An assessment of the nutraceutical potential of *Juglans regia* L. leaf powder in diabetic rats

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ABSTRACT

In this study, we evaluated the nutraceutical potential of *Juglans regia* L. (a dietary supplement and food- additive) by evaluating the *in-vitro* anti-diabetic potential and by assessing the *in-vivo* anti- hyperglycaemic, anti-hyperlipidaemic, and organ-protective effects of freshly-dried and powdered leaves of *J. regia* L. in diabetic rats. In the *in-vivo* experiments, dry powder of *J. regia* L. leaf (25, 50 and 100 mg/kg) was administered orally, twice daily (9.00 a.m. and 5 p.m.) to streptozocin-induced diabetic rats over a period of 28 days, during which body weight and blood glucose were monitored weekly. At the end of the experimental period, animals were sacrificed, blood was taken for assessment of lipid profile, antioxidant activity and liver/kidney biochemistry; while samples of the pancreas, liver and kidneys were fixed, processed, sectioned, and stained for general histology. Phytochemical evaluations of three extracts were carried out using HPLC-PDA validated procedures, while enzyme-inhibitory potentials were tested against a-amylase and a-glucosidase. *In-vivo* assays showed that twice-daily administration of *J. regia* L. leaf resulted in weight gain, glycaemic control, reversal of dyslipidaemia and biochemical evidences of liver/kidney injury, and protection against pancreas, liver and kidney tissue injury

Keywords: HPLC-PDA; Polyphenols; Flavonoids; *Juglans regia* L.; Diabetes

1. INTRODUCTION

In the past two decades, the concept of nutraceuticals, as well as the interest to functional foods has expanded tremendously (Salas-Salvadó et al., 2011). A food is regarded as ‘‘functional’’ if it has the ability to benefit one or more target functions in the body resulting in an improved state of health or a reduction of disease risk (Ballali and Lanciai, 2012), beyond their nutritional effects (Diplock et al., 1999). In this context, functional foods must demonstrate their effects at an intake level within normal dietary limits; while nutraceuticals exhibit their effects at doses much higher than the recommended dietary allowance. In recent years, researchers have focused on the properties of the various bioactive compounds found in food and the possible effects in the prevention and control of metabolic diseases such as diabetes mellitus (Diplock et al., 1999; Bahadoran et al., 2013; Giacco et al., 2010; Hussain et al., 2015). Nutraceuticals with anti-diabetic potentials include plants with a high phenolic content (Bahadoran et al., 2013), and high antioxidant activity.

Several studies have evaluated the antidiabetic potentials of *Juglans regia* L. extracts in humans (Kamyab et al., 2010; Hosseini et al., 2014a), and rodents (Asgary et al., 2008; Gholamreza and Hossein, 2008); however, there is a dearth of scientific evidence of the nutraceutical potential of whole parts of the *Juglans regia* L. leaf in the management of diabetes mellitus (either in humans or rodents).

Juglans regia L. (*J. regia*), also known as Persian or English walnut tree is a species in the genus Juglans and the family Juglandaceae (Hosseini et al., 2014a). The *J. regia* L. tree is native to a region extending from the Balkan sea eastward to the Himalayas and southwest China (Hosseini et al., 2014b). It is a deciduous tree reaching heights of up to 25e35 m, and a width of about 2 m (Zargari, 2012). Different parts of the *J. regia* L. tree have found use in foods and food preparations; the seed can be eaten raw or used as a flavoring-agent in confectioneries (Taha and Al-wadaan, 2011), and in sweet and savory dishes. The oil is used in salads or for cooking (Taha and Al-wadaan, 2011), the sap as a food-sweetener; the finely-ground shells added in the preparation of various dishes and as salad dressing, while the leaves as herbal tea (Hosseini et al., 2014a). Studies have reported beneficial effects of *J. regia* L. nuts extracts in the management of neurodegenerative diseases (Poulose et al., 2014) and reduction of cardiovascular disease risks (Sabate et al., 1993).

Several studies using aqueous (Gholamreza and Hossein, 2008), ethanolic (Asgary et al., 2008), methanolic or hydroalcholic (Mohammadi et al., 2012a) extracts of *J. regia* L. leaf have reported the beneficial effects of the leaves in controlling glucose levels, lipid profile and oxidant/antioxidant derangements in rodents and more recently in humans (Kamyab et al., 2010; Hosseini et al., 2014a). In a number of these studies, large bolus doses (greater than 200 mg/ kg) administered once daily were used (Asgary et al., 2008; Gholamreza and Hossein, 2008; Mohammadi et al., 2011, 2012a). However, in the present study, the possibility of using smaller (25, 50 and 100 mg/kg) doses, administered twice in 24 h in place of the conventional once-daily dosing (Asgary et al., 2008; Gholamreza and Hossein, 2008; Mohammadi et al., 2011, 2012a), was considered. The intention was to ascertain the efficacy and toxicologic profile of smaller divided doses of a possible nutraceutical in the control of diabetes mellitus. The rationale for this study was to determine the *in vitro* anti-diabetic potential of three *J. regia* L. leaf extracts (decoction, soxhlet and microwave extracts), and then the antidiabetic, antihyperlipidemic and organ-protective effects of freshly dried and powdered leaves of *J. regia* L. in a rodent model of

diabetes mellitus. *In-vivo*, we tested the hypothesis that twice daily repeated administration of *J. regia* L. leaf powder might significantly reduce blood sugar, restore plasma lipid balance, antioxidant levels and mitigate biochemical and morphologic evidences of renal, pancreatic and hepatic damage in streptozocin-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 CHEMICALS AND DRUGS

Streptozotocin (STZ), a-glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20) and a-amylase from porcine pancreas (E.C. 3.2.1.1) were purchased from Sigma Chemical Co. (Sigma St. Louis, USA). Metformin was obtained from Bristol-Myers Squibb (UK). All other chemicals and solvents were of analytical grade. All chemicals used for the HPLC-PDA analyses: gallic acid, catechin, chlorogenic acid, *p*-hydroxy-benzoic acid, vanillic acid, epicatechin, syringic acid, 3-hydroxy-benzoic acid, 3-hydroxy-4-methoxybenzaldehyde, *p*-coumaric acid, rutin, sinapinic acid, *t*-ferulic acid, naringin, 2,3-dimethoxy-benzoic acid, benzoic acid, *o*-coumaric acid, quercetin dihydrate, *t*-cinnamic acid, naringenin (all purity 98%) were obtained from Sigma Aldrich (Milan, Italy). Methanol and acetonitrile (HPLC-grade) were purchased from Sigma-Aldrich (Milan, Italy), while HPLC-grade acetic acid was bought from Carlo Erba Reagents (Milan, Italy). Double distilled water (Milli-Q system, Millipore, Bedford, USA) was used. Assay kits for liver function tests (Alanine transaminase and aspartate transaminase), kidney function tests (urea and creatinine) from Randox Laboratories (Crumlin, Co. Antrim, UK) and antioxidant kits (superoxide dismutase, glutathione peroxidase and catalase) from Biovision Inc. (Milpitas, CA,USA) were obtained and refrigerated until used. All other chemicals were analytical grade.

2.2 PLANT MATERIAL

Fresh leaves of *J. regia* L. were collected in Abruzzo region around the Chieti-Pescara area (Italy), from adult trees and air- dried. The dried leaves (500 g) were extracted by three different techniques: microwave extraction, soxhlet extraction and decoction by 60% MeOH/H_2O. The extracts were subjected to lyophilization. The dry extract obtained was analyzed by HPLC and tested *in vitro* enzymatic assays. For the *in-vivo* tests, fresh leaves were obtained, air-dried, ground to powder and sieved to remove plant debris.

