall Combi-plus ultracentrifuge and the resulted supernatants were used for the estimation of lens AR activity (Chauncey et al., 1988). Protein content was measured according to the method of (Lowry et al., 1951).

### 3.6. Statistical analysis

Results were expressed as mean  $\pm$  standard error of the mean (SEM) for six rats in each group. Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey–Kramer's Post hoc at  $P < 0.005$ .

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