Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: https://www.tandfonline.com/loi/iphb20

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**To cite this article:** Seham S. El Hawary, Soumaya Saad, Ali Mahmoud El Halawany, Zeinab Y. Ali & Mahitab El Bishbishy (2016) Phenolic content and anti-hyperglycemic activity of pecan cultivars from Egypt, Pharmaceutical Biology, 54:5, 788-798, DOI: 10.3109/13880209.2015.1080732

**To link to this article:** https://doi.org/10.3109/13880209.2015.1080732

PHARMACEUTICAL BIOLOGY, 2016
VOL. 54, NO. 5, 788–798
http://dx.doi.org/10.3109/13880209.2015.1080732

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RESEARCH ARTICLE

# Phenolic content and anti-hyperglycemic activity of pecan cultivars from Egypt

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**ABSTRACT**

*Context*: Pecans are commonly used nuts with important health benefits such as anti-hyperglycemic and anti-hyperlipidemic effects.

*Objective*: A comparative investigation of the antihyperglycemic and total phenolic content of the leaves and shells of four pecan cultivars growing in Egypt was carried out. The selected cultivars (cv.) were *Carya illinoinensis* Wangneh. K. Koch. cv. Wichita, cv. WesternSchely, cv. Cherokee, and cv. Sioux family Juglandaceae.

*Materials and methods*: Total phenolic and flavonoid contents of the leaves and shells of pecan cultivars were carried out using Folin–Ciocalteu's and aluminum chloride assays, respectively. Moreover, HPLC profiling of phenolic and flavonoid contents was carried out using RP-HPLC-UV. In addition, *in vivo* anti-hyperglycemic activity of the ethanolic extracts (125 mg/kg bw, p.o.) of *C. illinoinensis* cultivars was carried out using streptozotocin (STZ)-induced diabetes in Sprague–Dawley rats for 4 weeks.

*Results and discussion*: Phenolic contents were higher in shells than leaves in all studied cultivars, while flavonoids were higher in leaves. Leaves and shells of cv. Sioux showed the highest phenolics (251.7 μg gallic acid equivalent (GAE)/g), and flavonoid contents (103.27 μg rutin equivalent (RE)/g and 210.67 μg quercetin equivalent (QE)/g), respectively. The HPLC profiling of *C. illinoinensis* cultivars resulted in the identification of eight flavonoids (five of these compounds are identified for the first time from pecan), and 15 phenolic acids (six are identified for the first time from pecan). Leaves of cv. Sioux revealed the most potent decrease in blood glucose and glycated hemoglobin ($HbA_{1c}$%) (194.9 mg/dl and 6.52%, respectively), among other tested cultivars. Moreover, leaves of cv. Sioux significantly elevated serum total antioxidant capacity (TAC) and reduced glutathione (GSH) (0.33 mMol/l and 30.68 mg/dl, respectively), and significantly suppressed the markers of both lipid peroxidation (malondialdehyde, MDA) and protein oxidation (protein carbonyl, PC) (14.25 μmol/ml and 3.18 nmol/mg protein, respectively).

*Conclusion*: Different pecan cultivars showed significant variation in its phenolic and flavonoid contents and consequently their antioxidant and anti-hyperglycemic effects.

**ARTICLE HISTORY**
Received 8 November 2014
Revised 27 May 2015
Accepted 2 August 2015
Published online 7 October 2015

**KEYWORDS**
Antidiabetes, *Carya illinoinensis*, glycated hemoglobin, HPLC, phenolics, protein carbonyl

## Introduction

Recently, nuts have gained recognition as an important constituent of a healthy diet in human populations throughout the world; a regular consumption of a sufficient portion size is necessary for both prophylactic and clinical applications of nuts (Eagappan & Sasikumar, 2014; Nielsen et al., 2014). Nut consumption has been associated with improved nutrient intake, diet quality (Salas-Salvadó et al., 2014) and improved health outcomes including decreased incidence of ischemic heart and diabetes (Afshin et al., 2014; USDA, 2010). Diabetes mellitus is recognized by the World Health Organization (WHO) as a tremendously increasing global epidemic with more than 285 million people around the world afflicted in 2010, and it is estimated that the number of people with diabetes will increase to 439 million by 2030 (Shaw et al., 2010). Much of the increased mortality and morbidity seen in diabetic patients is a result of various complications, which develop with increasing duration of disease, particularly when glycemic control is poor.

Natural products have long been used as precious sources for the formulation of useful drugs. Pecan is among the most preferred, of all nuts, and an economically important crop in the United States and Mexico. Pecan ranks third in U.S. production of nuts but is tied with the walnut as the second most frequently consumed nut tree in the United States after almonds (de la Rosa et al., 2010). Pecan (*Carya illinoinensis* (Wangenh) K. Koch) (Juglandaceae) is a nut native to North

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America but can now be found across most of the world, including Europe, Africa, and Asia (Hal, 2000). It is excellent source of antioxidants including vitamin E in addition to other biologically active compounds such as sterols, flavonoids, and other phenolics (Ryan et al., 2006). These bioactive compounds synergistically play an important role in health promotion and the prevention of chronic diseases (Eagappan & Sasikumar, 2014). Most reports associate these benefits with healthful lipid profiles (i.e., MUFAs, PUFAs, α-tocopherol, and γ-tocopherol), but more recently attention has turned toward the favorable phenolic profiles that tree nuts possess. Characterization of these important classes is lacking in the literature (Robbins et al., 2014).

Although phenolic composition of pecans is complex and largely unknown (Hudthagosol et al., 2011), it was reported that pecans have been identified as a source of flavonoids, particularly the flavan-3-ol monomers (+)-catechin and (−)-epicatechin and their polymers, the proanthocyanidins (Gu et al., 2004). *In vitro* studies have classified flavan-3-ols as powerful antioxidants capable of scavenging both reactive oxygen and nitrogen species (Rice-Evans et al., 1996). The hepatoprotective effect of leaves (Gad et al., 2007), antioxidant, anti-inflammatory, and anti-hyperlipidemic effects of bark, stems, leaves, and fruits (Abdelrahman et al., 2008) were reported. Pecans increase plasma postprandial antioxidant capacity and decrease LDL oxidation in humans (Hudthagosol et al., 2011), in addition to the *in vitro* antihyperglycemic activity of its barks and leaves (Abdallah et al., 2011). Recently, it was reported that nut consumption decrease the risk of cardiovascular disease and type 2 diabetes (Zhou et al., 2014).