2.3 PRELIMINARY PHYTOCHEMICAL SCREENING OF PLANT FRACTIONS

2.3.1 HPLC ANALYSIS

Extracts of *J. regia* L. were analyzed for multicomponent pattern quantitative determination of polyphenols and flavonoids using a reverse phase HPLC-PDA in gradient elution mode. Analyses were carried out with a Waters liquid chromatograph equipped with a photodiode array detector, a C18 reversed-phase column (Prodigy ODS-3, 4.6 150 mm, 5 mm; Phemomenex, Torrance, CA), an *on- line* degasser (Biotech 4-CH DEGASI® compact, LabService, Anzola Emilia, Italy), a column oven set at 30 °C (±1 °C). The gradient elution was achieved by a solution of water-acetonitrile (93:7 ratios, with 3% of acetic acid) as initial settings, and the complete separation was achieved in 60 min by mean of a validated method (Locatelli et al.,

2017; Zengin et al., 2016); and herein applied after evaluation of analytical performances and the absence of matrix interferences.

2.4 In-vitro enzyme inhibition assays

a-amylase inhibitory activity was performed using Caraway-Somogyi iodine/potassium iodide (IKI) method (Zengin, 2016). Sample solution (25 mL, 2 mg/mL) was mixed with a-amylase solution (porcine pancreas, EC 3.2.1.1, Sigma, Saint Louis, Mo., USA) (50 mL) in phosphate buffer (pH 6.9 with 6 mM sodium chloride) in a 96-well microplate and incubated for 10 min at 37 °C. After preincubation, the reaction was initiated with the addition of starch solution (50 mL, 0.05%). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme solution.

The reaction mixture was incubated 10 min at 37 °C, then stopped by adding HCl (25 mL, 1 M). This is followed by the addition of the iodine-potassium iodide solution (100 mL). The sample and blank absorbances were recorded at 630 nm. The absorbance of the blank was subtracted from that of the sample and the a-amylase inhibitory activity was expressed as millimoles of acarbose equivalents (mmol ACAE/g extract).

α-glucosidase inhibitory activity was performed as previously described by Zengin (Zengin, 2016). Sample solution (50 μL) was mixed with glutathione (50 μL, 2 mg/mL), 50 μL a-glucosidase solution (from *Saccharomyces cerevisiae*, EC 3.2.1.20, Sigma) in phosphate buffer (pH 6.8), and 50 μL of 10 mM PNPG (4-N-trophenyl-a- D-glucopyranoside) (Sigma-Aldrich, Darmstadt, Germany) solution in a 96-well microplate and incubated for 15 min at 37 °C. Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme solution. The reaction was stopped with the addition of sodium carbonate (50 mL, 0.2 M). The sample and blank absorbances were recorded at 400 nm. The absorbance of the blank was subtracted from that of the sample and the a-glucosidase inhibitory activity was expressed as millimoles of acarbose equivalents (mmol ACAE/g extract).

2.5 In-vivo experiments

2.5.1 Animals

Healthy male Wistar rats from Empire Breeders, Osogbo, Osun State, Nigeria were used. Rats were housed in plastic cages measuring 24 × 18 × 12 inches (6 rats per cage). General housing was a temperature-controlled (22.5 °C ± 2.5 °C) quarters with 12 h of light (lights on at 7.00 a.m.). Rats had free access to food and water *ad libitum*. All animals were fed commercial standard chow (calories: 29% protein, 13% fat, 58% carbohydrate). All procedures were conducted in accordance with the approved institutional protocols and within the provisions for animal care and use pre- scribed in the scientific procedures on living animals, European Council Directive (EU2010/63).

2.5.2 Acute toxicity test

Acute oral toxicity studies were conducted using the Organization for Economic Co-operation and Development (OECD) guide- lines (OECD, 2008).

2.5.3 Induction of diabetes mellitus

Diabetes mellitus was experimentally induced in the animals by a single intraperitoneal injection of 70 mg/kg/body weight of streptozotocin (STZ), dissolved in ice-cold physiologic saline and administered at a volume of 2 ml/kg. Animals in the non-diabetic control group received an intraperitoneal injection of an equivalent volume of cold saline (vehicle). Rats were fasted overnight prior to administration of STZ or vehicle. 72 h after injection of STZ or vehicle, rats were fasted for 8 h and blood taken from the tail veins for glucose estimation. Rats having hyperglycemia (that is, blood glucose of 300 mg/dl) were considered diabetic and included in the study (Husain et al., 2015).

2.5.4 METHODOLOGY

Thirty-six rats weighing between 160 and 180 g each were randomly assigned into six groups of six (n 6) rats. Rats were administered vehicle, which was distilled water at 10 ml/kg [non-diabetic control (VEH group), and diabetes control (STZ)], metformin (MET), a standard anti-hyperglycemic agent at 25 mg/kg (Yanardag et al., 2005; Onaolapo & Onaolapo, 2012; Onaolapo et al., 2012) and one of three doses of *J. regia* leaf (L) powder at 25, 50 and 100 mg/kg. Vehicle, standard drug or herb was administered twice daily (9.00 a.m. and 5 p.m.) by gavage; gavage was used as method of drug delivery to simulate administration in humans. Doses used in the study were determined from the result of acute toxicity tests, and also guided by doses used in previous studies (Asgary et al., 2008; Gholamreza and Hossein, 2008; Mohammadi et al., 2011, 2012a) All treatments were administered for a period of 28 days and the animals were weighed weekly. Blood samples from the tail vein were taken weekly after an overnight fast for glucose estimation by the glucose oxidase method. At the end of the experimental period (day 28), rats were observed for changes in their physical characteristics, sacrificed by decapitation after an over- night fast. Plasma from blood taken via intracardiac puncture was used for estimation of lipid profile, cholesterol, antioxidant activity and biochemical parameters of liver/kidney injury. The pancreas, liver and kidneys were dissected out, observed grossly and fixed in 10% neutral buffered formalin. Samples of the pancreas, liver and kidney were processed for paraffin-embedding, sectioned at 5 mm and stained for general histological study.

2.5.5 BIOCHEMICAL ASSAYS

Blood was collected from each rat on the 28th day via intra- cardiac puncture after an overnight fast. Plasma was separated by centrifugation at 3500 rpm for 10 min using a general centrifuge (Uniscope SM 112, Surgifriend Medicals, England). The plasma was assayed either immediately or stored at —20 °C.

2.5.5.1 LIPID PROFILE, LIVER AND KIDNEY BIOCHEMISTRY.

Alanine transaminase (ALT) and aspartate transaminase (AST) levels were determined according to the spectrophotometric method described using appropriate assay kits. Plasma creatinine and urea levels were determined by a colorimetric reaction (Jaffe's Method), and (DAM Method) respectively using an autoanalyser (Astra 8 autoanalyser; Beckman Instruments, Fullerton, CA) (Yanardag et al., 2005; Onaolapo & Onaolapo, 2012; Onaolapo et al., 2012). Total cholesterol, triglycerides, HDL-C, LDL-C, and VLDL-C in plasma were analyzed using commercially available kits following the instructions of the manufacturer.

2.5.5.2 ANTIOXIDANT ASSAY.

Catalase (CAT) activity was estimated using CAT assay kit was measured using a UV vis spectrophotometer. Catalase reacts with a known quantity of hydrogen peroxide to form water and oxygen, the reaction is stopped after 5 min with a catalase inhibitor (potassium cyanide, bisulphate ion). Catalysed by peroxidase, the remaining hydrogen peroxide reacts with 3,5-dichloro-2-hydroxybenzene-sulfonic acid and 4-aminophenazone to form a coloured substrate; the absorbance was measured at 510 nm and is expressed as U/mg protein (Onaolapo et al., 2016). Superoxide dismutase activity was assayed based on the enzyme's ability to inhibit the phenazine methosulphate mediated reduction of nitro blue tetrazolium dye, the change in absorbance at 560 nm over 5 min was measured (Onaolapo et al., 2016, 2017). Glutathione peroxidase (GPX) activity was assessed by measuring the change in absorbance at 340 nm that follow NADPH consumption using the GPX assay kit. In this assay, NADPH consumption in the enzyme coupled reactions was measured and expressed as U/mg protein (Onaolapo et al., 2017).

2.5.6 PHOTOMICROGRAPHY AND IMAGE J MORPHOMETRIC ANALYSIS

Sections of the rat pancreas, liver and kidneys were examined using a Sellon-Olympus trinocular microscope (XSZ-107E, China) with a digital camera (Canon Powershot 2500) attached; and photomicrographs were taken. To quantify the cell density and % area covered by cells within the pancreas, liver and kidneys, an integrated morphology analysis was undertaken using the Image J (National Institute of Health U.S.) software. For each section, measurements were conducted on at least 5 representative slides from at least 5 animals per experimental group. Digital brightfield images were uploaded unto the software, and scale was set using a digital micrometer gauge reading (to convert measurements in pixels to microns); this was applied globally to all images. Cells were counted using the cell-counter plug-in available on the software, after a grid had been applied across the image.

2.6 STATISTICAL ANALYSIS

Data was analyzed using Chris Rorden's ezANOVA for windows. Hypothesis was tested using analysis of variance (ANOVA). We tested the hypothesis that oral administration of *J. regia* L. can significantly alter body weight, blood glucose, lipid profile, biochemical parameters of hepatic and renal function and morphology of the pancreas, liver and kidneys in streptozocin induced diabetic rats. Repeated measures ANOVA was used to test *J. regia* at 25, 50 and 100 mg/kg on body weight or blood glucose taken on day 1 and 28, while one way ANOVA was used to assess the effect of *J. regia* L. on lipid profile, antioxidant status, liver/ kidney biochemistry and morphometry of the pancreas, liver and kidneys. Tukey (HSD) test was used for posthoc analysis. Results were expressed as mean ± S.E.M, and $p < 0.05$ considered significant.

3. RESULTS

3.1 PHYTOCHEMICAL COMPOSITION AND IN-VITRO ENZYME INHIBITORY EFFECTS OF J. REGIA L.

The results of *in-vitro* anti-diabetic tests of *J. regia* L. leaves using a-amylase and a-glucosidase inhibitory assays are summarized in Table 1. All extracts of *J. regia* L. examined were positive for anti- amylase and anti-glucosidase activity. The microwave extract (0.27 mmolACAE/g extract) had showed the most potent anti-amylase activity, while the soxhlet extract exhibited the strongest anti-glucosidase activity, however the anti- glucosidase activity was overall much higher than the anti-amylase activity. HPLC characterization of the phenolic components of J. regia L. extracts (summarized in Table 2), revealed mainly the presence of rutin, catechin and 2,3-diMeO-benzoic acid as major components in the studied extracts.

3.2 Acute toxicity test of J. regia L.

Acute toxicity studies of powdered leaf of *J. regia* L. were carried out in rats by using the Organization for Economic Co-operation and Development (OECD) (2008) guideline. Rats were tested up to a dose of 2000 mg/kg body weight; and then observed for general behavioral changes, symptoms of toxicity and mortality after treatment for the first four (critical) hours, then over a period of 24 h, and thereafter for 14 days. At the end of the experiment, no mortalities, or any observable evidence of behavioral or tissue toxicity have been found.

3.3 Effects of J. regia L. on body weight

Fig. 1 (upper panel) represents the effect of *J. regia* on body weight. Repeated measures ANOVA revealed a significant effect of treatment ($F(5, 30) = 150$, $p < 0.001$), body weight ($F(1,30) = 41.4$, $p < 0.001$), and significant interactions between treatment x body weight ($F(5,30) = 77.3$, $p < 0.001$). Tukey (HSD) analysis revealed a significant decrease in body weight with STZ ($p < 0.001$, $p < 0.001$), MET ($p < 0.001$, $p < 0.001$), *J. regia* L. at 25 ($p < 0.001$, $p < 0.002$), 50 ($p < 0.001$, $p < 0.001$) and 100 mg/kg ($p < 0.001$, $p < 0.003$) compared to VEH on days 1 and 28 respectively. Compared to diabetic-control (STZ), body weight on day 28 increased significantly with MET ($p < 0.001$), *J. regia* L. at 25 ($p < 0.001$), 50 ($p < 0.001$) and 100 mg/kg ($p < 0.001$), while no significant dif- ference in weight was observed on day 1. Compared to MET, body weight on day 28 increased significantly with *J. regia* L. at 25 ($p < 0.001$), 50 ($p < 0.001$) and 100 mg/kg ($p < 0.001$) and showed no significant difference on day 1. Comparing body weight on day 1 to weight on day 28, revealed a significant increase in weight on day 28 with VEH ($p < 0.004$), *J. regia* L. at 25 ($p < 0.006$), 50 ($p < 0.002$) and 100 mg/kg ($p < 0.001$) and a significant decreased with STZ ($p < 0.001$) compared to day 1.

3.4 Effect of J. regia L. on blood glucose levels

Fig. 1 (lower panel) represents the effect of *J. regia* L. on blood glucose levels in all groups. Repeated measures ANOVA revealed a significant effect of treatment ($F(5,30) = 201$, $p < 0.001$), blood glucose ($F(1,30) = 596$, $p < 0.001$), and significant interactions be- tween treatment x blood glucose ($F(5,30) = 132$, $p < 0.001$). Tukey (HSD) analysis revealed a significant increase in blood glucose levels with STZ ($p < 0.001$, $p < 0001$), MET ($p < 0.001$, $p < 0.006$), *J. regia* L. at 25 ($p < 0.001$, $p < 0.001$), 50 ($p < 0.001$, $p < 0.001$) and 100 mg/kg ($p < 0.001$, $p < 0.001$) compared to VEH, on days 1 and 28 respectively. Compared to diabetic-control (STZ) blood glucose levels on day 28 decreased significantly with MET ($p < 0.001$), *J. regia* L. at 25 ($p < 0.001$), 50 ($p <$

0.001) and 100 mg/kg ($p < 0.001$) while no significant differences were seen with blood glucose levels on day 1. Compared to MET, no significant difference in blood glucose levels was observed at any of the doses of *J*. regia L. on either day 1or 28. Comparing blood glucose levels on day 1 to glucose levels on day 28, revealed a significant decrease in blood glucose levels on day 28 with MET ($p < 0.001$), *J. regia* L. at 25 ($p < 0.001$), 50 ($p < 0.001$) and 100 mg/kg ($p < 0.001$) compared to day 1.

Table 1
Enzyme inhibitory potentials of *Juglans regia* L. extracts.[a]

Samples	Amylase Inhibition (mmol ACAE/g extract)	Glucosidase Inhibition (mmol ACAE/g extract)
Soxhlet extract	0.18 ± 0.01	18.36 ± 0.10
Decoction	0.20 ± 0.02	8.06 ± 0.14
Microwave extract	0.27 ± 0.01	8.22 ± 0.13

[a] Three parallel experiments ± S.D. ACAE: acarbose equivalents.

Table 2
Phenolic components of *Juglans regia* L. (μg/mg extract).[a]

Compounds	Soxhlet	Decoction	Microwave
Gallic acid	nd	nd	nd
Catechin	18.27 ± 1.45	21.53 ± 1.87	22.80 ± 3.21
Chlorogenic acid	0.35 ± 0.08	0.12 ± 0.01	0.22 ± 0.03
p-OH benzoic acid	nd	nd	nd
Vanillic acid	0.31 ± 0.03	0.28 ± 0.05	0.96 ± 0.14
Epicatechin	3.72 ± 0.32	3.07 ± 0.54	2.80 ± 0.52
Syringic acid	nd	nd	0.09 ± 0.01
3-OH benzoic acid	0.14 ± 0.01	0.16 ± 0.02	0.74 ± 0.11
3-OH-4-MeO benzaldehyde	5.42 ± 0.58	0.10 ± 0.01	0.69 ± 0.08
p-coumaric acid	0.18 ± 0.03	0.12 ± 0.03	0.12 ± 0.01
Rutin	30.92 ± 2.95	40.47 ± 4.28	35.00 ± 3.21
t-Ferulic acid	1.26 ± 0.15	1.91 ± 0.23	1.69 ± 0.45
Naringin	nd	nd	nd
2,3-diMeO benzoic acid	24.41 ± 2.68	31.56 ± 2.82	nd
Benzoic acid	0.29 ± 0.05	0.30 ± 0.08	0.29 ± 0.09
o-Coumaric acid	0.58 ± 0.09	0.78 ± 0.10	0.61 ± 0.12
Quercetin	0.60 ± 0.07	0.08 ± 0.01	0.09 ± 0.02
t-Cinnamic acid	nd	nd	nd
Naringenin	nd	nd	nd

nd: not detected.
[a] Values expressed are means ± SD of three experiment.