In continuation with our previous study on pecan cultivars from Egypt (El Hawary et al., 2013), the present study was designed to carry out a comparative investigation of the total content and HPLC profiles of phenolics and flavonoids of the leaves and shells of four pecan cultivars growing in Egypt. In addition, *in vitro* antioxidant activity (DPPH assay) and *in vivo* antioxidant and anti-hyperglycemic activities were evaluated in STZ-induced diabetic rats. The selected cultivars (cv.) were *Carya illinoinensis* cv. Wichita, cv. WesternSchely, cv. Cherokee, and cv. Sioux. To the best of our knowledge, this is the first report concerning the determination of phenolic and flavonoid contents, and anti-hyperglycemic activity of these four pecan cultivars from Egypt. Therefore, this study attempts to establish the scientific basis for the use of pecan leaves and shells in management of diabetes and diabetic complications.

## Material and methods

### Plant material

Leaves and shells of *C. illinoinensis* (Wangenh) K. Koch cultivars Wichita, WesternSchely, Cherokee, and Sioux were collected from trees cultivated at El-Kanater El Khayreya, Giza, Egypt during April–May 2011–2012. The taxonomical identity was kindly verified by Prof. Dr. Shawky Mahmoud El Sharkawy, Head of Research Department, El Basateen Research Institute, Agricultural Research Center, Giza, Egypt. The plant material was authenticated and the genetic identity of the different cultivars was confirmed by DNA fingerprinting.

### Chemicals

Gallic acid, ascorbic acid, quercetin, and rutin were purchased from E-Merck, Darmstadt, Germany. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Folin–Ciocalteu and Streptozotocin were purchased from Sigma Co (St. Louis, MO). HPLC standards were kindly supplied by Agricultural Research center, Food Technology Research Institute, Giza, Egypt. Glucose GOD-PAP enzymatic colorimetric kit was purchased from Spinreact S.A., Sant Esteve de Bas, Spain. Hemoglobin A1C chromatographic–spectrophotometric ion exchange Kit was from Biosystems Reagents and Instruments Inc. Barcelona, Spain. Rat insulin ELISA kit was from ALPCO Diagnostics (Salem, NH). Total antioxidant capacity colorimetric method, glutathione reduced, and lipid peroxide (malondialdhyde) colorimetric method kits were from Biodiagnostic Inc. (River Falls, WI). Oxiselect® and protein carbonyl spectrophotometric assay kit was from Cell Biolabs, Inc., San Deigo, CA. Gliclazide, Diamicron® tablets were purchased from Servier Egypt Industries Ltd., Giza, Egypt.

### Spectroscopic assays

The powdered air-dried leaves and shells (1 g) of different studied cultivars of *C. illinoinensis* was defatted with petroleum ether followed by extraction with 95% ethanol (on cold) until exhaustion. The extracts were distilled to remove alcohol and were, separately, transferred to 100 ml measuring flasks and the volume was adjusted with distilled water for the determination of total phenolic content and ethanol for the determination of total flavonoid content. To determine the phenolic content, the method adopted was based on measuring the intensity of the color developed using Shimadzu UV (P/N 204-58000) UV–visible spectrophotometer. Phenolic compounds were complexed with

Folin–Ciocalteu's phenol reagent and the concentrations were calculated as gallic acid equivalent with reference to a pre-established standard calibration curve (Marinova et al., 2005), while the method adopted for the determination of flavonoids was based on measuring the intensity of the color developed using Shimadzu UV (P/N 204-58000) UV–visible spectrophotometer (Shimadzu Corporation, Kyoto, Japan), when flavonoids were mixed with aluminum chloride and the % was calculated as rutin (Karawya & Aboutabl, 1982) and as quercetin (Cook & Samman, 1996) with reference to pre-established standard calibration curves.

### HPLC profiles of phenolics and flavonoids

Five grams of each dried powdered leaves and shells of different studied cultivars of *C. illinoinensis* were mixed separately with methanol and centrifuged at 1000 rpm for 10 min and the supernatant was filtered through a 0.2 µm millipore membrane filter before injection.

Chromatographic separations were performed using HPLC Hewlett Packard (series 1050) (Hewlett Packard Inc., Palo Alto, CA) equipped with auto-sampler injector, solvent degasser, ultraviolet (UV) detector set at (280 nm for phenolics determination and 330 nm for flavonoids determination), and quaternary HP pump (series 1050). The column temperature was kept at 35 °C. Gradient separation was carried out using methanol and acetonitrile (2:1) as a mobile phase at a flow rate of 1 ml/min. Authentic phenolics and flavonoids were dissolved in the mobile phase and injected into HPLC. The retention time and the peak area were used to calculate the phenolic and flavonoids concentrations by the data analysis of Hewlett Packard software (Hewlett Packard Inc., Palo Alto, CA) (Goupy et al., 1999; Mattila et al., 2000).

### Free radical scavenging activity (DPPH assay)

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical 1,1-diphenyl-2-picrylhydrazine, (DPPH) adopting the method of Blois (1958). Briefly; different concentrations of tested hydro-ethanolic extracts or ascorbic acid (reference reagent) (0–100 µg/ml methanol) (2.5 ml, each) were added to methanolic solution of DPPH (0.3 mM, 1 ml) and incubated in dark at room temperature for 30 min. Methanol was used as a control in place of the sample. The absorbance values were measured against methanol at 517 nm using Unicam UV/vis spectrophotometer (Vision Engineering, Yokohama, Japan). Radical scavenged percent was calculated using the following formula

$$A\% = 100 - \left[\left[\left(A_{\text{sample}} - A_{\text{blank}}\right) \times 100\right)/A_{\text{control}}\right]$$

All tests were performed in triplicate and the graph was plotted with the average of the three determinations. $IC_{50}$ value, the concentration of the tested sample in µg/ml that scavenged 50% of free radicals, was calculated from the concentration response curve.

### Oral acute toxicity study

The median lethal dose ($LD_{50}$) of the leaves and shells of four pecan cultivars was determined according to the Organization for Economic Co-operation and Development (OECD)-423 guidelines, for the acute toxicity class method (OECD, 1994).

### In vivo evaluation of hypoglycemic activity

Dried powdered leaves and shells (100 g, each) were separately macerated in 70% ethanol until exhaustion; the hydro-ethanolic extracts were separately concentrated under reduced pressure for biochemical analysis.