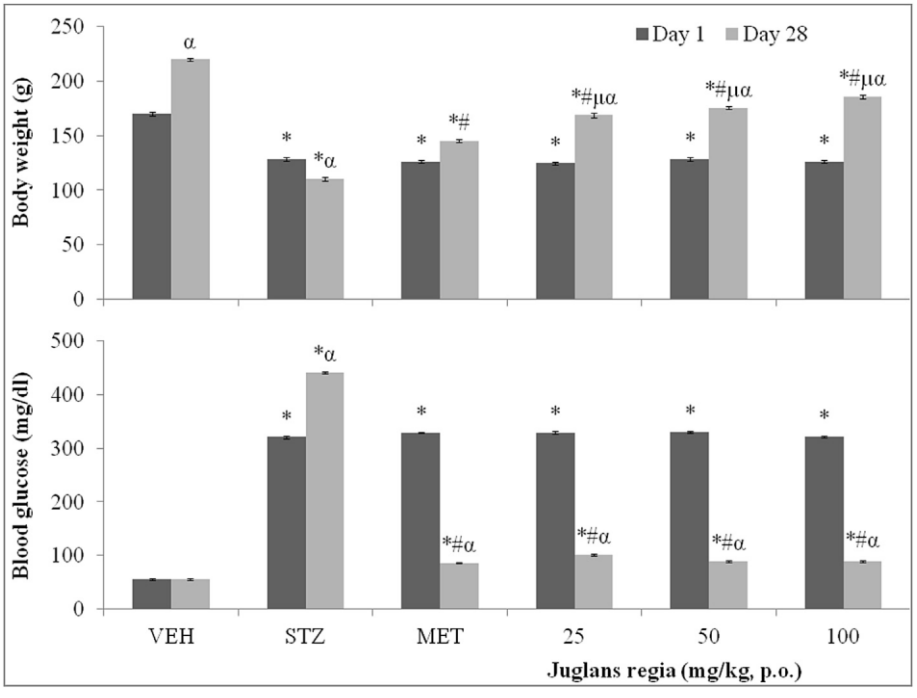

Fig. 1. Effect of *Juglans regia* L. on body weight (upper panel) and blood glucose levels (lower panel) in streptozocin-induced diabetic Wistar rats. Each bar represents mean ± S.E.M, $^{*}p < 0.05$ significantly different from VEH, $^{\#}p < 0.05$ significantly different from STZ, $^{m}p<0.05$ significantly different from MET, $^{a}p<0.05$ final significantly different from initial, number of rats per treatment group 6; VEH: Vehicle, STZ: Streptozocin, MET: Metformin, mg/kg, p.o.: milligrams per kilogram, per-oral.

3.5 Effect of J. regia L. on liver enzymes

¼Fig. 2 shows the effect of *J. regia* L. on liver enzymes in diabetic rats. Aspartate transaminase (AST) activity increased significantly (F(5,30) 117, p ≤¼ 0.001) with STZ, *J. regia* L. at 25, 50 and 100 mg/ kg and decreased with metformin (MET) compared to vehicle. Compared to STZ there was a significant decrease in AST activity with MET, *J. regia* L. at 25, 50 and 100 mg/kg. Compared to MET, aspartate transaminase activity increased significantly with *J. regia* L at 25, and 50 mg/kg. Alanine transaminase (ALT) activity increased significantly (F (5,30) 38.8, p < 0.001) with STZ, and *J. regia* L. at 25 mg/kg compared to vehicle. Compared to STZ, ALT activity decreased significantly with MET, *J. regia* L. at 25, 50 and 100 mg/kg, while compared to MET, alanine transaminase activity increased with *J. regia* L. at 25 mg/kg.

3.6 Effect of J. regia L. on kidney function tests

Fig. 3 shows the effect of *J. regia* L. on ¼levels of urea (upper panel) and creatinine (lower panel) in all rats. Urea levels increased significantly (F (3, 30) 344, p < 0.001) with STZ, MET and *J. regia* L. at 25 mg/kg compared to vehicle. Compared to STZ, urea levels decreased significantly with MET, *J. regia* L. at 25, 50 and 100 mg/kg. Compared to MET, urea levels decreased with *J. regia* L. at 50 and 100 mg/kg. Creatinine levels increased significantly (F (3,30) 46.3, p < 0.001) with STZ, *J. regia* L. at 25 mg/kg and decreased with MET and *J. regia* L. at 100 mg/kg compared to vehicle. Compared to the STZ there was a significant decrease in creatinine with MET and *J. regia* L. at 25, 50 and 100 mg/kg. Compared to MET, creatinine increased significantly with *J. regia* L. at 25 and 50 mg/kg.

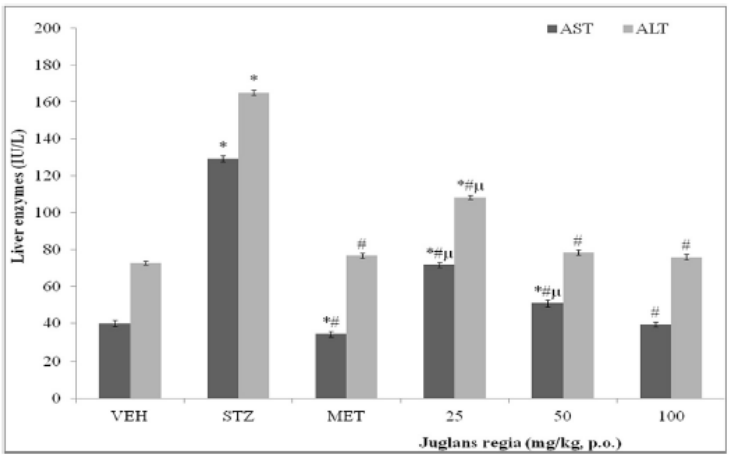

Fig. 2. Effect of *Juglans regia* L. on liver enzymes in streptozocin-induced diabetic Wistar rats. Each bar represents mean ± S.E.M, $^{*}p < 0.05$ significantly different from VEH, $^{\#}p < 0.05$ significantly different from STZ, $^{\mu}p<0.05$ significantly different from MET, number of rats per treatment group = 6; VEH: Vehicle, STZ: Streptozocin, MET: Metformin, mg/kg, p.o.: milligrams per kilogram, per-oral.

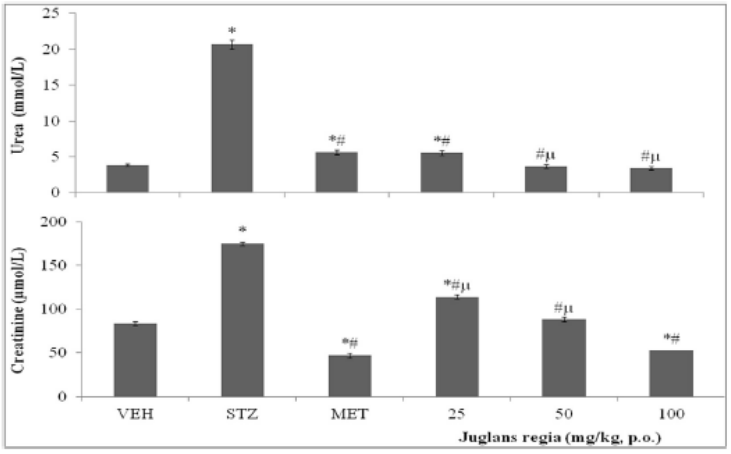

Fig. 3. Effect of *Juglans regia* L. on urea (upper panel) and creatinine (lower panel) levels in streptozocin-induced diabetic Wistar rats. Each bar represents mean ± S.E.M, $^{*}p < 0.05$ significantly different from VEH, $^{\#}p < 0.05$ significantly different from STZ, $^{\mu}p<0.05$ significantly different from MET, number of rats per treatment group = 6; VEH: Vehicle, STZ: Streptozocin, MET: Metformin, mg/kg, p.o.: milligrams per kilogram, per-oral.