### Induction of type 2 diabetes

Type 2 diabetes was induced in male Sprague–Dawley rats as previously described (Manautou et al., 2008). Animals fed a high-fat diet containing 58% kcal fat for a period of 2 weeks, then intraperitoneally injected with a single low dose of freshly prepared streptozotocin (STZ, 45 mg/kg i.p, in 0.01 M citrate buffer pH 4.3). The blood glucose level was determined on the 7th day and the animals with a fasting blood glucose level >250 mg/dl were served as diabetic rats and were used in this study (Srinivasan & Pari, 2012).

### Experimental design

Animals were divided into 11 groups each of eight rats as follows: group 1 served as normal control and received vehicle, group 2 was untreated STZ-induced diabetic rats for 4 weeks, groups 3–6: diabetic rats treated with the hydro-ethanolic extracts of the leaves of the four tested cultivars of *C. illinoinensis* (125 mg/kg bw/d, p.o.) for 4 weeks, groups 7–10: diabetic rats treated with the hydro-ethanolic extracts of the shells of the four tested cultivars of *C. illinoinensis* (125 mg/kg bw/d, p.o.) for 4 weeks and group 11: diabetic rats treated with gliclazide at a dose of 0.5 mg/kg bw/d, p.o. (equivalent to human therapeutic dose) for the same period (Paget & Barnes, 1964).

### Sample collection

On the last day, 28th day of the experiment, overnight fasted animals were killed by cervical dislocation after exposing them to mild ethyl ether anesthesia. Blood samples were collected and allowed to clot at ambient temperature for 30 min. Serum was separated by centrifugation at 3000 rpm for 10 min and stored at 4 °C until used for different biochemical estimations.

### Biochemical analysis

For the assessment of anti-hyperglycemic activity, the fasting blood glucose levels were measured by the glucose oxidase/peroxidase method (Trinder, 1969), glycated hemoglobin ($HbA_{1C}$%) levels were determined according to the method of Beutler et al. (1977) and insulin levels was assayed by rat ELISA kit according to Findlay and Dillard (2007).

### In vivo *antioxidant activity*

*In vivo* antioxidant activity was evaluated by the assessment of serum total antioxidant capacity (TAC) (Aebi, 1984) and reduced glutathione (GSH) levels (Beutler et al., 1963). In addition, malondialdehyde (MDA) (Buege & Aust, 1978) and protein carbonyl (PCO) levels (Reznick & Packer, 1994) were measured as a marker of the oxidative stress.

### Statistical analysis

The biochemical results were expressed as mean ± SE. The biochemical parameters were statistically analyzed using one-way analysis of variance (ANOVA) version 17, followed by Duncan's multiple range test (DMRT), to calculate the statistical significance between various groups and a value of $p < 0.05$ was considered as statistically significant.

## Results

### Total phenolic and flavonoid content

The percentage of total phenolics calculated as gallic acid equivalent (GAE) was found highest in the shells of cv. Sioux (251.7 µg GAE/g) followed by cv. Wichita (197.28 µg GAE/g, Table 1). These values varied significantly from the shells of the other two studied cultivars: cv. WesternSchely and cv. Cherokee (14.97 and 56.47 µg GAE/g). Also, the leaves of cv. Sioux showed a relatively high amount of phenolics (98.64 µg GAE/g), followed by cv. Cherokee (72.79 µg GAE/g), while the other two studied cultivars cv. WesternSchely and cv. Wichita showed relatively lower amounts of phenolics (32.25 and 43.47 µg GAE/g).

Table 1. Total phenolic and flavonoid contents of leaves and shells of different studied cultivars of *C. illinoinensis*.

| *C. illinoinensis* cultivars | Total phenolic content concentration, µg GAE/g[a] | Total flavonoids content concentration, µg/g[b] | |
|---|---|---|---|
| | | RE[c] | QE[d] |
| Leaves | | | |
| Wichita | 43.47 ± 0.24 | 84.64 ± 0.65 | 172.67 ± 1.33 |
| WesternSchely | 32.25 ± 0.31 | 63.73 ± 0.57 | 130 ± 1.15 |
| Cherokee | 72.79 ± 0.68 | 88.89 ± 0.86 | 181.33 ± 1.76 |
| Sioux | 98.64 ± 0.68 | 103.27 ± 0.33 | 210.67 ± 0.67 |
| Shells | | | |
| Wichita | 197.28 ± 1.8 | 28.89 ± 0.28 | 58.93 ± 0.58 |
| WesternSchely | 14.97 ± 0.07 | 5.95 ± 0.07 | 12.13 ± 0.13 |
| Cherokee | 56.46 ± 0.34 | 20.92 ± 0.17 | 42.67 ± 0.35 |
| Sioux | 251.7 ± 0.68 | 33.01 ± 0.33 | 67.33 ± 0.67 |

[a]Calculated as gallic acid equivalent (GAE), values are presented as the mean of triplicates ± SE.
[b]Values are presented as the mean of triplicates ± SE.
[c]Calculated as rutin equivalent (RE).
[d]Calculated as quercetin equivalent (QE).

Concerning the flavonoid content, the percentage of total flavonoids calculated as rutin equivalent (RE) and quercetin equivalent (QE) was found highest in the leaves than the shells of all studied cultivars (Table 1). The highest concentration of flavonoids was discovered in the leaves of cv. Sioux (103.27 µgRE/g and 210.67 µgQE/g), while the lowest concentration was found in the leaves of cv. WesternSchely (63.73 µgRE/g and 130 µgQE/g). The shells showed not only relatively low concentration of flavonoids but also the highest amount was present in the shells of cv. Sioux (33.01 µgRE/g and 67.33 µgQE/g), while the lowest concentration was found in the shells of cv. WesternSchely (5.95 µgRE/g and 12.13 µgQE/g) (Table 1).

### HPLC profiles of phenolics and flavonoids

Phenolic profiles of the leaves and shells of *C. illinoinensis* cultivars were determined by RP-HPLC (Figures S1–S8; Supporting information). Twenty-three phenolic compounds (eight flavonoids and 15 phenolic acids) were identified. The different identified compounds are presented in Tables 2 and 3.

The leaves of all studied cultivars showed higher percentages of flavonoids than all shells, *C. illinoinensis* cv. Sioux were the highest in flavonoid content (173.18 mg/100 g) followed by cv. Wichita leaves (158.26 mg/100 g), then the other two cultivars cv. WesternSchely and cv. Cherokee (141.9 mg/100 g and 107.3 mg/100 g), respectively.

Quercetrin was the major flavonoid in the leaves of cv. Sioux cv. and WesternSchely (108 mg/100 g and 88.11 mg/100 g), respectively. Rutin was the major

Table 2. Results of determination of flavonoids in the leaves and Shells of *C.illinoinensis* cultivars using HPLC analysis.

| | Concentrations of flavonoids (mg/100 g) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Flavonoid | Wichita leaves | Shells | WesternSchely leaves | Shells | Cherokee leaves | Shells | Sioux leaves | Shells |
| Hesperidin | 19.61 ± 0.2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rutin | 59.93 ± 1.3 | 6.04 ± 0.1 | 21.5 ± 0.1 | 3.84 ± 0.07 | 46.14 ± 3.1 | 0 | 40.05 ± 0.3 | 14.38 ± 0.1 |
| Quercitrin | 56.91 ± 1.1 | 0.8 ± 0.05 | 88.11 ± 2.3 | 0 | 26.33 ± 1.4 | 4.01 ± 0.03 | 108.0 ± 4.2 | 1.94 ± 0.01 |
| Quercetin | 1.9 ± 0.03 | 0 | 0 | 1.75 ± 0.03 | 11.73 ± 0.4 | 5.45 ± 0.09 | 8.94 ± 0.2 | 2.49 ± 0.03 |
| Naringenin | 6.52 ± 0.2 | 1.73 ± 0.02 | 8.42 ± 0.2 | 0 | 17.29 ± 0.6 | 5.99 ± 0.1 | 13.28 ± 0.1 | 0 |
| Hesperitin | 12.2 ± 0.3 | 0 | 21.04 ± 0.5 | 0 | 5.81 ± 0.2 | 0 | 2.5 ± 0.06 | 7.28 ± 0.2 |
| Apigenin | 1.19 ± 0.1 | 0 | 2.0 ± 0.06 | 0 | 0 | 0 | 0 | 0 |
| Kaempferol | 0 | 0.74 ± 0.01 | 0.8 ± 0.01 | 2.28 ± 0.1 | 0 | 5.49 ± 0.03 | 0.41 ± 0.02 | 4.46 ± 0.03 |
| Total identified flavonoids | 158.26 | 9.31 | 141.9 | 7.87 | 107.3 | 20.94 | 173.18 | 30.55 |
| Percentage of total identified flavonoids (%) | 0.16 | 0.01 | 0.14 | 0.01 | 0.11 | 0.02 | 0.17 | 0.03 |

Table 3. Phenolic contents in the leaves of *C. illinoinensis* Wangneh. K. Koch. cultivars using HPLC analysis.

| | Concentrations of phenolic compounds (mg/100 g) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Phenolic compound | Wichita leaves | Shells | WesternSchely Leaves | Shells | Cherokee Leaves | Shells | Sioux leaves | Shells |
| Pyrogallic acid | 52.58 ± 5.3 | 676.3 ± 9.8 | 45.0 ± 0.4 | 0 | 160.14 ± 8.9 | 0 | 88.06 ± 1.2 | 4287.6 ± 2.2 |
| Gallic acid | 8.35 ± 0.08 | 0 | 9.85 ± 0.7 | 17.85 ± 1.4 | 34.0 ± 2.8 | 41.79 ± 2.4 | 17.13 ± 2.1 | 216.5 ± 5.3 |
| *p*-Hydroxy benzoic acid | 2.85 ± 0.2 | 0 | 2.45 ± 0.2 | 0 | 3.91 ± 0.2 | 0 | 3.97 ± 0.02 | 0 |
| Protocatechuic acid | 27.12 ± 1.3 | 25.22 ± 3.1 | 35.64 ± 3.7 | 9.56 ± 1.6 | 71.34 ± 4.3 | 62.2 ± 3.1 | 83.33 ± 4.5 | 199.48 ± 2.2 |
| Catechol | 28.13 ± 2.6 | 53.83 ± 4.3 | 14.18 ± 0.6 | 78.27 ± 6.1 | 25.75 ± 1.9 | 170.33 ± 6.2 | 45.39 ± 2.5 | 221.0 ± 3.6 |
| Caffeic acid | 0 | 0 | 6.96 ± 0.1 | 0 | 4.07 ± 0.9 | 0 | 13.24 ± 1.2 | 0 |
| Vanillic acid | 76.43 ± 6.7 | 5.12 ± 0.6 | 68.95 ± 4.8 | 1.47 ± 0.4 | 29.79 ± 0.3 | 21.14 ± 2.1 | 135.82 ± 9.3 | 27.08 ± 1.2 |
| Syringic acid | 4.57 ± 0.3 | 0 | 0 | 0 | 4.82 ± 0.2 | 0 | 0 | 0 |
| Chlorogenic acid | 0 | 35.19 ± 3.2 | 0 | 8.82 ± 0.9 | 0 | 72.78 ± 5.8 | 0 | 201.36 ± 7.5 |
| Ferulic acid | 1.66 ± 0.2 | 0 | 8.4 ± 1.0 | 1.34 ± 0.8 | 2.99 ± 1.5 | 12.64 ± 1.8 | 13.59 ± 2.6 | 0 |
| p-Coumaric acid | 0 | 0 | 0.65 ± 0.08 | 0 | 0 | 0 | 0 | 0 |
| Ellagic acid | 59.54 ± 4.3 | 0 | 86.57 ± 6.8 | 0 | 125.72 ± 1.2 | 0 | 180.76 ± 2.2 | 0 |
| Salicylic acid | 2.21 ± 0.3 | 0 | 7.63 ± 0.4 | 1.43 ± 0.4 | 7.32 ± 0.2 | 0 | 18.93 ± 0.1 | 0 |
| Cinnamic acid | 1.16 ± 0.1 | 0 | 2.44 ± 0.05 | 0 | 1.96 ± 0.08 | 0 | 3.88 ± 0.2 | 0 |
| Benzoic acid | 0 | 17.87 ± 3.7 | 0 | 12.18 ± 1.2 | 0 | 0 | 0 | 0 |
| Total identified phenolic compounds | 264.6 | 813.53 | 288.72 | 130.92 | 471.81 | 380.88 | 604.1 | 5153.1 |
| Percentage of total identified phenolic compounds (%) | 0.26 | 0.81 | 0.29 | 0.13 | 0.47 | 0.38 | 0.6 | 5.15 |

flavonoid in the leaves of cv. Wichita and cv. Cherokee (59.93 mg/100 g and 46.14 mg/100 g), respectively, and the shells of cv. Sioux, cv. Wichita, cv. WesternSchely (14.38 mg/100 g, 6.04 mg/100 g and 3.84 mg/100 g), respectively. Naringenin was the major identified flavonoid in cv. Cherokee shells (5.99 mg/100 g).