3.7 Effect of J. regia L. on lipid profile

Table 3 shows the effect of *J. regia* L. on lipid parameters in all groups. Total cholesterol (TC) increased significantly ($F(5, 30) 253$, $p < 0.001$) with STZ and *J. regia* L. at 25 mg/kg, and decreased with MET and *J. regia* L. at 100 mg/kg compared to vehicle. Compared to STZ group there was a significant decrease in TC with MET, *J. regia* L. at 25, 50 and 100 mg/kg. Compared to MET, total cholesterol levels increased significantly with *J. regia* L. at 25 and 50 mg/kg. Triglyceride (Tg) levels increased significantly ($F(5, 30) 250$, $p < 0.001$) with STZ and with *J. regia* L. at 25 mg/kg and decreased significantly with MET and *J. regia* L. at 100 mg/kg compared vehicle. Compared to the STZ triglyceride levels decreased significantly with MET and with *J. regia* L. at 25, 50 and 100 mg/kg. Compared to MET there was a significant increase in Tg levels with *J. regia* L. at 25 and 50 mg/kg. High-density lipoprotein (HDL) levels decreased significantly ($F(3, 30) 76.8$, $p < 0.001$) with STZ, MET and *J. regia* L. at 25 and 50 mg/kg compared to vehicle. Compared to STZ, there was significant increase in HDL levels with MET and with *J. regia* L. at 25, 50 and 100 mg/kg. Compared to MET, HDL levels increased significantly with *J. regia* L. at 100 mg/kg. Low-density lipoprotein (LDL) levels increased significantly ($F(3, 30) 372$, $p < 0.001$) with STZ, and with *J. regia* L. at 25 mg/kg, and decreased significantly with MET and with *J. regia* L. at 50 and 100 mg/kg compared to vehicle. Compared to STZ, LDL levels decreased significantly with MET, *J. regia* L. at 25, 50 and 100 mg/kg.

Compared to MET, LDL levels increased significantly with *J. regia* L. at 25 and 50 mg/kg.

3.8 Effect of J. regia L. on antioxidant activity

Table 4 shows the effect of *J. regia* L. leaf powder on plasma catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) activity. CAT activity decreased significantly ($F(5, 30)$ 36.7, $p < 0.001$) with STZ, MET, *J. regia* L. at 25, 50 and 100 mg/ kg compared to vehicle. Compared to STZ, CAT activity increased significantly with MET, *J. regia* L. at 50 and 100 mg/kg. Compared to MET, CAT activity decreased with J. *regia* at 25 mg/kg and increased with *J. regia* L. at 100 mg/kg. Super oxide dismutase activity decreased significantly ($F(5, 30)$ 56, $p < 0.001$) with STZ, MET,*J. regia* L. at 25, 50 and 100 mg/kg compared to vehicle. Compared to STZ there was a significant increase in superoxide dismutase activity with MET, *J. regia* L. at 25, 50 and 100 mg/kg. Compared to MET, superoxide dismutase activity increased significantly with
J. regia L. at 50 and 100 mg/kg. Glutathione peroxidase activity decreased significantly ($F(5, 30)$ 23, $p < 0.001$) with STZ, MET, *J. regia* L. at 25, 50 and 100 mg/kg compared to vehicle. Compared to STZ there was a significant increase in glutathione peroxidase activity with MET, *J. regia* L. at 25, 50 and 100 mg/kg. Compared to MET, glutathione peroxidase activity increased significantly with *J. regia* L. at 50 and 100 mg/kg.

Table 3
Effect of *J. regia* L. on lipid parameters.

Groups	TC (mmol/L)	Tg(mmol/L)	HDL(mmol/L)	LDL(mmol/L)
VEH	3.72 ± 0.18	1.68 ± 0.10	1.43 ± 0.04	2.39 ± 0.10
STZ	12.65 ± 0.34*	12.18 ± 0.27*	0.16 ± 0.03*	13.34 ± 0.51*
MET	2.35 ± 0.10*#	1.01 ± 0.17*#	0.66 ± 0.10*#	1.33 ± 0.20*#
25	7.86 ± 0.45*#μ	7.70 ± 0.65*#μ	0.46 ± 0.010*#	4.80 ± 0.20*#μ
50	3.84 ± 0.16#μ	2.54 ± 0.13*#μ	0.78 ± 0.10*#	2.08 ± 0.10*#μ
100	2.32 ± 0.59*#	1.18 ± 0.12*#	1.52 ± 0.10#μ	1.34 ± 0.10*#

Results presented as mean ± SE.M. Comparisons are: *p < 0.05 significantly different from VEH, #p < 0.05 significantly different from STZ, μp < 0.05 significantly different from MET, VEH: Vehicle, STZ: Streptozocin, MET: Metformin, TC: Total cholesterol, Tg: Triglyceride, HDL: High density lipoprotein, LDL: Low density lipoprotein, number of animals per group = 6.

Table 4
Effect of *J. regia* L. on antioxidant activity.

Groups	CAT (U/mg protein)	SOD (U/mg protein)	GPX (U/mg protein)
VEH	8.11 ± 1.2	5.3 ± 0.02	200.2 ± 2.3
STZ	3.22 ± 1.6*	1.18 ± 0.03*	62.0 ± 2.4*
MET	5.20 ± 1.3*#μ	2.16 ± 0.02*#μ	101.4 ± 2.1*#μ
J. regia 25	3.88 ± 1.15*μ	1.91 ± 0.02*#μ	98.2 ± 2.1*#
J. regia 50	5.82 ± 1.12*#	2.12 ± 0.02*#	120 ± 2.5*#μ
J. regia 100	7.20 ± 1.60*#μ	3.22 ± 0.01*#μ	189 ± 2.2*#μ

Results presented as mean ± SE.M. Comparisons are: *p < 0.05 significantly different from VEH, #p < 0.05 significantly different from STZ, μp < 0.05 significantly different from MET, VEH: Vehicle, STZ: Streptozocin, MET: Metformin, CAT: catalase, GPX: glutathione peroxidase, SOD: superoxide dismutase, number of animals per group = 6.

3.9 Morphology

3.9.1 Effect of J. regia L. administration on morphology of the pancreas

Fig. 4 shows representative photomicrographs of hematoxylin and eosin stained sections of the pancreas in normal and STZ- induced diabetic Wistar rats administered metformin or increasing doses of *J. regia* L. (Fig. 4aee). Examination of slides from rats administered vehicle (Fig. 4a) revealed normal pancreatic islet cells with deeply-staining nuclei, normal sized blood vessels and pancreatic ducts. The exocrine pancreas also showed normal glandular cells with deeply staining nuclei. Slides from the STZ control group (Fig. 4b) showed edematous pancreatic tissue with widening of the interglandular spaces and engorged blood vessels. The islets of Langerhans appeared shrunken with numerous pale staining nuclei. In groups administered metformin (Fig. 4c) a standard anti-hyperglycemic agent, there was minimal swelling of the pancreatic tissue, evidenced by decrease in the spaces between individual glands. The pancreatic duct showed some dilation, while only a few engorged blood vessels were seen. In groups administered *J. regia* L., there was edema within the pancreatic tissue (with minimally engorged blood vessels), shrinkage of the islet of Lang- erhans cells with a mixture of pale and deeply-staining nuclei, at 25 (Fig. 4d) and 50 mg/kg (Fig. 4e); while at 100 mg/kg (Fig. 4f), normal looking pancreatic islet cells with deeply staining nuclei and very few pale staining nuclei were observed.