Protocatechuic acid, catechol, and vanillic acid represented an important share of the detected phenolic compounds of the leaves and shells of all studied cultivars. *p*-Hydroxy benzoic acid, caffeic acid, syringic acid, ellagic acid, and cinnamic acid were identified in the leaves, but absent in the shells of all cultivars, while chlorogenic acid and benzoic acid were identified in the shells but absent in the leaves of all cultivars.

Among the detected phenolics, pyrogallic acid was the main phenolic compound in the leaves of cv. Cherokee (160.14 mg/100 g), the shells of cv. Wichita and cv. Sioux (676.3 mg/100 g and 4287.68 mg/100 g), respectively, catechol was the main phenolic compound in the shells of cv. WesternSchely and cv. Sioux (78.27 mg/100 g and 170.33 mg/100 g), respectively. Vanillic acid was the major phenolic compound in the leaves of cv.Wichita (76.43 mg/100 g), while ellagic acid was the main phenolic compound in the leaves of cv. WesternSchely and cv. Sioux (86.57 mg/100 g and 180.76 mg/100 g).

Among the identified flavonoids, hesperidin, naringenin, kaempferol, hespertin, and apigenin were reported for the first time from pecan. In addition, pyrogallic acid, cetechol and ferulic, benzoic, salicylic, and cinnamic acids are first time to be detected in pecan.

## Free radical scavenging activity (DPPH assay)

The *in vitro* radical scavenging activity of the leaves extracts of the studied cultivars surpassed those of the shells compared with ascorbic acid ($IC_{50}$ value = 7.99 ± 0.46) (Table 4). The most potent antioxidant extract was found to be the leaves of cv. Sioux ($IC_{50}$ value = 21.8 ± 1.72) followed by cv. Cherokee, cv. WesternSchely and cv. Wichita ($IC_{50}$ value = 23.7 ± 1.86, 26.2 ± 1.76, and 35.4 ± 2.34), respectively, while the least antioxidant activity was that of the shells of cv. Wichita

Table 4. The anti-oxidant potential of various concentrations of the ethanolic extracts of leaves and Shells of *C. illinoinensis* cultivars by DPPH radical scavenging assay.

| | DPPH radical-scavenging activity %[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *C. illinoinensis* cultivars | | | | | | | | |
| Concentration (µg/ml) | Wichita leaves | Shells | WesternSchely leaves | Shells | Cherokee leaves | Shells | Sioux leaves | Shells | Ascorbic acid |
| 6.25 | 12.3 ± 0.95 | 10.3 ± 0.86 | 15.1 ± 0.85 | 10.6 ± 0.58 | 15.8 ± 0.94 | 13.9 ± 0.75 | 20.9 ± 1.34 | 13.7 ± 1.05 | 39.1 ± 1.25 |
| 12.5 | 21.6 ± 1.35 | 17.1 ± 1.28 | 28.9 ± 1.78 | 20.5 ± 1.42 | 31.2 ± 2.61 | 22.6 ± 1.53 | 32.1 ± 1.98 | 25.7 ± 1.28 | 75.4 ± 2.81 |
| 25 | 35.3 ± 1.69 | 30.7 ± 2.75 | 47.6 ± 3.69 | 35.0 ± 2.46 | 52.7 ± 2.48 | 38.6 ± 2.61 | 57.3 ± 3.67 | 46.4 ± 3.13 | 93.1 ± 4.26 |
| 50 | 68.1 ± 3.95 | 44.2 ± 1.98 | 77.5 ± 5.28 | 49.8 ± 3.61 | 79.8 ± 4.59 | 56.5 ± 4.59 | 82.4 ± 4.58 | 62.5 ± 4.28 | 96.8 ± 4.34 |
| 100 | 78.9 ± 5.36 | 70.5 ± 5.77 | 89.4 ± 5.94 | 74.4 ± 5.13 | 89.1 ± 5.81 | 78.2 ± 4.32 | 92.2 ± 6.34 | 81.8 ± 5.36 | 98.3 ± 4.19 |
| $IC_{50}$ | 35.4 ± 2.34 | 56.6 ± 3.46 | 26.2 ± 1.76 | 50.2 ± 4.55 | 23.7 ± 1.86 | 32.4 ± 2.47 | 21.8 ± 1.72 | 26.9 ± 1.48 | 7.99 ± 0.46 |

[a]Values are presented as mean ± SE of triplicate observations.

($IC_{50}$ value = 56.6 ± 3.46), followed by cv. WesternSchely, cv. Cherokee and cv. Sioux ($IC_{50}$ value = 50.2 ± 4.55, 32.4 ± 2.47, and 26.9 ± 1.48), respectively.

## Oral acute toxicity study

The tested extracts up to a dosage of 5 g/kg (i.e., $LD_{50}$ value > 5 g) showed no mortality and no adverse effects on the behavior or appearance of the rats. All the rats survived during the experimental period and their body weights and food consumption were normal when compared with control rats. Given these results, it can be concluded that all tested extracts are non-toxic and have a wide safety margin according to (OECD, 1994). Therefore, one-twentieth of the $LD_{50}$ value (125 mg/kg bw/d, p.o.) of the extracts were selected for biochemical studies.

## Anti-hyperglycemic activity

As shown in Table 5, non-treated diabetic rats showed high serum glucose levels (275.2 ± 6.62 mg/dl) while diabetic rats treated with gliclazide showed relatively low serum glucose levels (94.6 ± 1.9 mg/dl). Administration of *C. illinoinensis* extracts showed significant decrease in serum glucose levels. The leave extracts decreased the serum glucose levels more than that of the shells of all studied cultivars. The hydro-alcoholic extract of the leaves of cv. Sioux showed the most potent activity (194.9 ± 3.91 mg/dl) followed by cv. Cherokee, cv. WesternSchely, then, cv. Wichita (199.9 ± 4.02, 203.2 ± 4.08, and 208.8 ± 4.19 mg/dl), respectively.

In the diabetic group, the significant increase in $HbA_{1c}$% level (9.31 ± 0.25%) was reversed by the action of gliclazide (5.59 ± 0.15%), while in the groups treated with the hydro-ethanolic extracts of the leaves and shells of all cultivars, a marked decrease in $HbA_{1c}$% levels was observed when compared with that of diabetic animals. The highest activity was observed by the leaves extracts of cvs. Cherokee and Sioux (6.61 ± 0.18 and 6.52 ± 0.17%), respectively, followed by the leaves extracts of the other two cultivars, cv. Wichita and cv. WesternSchely (6.99 ± 0.19, 6.82 ± 0.18%), respectively. Concerning serum insulin levels, compared with diabetic rats (6.04 ± 0.46 µU/ml), gliclazide showed a significant increase in insulin levels (11.2 ± 0.85 µU/ml), while all the studied extracts showed insignificant increase in insulin levels.

## Antioxidant activity

Administration of STZ significantly increased serum oxidative stress markers, malondialdehyde (MDA) to (20.08 ± 0.61 µmol/ml) and protein carbonyl (PCO) levels to (4.46 ± 0.23 nmol/mg protein), associated with significant depletion of total antioxidant capacity (TAC) to (0.25 ± 0.02 mMol/l) and reduced glutathione (GSH) (23.39 ± 0.98 mg/dl) as compared with the control group, indicating the generation of oxidative stress as shown in Table 6. Administration of gliclazide reversed the changes in the previously mentioned parameters significantly, showing a decrease in MDA and PCO levels to 12.26 ± 0.37 µmol/ml and 2.85 ± 0.15 nmol/mg protein, respectively, and an increase in TAC and GSH levels to 0.36 ± 0.02 mMol/l and 33.45 ± 1.41 mg/dl, respectively.

All the studied extracts of pecan showed a significant increase in the serum TAC and GSH levels. The effects of the leaves extracts of all cultivars exceeded those of the shells, whereas the most effective extract was the leaves of *C. illinoinensis* cv. Sioux, showing TAC (0.33 ± 0.02 mMol/l) and GSH (30.68 ± 1.29 mg/dl). Also, all the studied extracts demonstrated significant decrease in serum oxidative stress markers, and yet again, the effects of the leaves extracts of all cultivars surpassed those of the shells, the most effective extract was the leaves of *C. illinoinensis* cv. Sioux, showing a decrease in MDA level (14.25 ± 0.43 µmol/ml) and PCO level (3.18 ± 0.17 nmol/mg protein).

## Discussion

### Total phenolic and flavonoid contents

Phenolic acids and flavonoid compounds are the major active nutraceutical ingredients in plants. Both classes exhibited good antioxidant potential. In this study, the total phenolic and flavonoid contents of the leaves and shells of four pecan cultivars were determined using the spectrophotometric method. Furthermore, analysis of the studied extracts using RP-HPLC, demonstrated the presence of different phenolics and flavonoids, which are known for their antidiabetic and antioxidant potential (El-Baz et al., 2014; Niture et al., 2014). The data showed that both leaves and shells of cv. Sioux showed higher phenolics and flavonoid contents than other tested extracts which explains their antioxidant potential. The previous studies showed that flavonoid-rich extracts have a remarkable dual antioxidant and hypoglycemic potential (Irondi et al., 2014; Sharma & Pooja, 2011). According to RP-HPLC analysis of phenolics and flavonoids profiles of all tested leaves and shells of *C. illinoinensis* cultivars, 23 phenolic compounds were identified (Tables 2 and 3), involving eight flavonoids (hesperidin, rutin, quercitrin, quercetin, naringenin, hesperitin, apigenin, and kaempferol) and 15 phenolic acids (pyrogallic, gallic, *p*-hydroxy benzoic, protocatechuic, catechol, caffeic, vanillic, syringic, chlorogenic, ferulic, *p*-coumaric, ellagic, salicylic, cinnamic, and benzoic acid).

Concerning the phenolic acids, it is worth-mentioning that the concentration of phenolic compounds in *C. illinoinensis* cv. Sioux shells exceeds by 5-fold the concentration of phenolic compounds in the leaves and shells of all studied cultivars. In addition, flavonoid contents in leaves of the cv. Sioux was higher than the other cultivars. Furthermore, all studied extracts of the leaves and shells of the four pecan cultivars showed dose-dependent scavenging of DPPH radicals. The obtained results revealed that the ability of different samples to act as DPPH radical scavenger was in strong positive correlation with their phenolics and flavonoid contents (Table 7). Moreover, quercitrin, a major flavonoid (Babujanarthanam et al., 2011), and ellagic acid, a major phenolic acid (Yan-zhi & Xue-jun, 2013) in leaves of cv. Sioux are known with their antihyperglycaemic and antioxidant activity in diabetic rats.

Experimental animal models are one of the best strategies for understanding of the pathophysiology of diseases in order to design and develop new drugs. In the present study, the acute oral toxicity test showed a large margin of safety of all tested pecan extracts in rats. Type 2 diabetes was induced in Sprague–Dawley rats by interprotenial injection of STZ because STZ is a cytotoxic glucose analogue; selectively destroy insulin secreting cells (pancreatic β-cell). The glucose moiety in its

Table 5. Effect of different treatments on serum glucose, glycated hemoglobin ($HbA_{1c}$) and insulin levels.