3.9.2 Effect of J. regia L. administration on liver morphology

Fig. 5 shows representative photomicrographs of hematoxylin and eosin-stained sections of the liver in normal and diabetic Wistar rats. In rats, administered vehicle (Fig. 5a) sheets of radially arranged hepatocytes having deeply staining nuclei, with intervening sinusoidal spaces around the central vein, are observed. Scattered within the liver parenchyma are few Kupffer cells with deeply staining nuclei. A few Kupffer cells are seen surrounding the central vein; features are in keeping with normal histology. Slides from sections of the liver of the STZ group (Fig. 5b) showed marked disruption of liver parenchyma, with loss of intervening sinusoidal spaces and swollen hepatocytes; numerous pale-staining shrunken hepatocyte nuclei and Kupffer cells are scattered throughout the hepatic parenchyma. Also obvious, is a markedly dilated central vein; features are in keeping with hepatic injury. Examination of liver slides from the metformin group (Fig. 5c) revealed sheets of radially arranged hepatocytes with intervening sinusoids. Hepatocytes with deeply staining nuclei and few pale staining shrunken hepatocyte nuclei are also observed. Numerous inflammatory cells and/or Kupffer cells are seen scattered around a dilated central vein and hepatic vein. In the groups administered *J. regia* L. at 25 (Fig. 5d), 50(Fig 5e) and 100 mg/kg (Fig. 5f), mild to moderate loss of liver architecture with some chords of radially-arranged hepatocytes with deeply staining and pale staining hepatocyte nuclei, a few Kupffer cells are also seen.

3.9.3 Effect of J. regia L. administration on kidney morphology

Fig. 6(ael) representative photomicrographs of hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) stained sections of the right kidney of normal and diabetic Wistar rats respectively. Kidney sections of animals administered vehicle (Fig. 6a and g) revealed well-demarcated cortex and medulla. Bowman's capsule, Bow- man's space, glomeruli, proximal/distal renal tubules (with intact epithelial lining of the glomerular basement membrane and tubular epithelium) and

blood vessels appear within normal limits. Deeply staining-nuclei of the glomeruli and tubular epithelium are also seen. Examination of slides of the STZ group (Fig. 6b and h) showed disruption of normal renal architecture with markedly contracted renal glomeruli, degenerating shrunken glomeruli, widening of the Bowman's space and swollen renal tubules. Glomerular and renal tubular epithelial cell nuclei are pale- staining, shrunken and pyknotic; also present are numerous inflammatory cells scattered all through the renal parenchyma. In animals administered MET (Fig. 6c and i), swollen and crumpled renal glomeruli, with dilated Bowman's space is observed. Also obvious are swelling of the renal tubules, as evidenced by constriction of lumen of both the proximal and distal tubules. The nuclei of the renal glomerular and renal tubular epithelium were a mixture of deeply and pale staining. In the groups administered *J. regia* L. at 25 (Fig. 6d, j), 50 (Fig. 6e, k) and 100 mg/kg (Fig. 6f, l) varying levels of protection from renal injury is observed. Loss of normal renal architecture, with very few degenerating or shrunken renal cell nuclei, mild to moderate swelling of the proximal and distal renal tubules; minimal dilatation of the Bowman's space is also observed.

3.10 Effect of J. regia L. administration on morphometry of the pancreas, liver and kidney

The effect of *J. regia* L. leaf powder on the morphometry of the pancreas, liver and kidney are summarized in Table 5. In the pancreas there was a significant ($F(5, 20) = 378$, $p < 0.001$) increase in total cell count with STZ, MET and *J. regia* L. at 100 mg/kg and a significant decrease with *J. regia* L. at 25 and 50 mg/kg compared to vehicle. Compared to STZ, total cell count decreased with MET, *J. regia* L. at 25, 50 and 100 mg/kg; while compared to MET, total cell count decreased with *J. regia* L. at 25 and 50 mg/kg. Average cell size and total area covered by cells did not differ significantly with treatment compared to vehicle. In the liver there was a significant ($F(5, 20) = 47.9$, $p < 0.001$) increase in total cell count with STZ and a significant decrease with *J. regia* L. at 25 and 50 mg/kg compared to vehicle. Compared to STZ total cell count decreased with MET, *J. regia* L. at 25, 50 and 100 mg/kg; while compared to MET total cell count decreased with *J. regia* L. at 25 and 50 mg/kg. Average cell size decreased significantly ($F(5, 20) = 23.0$, $p < 0.001$) with STZ, MET and *J. regia* L. at 25, 50 and 100 mg/kg compared to vehicle. Compared to STZ, average cell size increased with MET, and *J. regia* L. at 50 and 100 mg/kg; while compared to MET, average cell size decreased with *J. regia* L. at 25 mg/kg. There was no significant difference in total area covered by cells with treatment compared to vehicle. In the kidney there was a significant ($F(5, 20) = 22.9$, $p < 0.001$) decrease in total cell count with STZ, MET and with *J. regia* L. at 25, 50 and 100 mg/kg compared to vehicle. Compared to STZ, total cell count increased with MET, *J. regia* L. at 100 mg/kg and decreased with *J. regia* L. at 25 and 50 mg/kg; while compared to MET, total cell count decreased with *J. regia* L. at 25 and 50 mg/kg. Average cell size decreased significantly ($F(5, 20) = 23.0$, $p < 0.001$) with STZ, and MET and increased with *J. regia* L. at 50 mg/kg compared to vehicle. Compared to STZ average cell size increased with MET and *J. regia* L. at 25, 50 and 100 mg/kg; while compared to MET, average cell size increased with *J. regia* L. at 25 and 50 mg/kg. There was no significant difference in total area covered by cells with treatment compared to vehicle.

4. Discussion

The anti-hyperglycaemic (Hosseini et al., 2014a, 2014b) and anti-hyperlipidaemic (Ebrahim

et al., 2012; Iwamoto et al., 2000) effects of various extracts of the *J. regia* L. tree have been previously reported. However, in this paper we verified the *in-vitro* anti- diabetic potentials of *J. regia* L. extracts and assessed the *in-vivo* nutraceutical potential of *J. regia* L. leaf powder in the management of diabetes mellitus by daily administration in diabetic rodent model. HPLC analysis of plant extracts revealed large quantities of phenols, like quercetin-3-O-rutinoside (rutin) which is a glycoside of the flavonoid quercetin, 2,3-diMeO-benzoic acid, cathechins, and smaller quantities of other phenolic compounds like epicatechins, 3-OH benzoic acid, chlorogenic acid, coumaric acid and vanillic acid. Plant polyphenols are naturally-occurring phytochemical compounds that may hold promise as unique nutraceuticals and supplementary treatments for the management of diabetes mellitus (Bahadoran et al., 2013). A number of papers have reported the presence of large quantities of phenols and flavonoids in the leaves of *J. regia* L. (Amaral et al., 2003; Pereira et al., 2007) among which rutin is the predominant flavonoid observed in *J. regia* L., in accordance with literature data (Colaric et al., 2005; Nour et al., 2012). Rutin is a flavonoid with high antioxidant activity that inhibits DNA oxidation (Nour et al., 2012) and aldose reductase activity (Reddy et al., 2011), preventing the deposition of glycation endeproducts in body organs. Enzyme inhibition assays demonstrated moderate inhibition of a-amylase and a strong activity to- ward a-glucosidase. a-Amylase and a-glucosidase inhibition has been linked to the regulation of blood glucose after a high carbohydrate meal (Kwon et al., 2008).