| Groups | Serum glucose levels (mg/dl) | $HbA_{1c}$% | Insulin (μU/ml) |
|---|---|---|---|
| Control | $83.84 \pm 1.82^{a}$ | $5.56 \pm 0.26^{a}$ | $14.1 \pm 0.58^{c}$ |
| Diabetic rats treated with Gliclazide | $94.64 \pm 1.90^{a}$ | $5.59 \pm 0.15^{a}$ | $11.2 \pm 0.85^{b}$ |
| Diabetic rats treated with alcoholic extracts of the leaves of | | | |
| Wichita | $208.76 \pm 4.19^{c,d}$ | $6.99 \pm 0.19^{b,c,d}$ | $7.55 \pm 0.57^{a}$ |
| WesternSchely | $203.2 \pm 4.08^{b,c}$ | $6.82 \pm 0.18^{b,c}$ | $7.67 \pm 0.58^{a}$ |
| Cherokee | $199.85 \pm 4.02^{b,c}$ | $6.61 \pm 0.18^{b}$ | $7.73 \pm 0.59^{a}$ |
| Sioux | $194.85 \pm 3.91^{b}$ | $6.52 \pm 0.17^{b}$ | $7.85 \pm 0.6^{a}$ |
| Diabetic rats treated with alcoholic extracts of the shells of | | | |
| Wichita | $236.6 \pm 4.75^{g}$ | $7.92 \pm 0.22^{f}$ | $6.95 \pm 0.53^{a}$ |
| WesternSchely | $231 \pm 4.64^{f,g}$ | $7.65 \pm 0.21^{e,f}$ | $7.13 \pm 0.54^{a}$ |
| Cherokee | $222.66 \pm 4.46^{e,f}$ | $7.45 \pm 0.2^{d,e,f}$ | $7.25 \pm 0.55^{a}$ |
| Sioux | $217.11 \pm 4.36^{d,e}$ | $7.27 \pm 0.2^{c,d,e}$ | $7.37 \pm 0.56^{a}$ |
| Diabetic non-treated rats | $275.21 \pm 6.62^{h}$ | $9.31 \pm 0.25^{g}$ | $6.04 \pm 0.46^{a}$ |

Each value represents the mean of eight rats ± S.E. In the same column, the presence of different letters indicating a significant difference between groups by using one way ANOVA followed by Duncan's multiple comparison test (DMRT) at $p < 0.05$.

Table 6. Serum Total Antioxidant Capacity (TAC), reduced glutathione (GSH), malondialdehyde (MDA) and protein carbonyl (PCO) levels of the hydro-ethanolic extracts of the leaves and shells of *C. illinoinensis* cvs ($n = 8$).

| Groups | Antioxidant defense TAC (mMol/L) | Oxidative stress markers GSH (mg/dL) | MDA (μmol/ml) | PCO (nmol/mg protein) |
|---|---|---|---|---|
| Control | $0.46 \pm 0.03^{d}$ | $34.64 \pm 1.21^{d}$ | $11.14 \pm 0.38^{a}$ | $2.86 \pm 0.12^{a}$ |
| Diabetic rats treated with Gliclazide | $0.36 \pm 0.02^{c}$ | $33.45 \pm 1.41^{c,d}$ | $12.26 \pm 0.37^{a}$ | $2.85 \pm 0.15^{a}$ |
| Diabetic rats treated with alcoholic extracts of the leaves of | | | | |
| Wichita | $0.32 \pm 0.02^{b,c}$ | $29.25 \pm 1.23^{b}$ | $15.06 \pm 0.45^{b,c}$ | $3.35 \pm 0.17^{a,b,c}$ |
| WesternSchely | $0.32 \pm 0.02^{b,c}$ | $29.6 \pm 1.22^{b}$ | $14.66 \pm 0.45^{b}$ | $3.27 \pm 0.17^{a,b,c}$ |
| Cherokee | $0.32 \pm 0.02^{b,c}$ | $30.19 \pm 1.27^{b,c}$ | $14.28 \pm 0.44^{b}$ | $3.17 \pm 0.17^{a,b}$ |
| Sioux | $0.33 \pm 0.02^{b,c}$ | $30.68 \pm 1.29^{b,c}$ | $14.25 \pm 0.43^{b}$ | $3.18 \pm 0.17^{a,b}$ |
| Diabetic rats treated with alcoholic extracts of the shells of | | | | |
| Wichita | $0.29 \pm 0.017^{a,b}$ | $27.15 \pm 1.14^{b}$ | $17.08 \pm 0.51^{d}$ | $3.82 \pm 0.2^{c}$ |
| WesternSchely | $0.3 \pm 0.018^{a,b,c}$ | $27.63 \pm 1.16^{b}$ | $16.55 \pm 0.5^{d}$ | $3.67 \pm 0.19^{b,c}$ |
| Cherokee | $0.3 \pm 0.018^{a,b,c}$ | $28.14 \pm 1.18^{b}$ | $16.1 \pm 0.49^{c,d}$ | $3.55 \pm 0.19^{b,c}$ |
| Sioux | $0.31 \pm 0.018^{a,b,c}$ | $28.45 \pm 1.2^{b}$ | $15.68 \pm 0.47^{b,c,d}$ | $3.5 \pm 0.18^{b,c}$ |
| Diabetic non-treated rats | $0.25 \pm 0.02^{a}$ | $23.39 \pm 0.98^{a}$ | $20.08 \pm 0.61^{e}$ | $4.46 \pm 0.23^{d}$ |

Each value represents the mean of eight rats ± S.E. In the same column, the presence of different letters indicating a significant difference between groups by using one way ANOVA followed by Duncan's multiple comparison test (DMRT) at $P < 0.05$.

Table 7. Pearson correlation matrix.

| Parameters | T. flavonoid | Glucose | HbAc% | Insulin | TAC | GSH | MDA | PC |
|---|---|---|---|---|---|---|---|---|
| T. flavonoid | 1 | | | | | | | |
| Glucose | −0.770** | 1 | | | | | | |
| HbAc% | −0.653** | 0.764** | 1 | | | | | |
| Insulin | 0.233 | −0.799** | −0.534** | 1 | | | | |
| TAC | 0.414* | −0.658** | −0.642** | 0.724** | 1 | | | |
| GSH | 0.447* | −0.641** | −0.420** | 0.372** | 0.474** | 1 | | |
| MDA | −0.787** | 0.751** | 0.892** | −0.482** | −0.520** | −0.569** | 1 | |
| PC | −0.415* | 0.589** | 0.495** | −0.684** | −0.409** | −0.379** | 0.504** | 1 |

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

chemical structure enables STZ to enter the beta cell via the low affinity glucose 2 transporter in the plasma membrane because the β-cells of the pancreas are more active than other cells in taking up glucose and so are more sensitive than other cells to STZ challenge (Elsner et al., 2000). In addition, STZ-diabetes model mimics many of the acute and chronic complications of human diabetes and given the established similarities of some of the structural, functional, and biochemical abnormalities to human disease; it is an appropriate model to assess the mechanism of diabetes (Eleazu & Okafor, 2013).

In the present study, STZ-induced diabetic group exhibited a significant decrease in serum insulin and marked increase in the blood glucose and glycosylated hemoglobin (Hb A1C) compared with the normal control group. These results are in agreement with the previous studies (Suchithra & Subramanian, 2014). However, daily oral administration of pecan extracts at a dose of 125 mg/kg bw and gliclazide for 4 weeks significantly decreased the blood glucose and HbA1c levels in the STZ-diabetic rats indicating their hypoglycemic activity. The effect of pecan extracts on blood glucose was slowly generated after 4 weeks; in contrast, gliclazide was more potent suggesting the involvement of an extra-pancreatic effect of pecan on glucose production or glucose clearance. The present study suggested that the hypoglycemic effect of the hydro-ethanolic extract of the leaves and shells of four pecan cultivars could be attributed to its bioactive ingredients such as phenolic and flavonoid compounds which affect glucose metabolism by different mechanisms.

It was reported that plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances, inhibit insulinase activity, stimulate β-cells to produce more insulin, and others may increase β-cells in the pancreas by activating regeneration of pancreatic cells (Sharma & Pooja, 2011). Cv. Sioux leaves showed the most potent anti-hyperglycemic activity among all the tested cultivars, which could be attributed to its highest flavonoid contents, while shells of the same cultivar that with high phenolic contents showed lower activity. One of the therapeutic approaches to treat diabetes is to decrease the postprandial hyperglycemia by retarding absorption of glucose. Inhibition of carbohydrate-hydrolyzing enzymes, such as α-amylase and α-glucosidase, is considered a possible pathway because these enzymes play a key role in digesting carbohydrates (American Diabetes Association, 2008; Kamtekar et al., 2014). Polyphenols have received wide attention because of their antioxidant properties which refers to their ability to prevent damage from reactive oxygen through free radical scavenging or prevent the generation of these species by iron chelation as well as bind and inhibit the enzymes α-amylase and α-glucosidase (Oboh et al., 2014). In consistence with our results, recent study demonstrated that a well-defined hydro-alcoholic Ceylon cinnamon bark extract (CCE) reduces the glycemic response to starch in normal rats and healthy male and female subjects by inhibiting pancreatic α-amylase starch digestion, and suggested that CCE may provide a natural and safe solution for the reduction of postprandial hyperglycemia (Beejmohun et al., 2014). The potent α-amylase inhibitory activity contributed to antidiabetic activity of Rosmary that depends on its total phenolics and flavonoids contents (Ali et al., 2014).

From the current results, flavonoid content might be more significant in the control of hyperglycemia than other phenolics in the used model. The main flavonoids detected in the leaves of pecan cultivars such as naringenin and rutin are known of their anti-hyperglycemic activities through inhibition of intestinal alpha glucosidase or intestinal and renal $Na^+$-glucose co-transporter (Brahmachari, 2011). The present findings support that the studied extracts reversed the diabetic effects caused by STZ tending to bring the parameters significantly towards the normal compared with the antihyperglycemic drug (gliclazide) in diabetic animals.

Glycosylated hemoglobin ($HbA_{1c}$%) is formed as a result of reaction of excess glucose with normal blood hemoglobin. World Health Organization (2011) recommended that HbA1c can be used as a diagnostic test for diabetes. The concentration of glycosylated hemoglobin strongly predicts for the diagnosis and prognosis of diabetes-related complications (Suchithra & Subramanian,

2014). Therefore, the decreased level of HbA1c% after treatment with pecans extracts is a reliable index of glycemic control.

Several reaction mechanisms are thought to be involved in the genesis of oxidative stress in both diabetic patients and diabetic animals include glucose auto-oxidation, protein glycation, formation of advanced glycation products, and the polyol pathway (Eleazu & Okafor, 2013). The excessive production of reactive oxygen species (ROS) and increased oxidative stress contributes to the development and progression of diabetes and its complications (Niture et al., 2014; Zheng et al., 2013). The increased ROS may react with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation, a deleterious effect on β-cells and inactivation of antioxidant enzymes that are specifically protective against oxidative stress damage. Consequently, intracellular antioxidants or ROS scavengers substantially can attenuate STZ toxicity. In the present study, the elevated level of serum MDA is a clear manifestation of excessive formation of free radicals and activation of LPO result in enhancement of the oxidative modification of proteins (PC) that may leading to the structural alteration and functional inactivation of many enzyme proteins (Jomova & Valko, 2011). Furthermore, the depletion of serum total antioxidant defenses (TAC) along with increased LPO followed by an imbalance in the redox status indicated enhanced oxidative stress. In addition, the decreased level of GSH in STZ-induced diabetic rats could be a result of decreased synthesis or increased utilization due to increased oxidative stress in the untreated diabetic group. These results were in agreement with that of Eleazu and Okafor (2013). However, the marked increase in TAC and GSH content in both pecan extract and gliclazide-treated animals could be attributed to either increased biosynthesis of GSH or reduced oxidative stress.

Supplementation of the tested extracts for 4 weeks significantly prevented these biochemical changes confirming their antidiabetic potential that attributed to antioxidant potential of their constituents. The antioxidant and hypoglycemic activities of pecans extracts were significantly correlated with their total phenolics and flavonoids content (Table 7). All leaves of studied cultivars exhibited higher antioxidant activities than all studied shells that may be attributed to their higher flavonoids content. The highest concentration of phenolics and flavonoids was observed in the leaves of cv. Sioux which possess the highest antioxidant activity

Furthermore, a calculated coefficient of correlations between different variables (Table 7) reveals a significant negative correlation between total flavonoid and blood glucose, HbA1c, MDA, and PC. In contrast, a significant positive correlation was observed between total flavonoid and TAC & GSH. Therefore, the present study concluded that diabetes is associated with oxidative stress and suggested that the leaves and shells of the four pecan cultivars show hypoglycemic activity through their antioxidant potential, consequently, preserving the β-cells from the progressive destructive action of STZ. The leaves of cv. Sioux possess the highest antioxidant and hypoglycemic potential.

## Conclusion

Results of our study have highlighted the fact that the different studied cultivars imparted significant antihyperglycemic and antioxidant activities. The cultivar variation has an influence on the content of phenolic and flavonoid compounds. Leaves and shells of the studied pecan cultivars may be a good candidate for employment as a source of flavonoids and phenolic antioxidants. Also, these findings encourage the recovery of phenolic compounds from pecans by-products and their exploitation as natural antioxidants. Finally, further studies are still necessary to delineate the precise mechanisms underlying the effects of pecans. Human clinical trials will be required to confirm these results on postprandial glycemia and in order to directly assess the other health benefits.

## Declaration of interest

The authors have declared that there is no conflict of interest.

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**Supplementary material available online**

Supporting Figure S1–S8