Results of the *in-vivo* experiments revealed that twice-daily administration of increasing doses of *J. regia* L. leaf (compared to diabetic control) was associated with 1) weight gain 2) significant reduction in blood glucose levels 3) reversal of diabetes induced dyslipidemia, oxidative stress and biochemical parameters of liver and kidney injury 4) and varying degrees of renal, hepatic and pancreatic tissue protection. In this study, streptozotocin-induced diabetes was associated with weight loss, compared to non- diabetic controls; this is consistent with the results of a number of studies (Locatelli et al., 2017; Onaolapo et al., 2011; Patel and Sharma, 2015) that have reported weight loss with induction of experimental diabetes. Weight loss in diabetes has been linked to excessive tissue protein breakdown (Andallu and Varadacharyulu, 2003). Repeated, oral, twice-daily administration of *J. regia* L. leaf powder was associated with a dose-related increase in weight (compared to diabetic control and metformin group), consistent with previous studies (Locatelli et al., 2017; Mohammadi et al., 2012b), but less weight gains than was observed with non- diabetic control. Glycemic control is crucial to the successful management of diabetes mellitus. In the present study, twice daily administration of increasing doses of *J. regia* L. leaf powder was associated with significant blood glucose lowering effects; and effects observed were comparable to metformin. Studies have shown that different parts of *J. regia* L. plant have significant glucose lowering ability (Kavalah et al., 2002; Mirbadalzadeh and Shirdel, 2010). The glucose lowering effects of *J. regia* L. have been attributed to 1) the inhibition of a-glucosidase (Rahimzadeh et al., 2014) (a feature also observed in this study), 2) the inhibition of glycation and oxidation reactions (Ahmad et al., 2012), and 3) the inhibition of protein tyrosine phosphatase 1B (PTP1B) (Pitschmann et al., 2014). The presence of rutin in significant quantities may also be important in the control of blood sugar, since rutin has strong antioxidant po- tential which may help in the preservation of pancreatic beta cell (as was observed with pancreatic morphology in this study) from oxidative damage; thereby ensuring continued insulin secretion. In this study, the glucose lowering abilities of *J. regia* L. are

comparable to metformin. Metformin alters blood glucose levels through the suppression of hepatic gluconeogenesis (Kirpichnikov et al., 2002). It also stimulates the adenosine monophospate-activated protein kinase (AMPK) (Towler and Hardie, 2007).

Diabetes mellitus is also associated with dyslipidemia, which occurs as a result of mobilization of free fatty acids from the peripheral fat stores (Mitra et al., 1995). In this study, streptozotocin-induced diabetes was associated with alterations of lipid levels that were reversed by metformin and by increasing doses *J. regia* L. leaf. Few studies have reported lipid lowering ability of *J. regia* L. in rodents (Asgary et al., 2008; Locatelli et al., 2017). The lipid lowering potential of *J. regia* L. has been attributed to its high polyphenol- content. Shimoda et al. (Shimoda et al., 2009), demonstrated the triglyceride lowering effect of a polyphenol-rich extract of walnut and suggested that this effect is achieved through the enhancement of peroxisomal fatty acid b-oxidation in the liver.

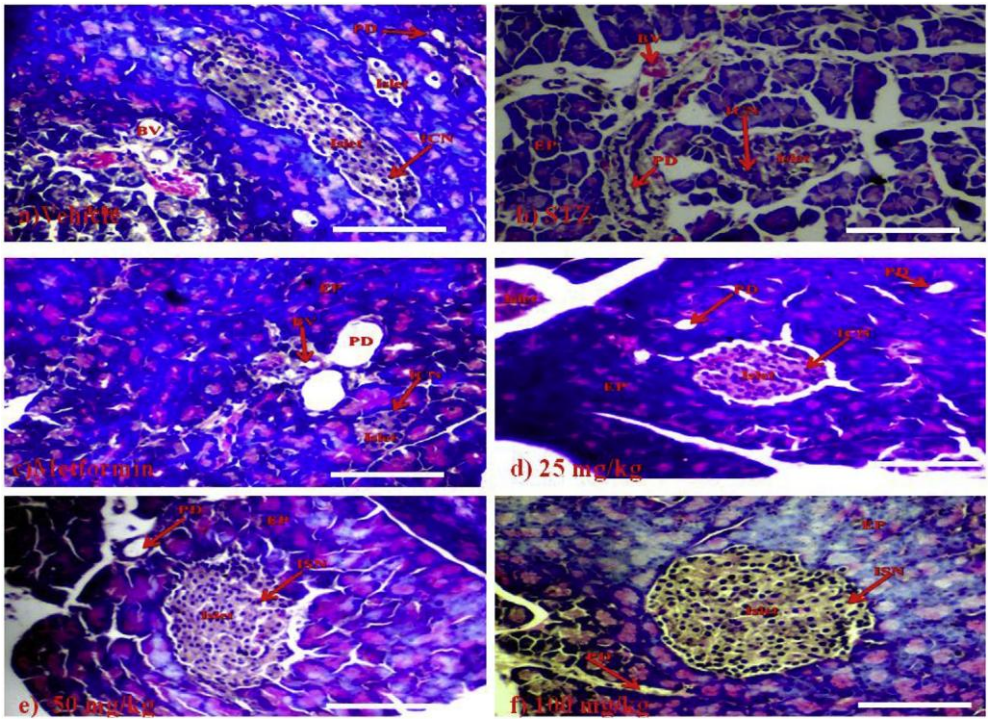

Fig. 4. aef: Effect of *Juglans regia* L. on the pancreas of STZ-induced diabetic Wistar rats. 4a) Vehicle, 4b) STZ 4c) Metformin 4d) *Juglans regia* L. 25 mg/kg, 4e) *Juglans regia* L. 50 mg/kg, 4f) *Juglans regi*a L. 100 mg/kg. Representative photomicrographs of hematoxylin and eosin (H&E) stained sections of the rat pancreas showing islet of Langerhans (Islet) with deeply staining nuclei (ICN), exocrine pancreas (EP), blood vessels (BV), and pancreatic duct (PD) STZ: Streptozocin. H&E ×160, Scale bar-29 mm.

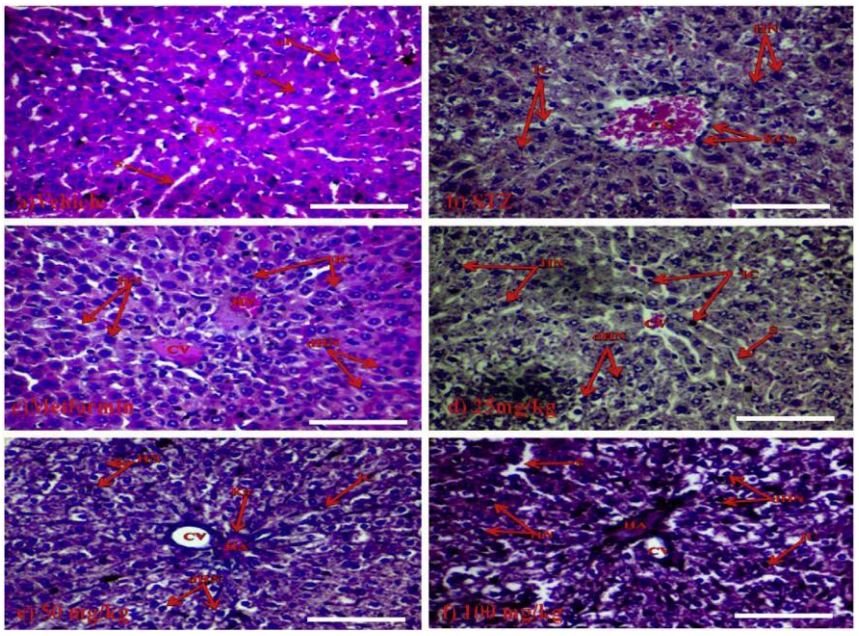

Fig. 5. a-f: Effect of *Juglans regia* L.on the liver of STZ-induced diabetic Wistar rats. 5a) Vehicle, 5b) STZ 5c) Metformin 5d) *Juglans regia* L. 25 mg/kg, 5e) *Juglans regia* L. 50 mg/kg, 5f) *Juglans regia* L. 100 mg/kg. Representative photomicrographs of hematoxylin and eosin (H&E) stained sections of the rat liver showing central vein (CV), nucleus of the hepatocytes (HN), Hepatic artery (HA) Hepatic vein (HV)Portal vein (PV), nucleus of Kupfer cells (KCn), degenerating hepatic nucleus (dHN), inflammatory cells (IC) STZ: Streptozocin. H&E ×160, Scale bar-29 mm.

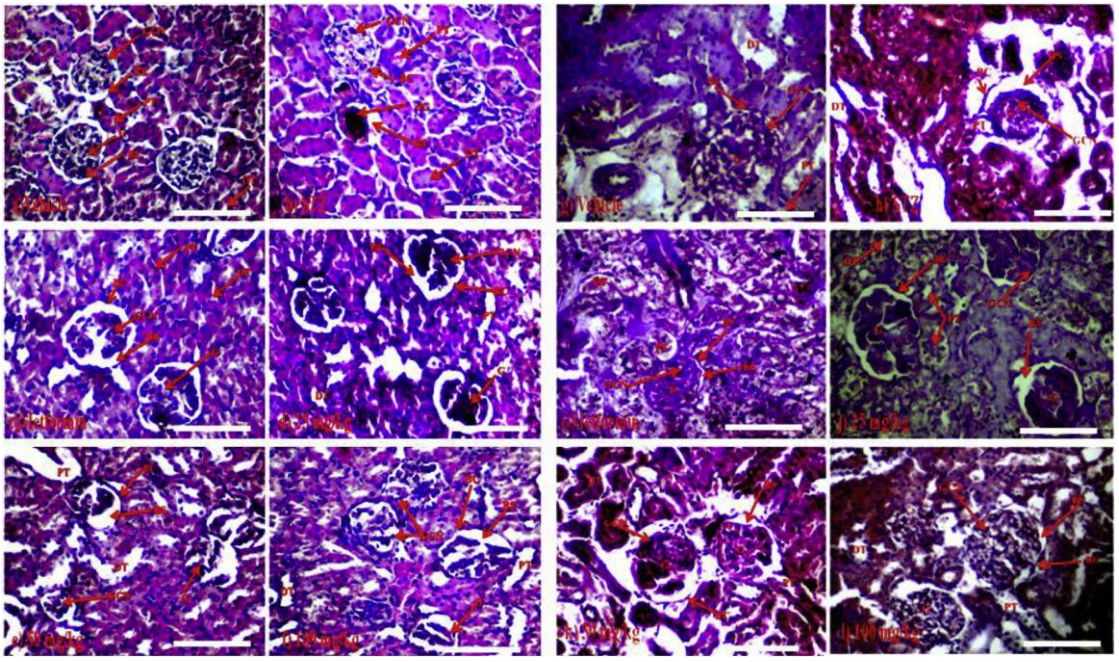

Fig. 6. a-l: Effect of *Juglans regia* L. on hematoxylin and eosin [H&E] (6a-f) and periodic acidic Schiff [PAS] (6g-l) stained sections of the kidney of STZ-induced diabetic Wistar rats. 6a, 6g) Vehicle, 6b, 6h) STZ, 6c, 6i) Metformin 6d, 6j) *Juglans regia* L. 25 mg/kg, 6e, 6k)

Juglans regia L. 50 mg/kg, 6f, 6l) *Juglans regia* L. 100 mg/kg. Representative photomicrographs showing glomerulus (G), glomerula cell nucleus (GCN), degenerating glomerulus (dG), Bowman's capsule (BC), Bowman's space (BS), proximal tubule (PT), distal tubule (DT) STZ: Streptozocin. H&E, PAS ×160, Scale bar-18 mm.

Table 5
Effect of *J. regia* L. administration on morphometry of the pancreas, liver and kidney.

Pancreas	Groups	Mean total cell count	Average cell size (μm)	% area covered by cells
	VEH	1155 ± 17.88	0.003 ± 0.01	5.357 ± 0.01
	STZ	2380 ± 16.49*	0.001 ± 0.01	5.619 ± 0.01
	MET	1510 ± 16.22*#	0.002 ± 0.01	5.340 ± 0.01
	25	996.0 ± 17.00*#μ	0.001 ± 0.01	5.512 ± 0.01
	50	878.0 ± 16.10*#μ	0.002 ± 0.03	5.952 ± 0.01
	100	1218 ± 17.11*#	0.003 ± 0.01	5.422 ± 0.01
Liver	VEH	2037 ± 20.00	0.005 ± 0.01	11.180 ± 0.01
	STZ	2886 ± 22.10*	0.001 ± 0.01*#	10.890 ± 0.01
	MET	1900 ± 20.82#	0.003 ± 0.01*#	11.180 ± 0.02
	25	819 ± 20.21*#μ	0.001 ± 0.01*μ	11.178 ± 0.02
	50	1499 ± 23.00*#μ	0.003 ± 0.01*#	11.190 ± 0.02
	100	1988 ± 22.11#	0.004 ± 0.01*#	11.098 ± 0.02
Kidney	VEH	1557 ± 25.00	0.020 ± 0.01	20.135 ± 0.01
	STZ	1020 ± 25.00*	0.001 ± 0.01*	20.114 ± 0.01
	MET	1148 ± 20.49*#	0.013 ± 0.01*#	20.058 ± 0.02
	25	800 ± 24.31*#μ	0.019 ± 0.01*#μ	20.150 ± 0.02
	50	982.80 ± 22.32*#μ	0.031 ± 0.01*#μ	20.120 ± 0.02
	100	1242 ± 23.00*#	0.016 ± 0.01#	20.098 ± 0.02

Table represents Mean ±S.E.M, *$p < 0.05$ significantly different from vehicle, #$p < 0.05$ significantly different from STZ, μ$p < 0.05$ significantly different from MET, VEH: Vehicle, STZ: Streptozocin, MET: Metformin, number of mice per treatment group = 5.

Histological and histomorphometric examination of the pancreas, liver and kidneys of rats in the diabetic control group was consistent with organ injury which was similar to those reported in previous studies (Onaolapo & Onaolapo, 2012; Onaolapo et al., 2012). Administration of metformin or increasing doses of *J. regia* L. reversed (to varying degrees) the organ injury. Biochemical parameters of liver (ALT and AST) and kidney (urea and creatinine) injury were also elevated in the diabetic control, with reversal of this trend with treatment (Metformin or *J. regia* L.). Other studies have demonstrated the protective effects of *J. regia* L. to liver (Shimoda et al., 2009) and pancreas (Jelodar et al., 2007); these effects can be attributed to the presence of flavonoids like rutin which have antioxidant properties (Nour et al., 2012) and help reduce oxidative damage or prevent the formation of glycation products. There are also suggestions that the presence of poly- phenolics constituents like, tellimagrandins I and II, rugosin C and casuarictin have been linked to the hepatoprotective activity of *J. regia* L. (Taha and Al-wadaan, 2011).

Oxidative stress has been considered an important factor in the pathophysiology of diabetes mellitus, reactive oxygen species have been reported to alter metabolism and beta cells function (Giugliano et al., 1996; Simmons, 2006). Studies have shown evidence of derangement in the oxidant-antioxidant balance due to hyperglycaemia-induced decrease in the activity of free radical scavenging enzymes and increase free radicals formation (Giacco and Brownlee, 2010; Fiorentino et al., 2013). In this study diabetes mellitus was associated with decrease in activities of SOD, CAT and GPX which is consistent with the literature data (Usman et al., 2016; Milani et al., 2005) and indicative of increased oxidative stress. Twice daily administration of *J. regia* L. however, resulted in a dose related reversal of oxidative stress. These data are in full agreement with the previously reported antioxidant potential of different extracts of the *juglans regia* L. (Amaral et al., 2003; Pereira et al., 2007; Cheniany et al., 2013). The ability of *J. regia* L. leaf powder to reverse oxidative stress may also be related to its effect on glycaemic control, since

uncontrolled hyperglycaemia also increases oxidative stress.

CONCLUSION

J. regia L. extracts have been analyzed by HPLC-DAD and several active phytochemicals have been found. The *in-vitro* studies on extracts revealed a strong inhibition activity toward a-glucosidase and in less extent to a-amylase, two key enzymes involved in the digestion of starch into glucose. Thus, the large presence of rutin and other polyphenols have been related to the antidiabetic, anti- hyperglycemic and antioxidant effect observed in the following *in- vivo* tests. The *in vivo* experiments, clearly shows that *J. regia* L. leaf powder, administered daily, as food supplement to STZ induced diabetic-rats was able to revert hyperglycemia, hypercholesterolemia and multi-organs failure commonly related to diabetes mellitus.

These evidences, taking together, are in full agreement with the previously reported studies on the antidiabetic potential of the *J. regia* L. extracts (Kamyab et al., 2010; Hosseini et al., 2014a; Asgary et al., 2008; Gholamreza and Hossein, 2008) and even more they provide scientific basis on the nutraceutical potential of the *J. regia* L. leaf in the management of diabetes mellitus. However further studies are needed to establish a safe protocol for human clinical trials.

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