Mohammed et al. The Journal of Basic and Applied Zoology (2018) 79:3
DOI 10.1186/s41936-018-0024-8

The Journal of Basic and Applied Zoology

RESEARCH Open Access

Influences of olive leaf extract in the kidney of diabetic pregnant mice and their offspring

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Abstract

Background: Phytotherapeutic treatment is used in the treatment of diabetes and its complications. The current study aims to evaluate the significant effect of supplementation of *aqueous* olive leaf extract (OLE) (*Olea europaea*) in the kidney of diabetic pregnant mice and their fetuses. Forty pregnant mice were divided into four groups contained 10 mice each after mating. The first group was the control (G1). The second group (GII) was intraperitoneally injected by a single dose of (240 mg/kg body weight) of streptozotocin (STZ). The third group (GIII) was administrated with a daily oral dose of extract of olive leaf extract (100 mg/kg) from days 1 to 18 of gestation. The fourth group (GIV) was injected intraperitoneally by a single dose of (240 mg/kg body weight) of STZ and post-treatment with oral dose of extract of olive leaves from days 1 to day 18 of gestation.

Results: Both mothers and their fetuses of STZ-induced diabetic group showed a decrease in weight compared to control and diabetic group supplemented OLE extract. According to the biochemical and histopathological observations, the STZ-induced diabetic group showed a significant ($P < 0.05$) increase in serum urea and creatinine levels parallel with detectable histopathological changes in kidney tissues of pregnant mice and their fetuses. Moreover, there was a significant decrease in serum urea and creatinine ($P < 0.05$) of diabetic mother group under treatment with OLE as compared to diabetic mice. Also, histological findings showed improved renal architecture as reflected by reduced glomerular and tubular necrosis in pregnant mice and their fetuses when compared with control group. Also, there was an increase in the anti-angiotensin II (Ang II) immunoreactivity in renal tubules, intra-glomerular, and interstitial cells in the kidney tissue of STZ-induced diabetic group which was markedly improved by treatment with OLE.

Conclusion: Oral administration of aqueous olive leaf extract to diabetic pregnant mice and their fetuses has ameliorative effect on weight gain as well as kidney functions and has the ability to minimize the damage in the kidney and placental tissue caused by hyperglycemia, and this effect may be attributed to its antioxidant activity.

Keywords: Diabetes mellitus, Kidney function, Olive (*Olea europaea*)

Background

Diabetes mellitus is a disease resulting from a variable interaction of genetic and environmental factors. It is caused by relative lack of insulin and/or malfunction of insulin action (Rasineni, Bellamkonda, Singareddy, & Desireddy, 2010). It is characterized by hyperglycemia, disturbances in carbohydrate, protein, and fat metabolisms, in addition to long-term complications affecting the eyes, kidneys, nerves, heart, and blood vessels (Gupta, Bajpai, Johri, & Saxena, 2008). However, the kidney disease is a known complication of diabetes where the diabetic kidney disease occurs in 20–40% of patients with diabetes and is the leading cause of end-stage renal disease (Rossing, Rossing, Jacobsen, & Parving, 1995). The end stages of kidney disease are nephropathy, renal failure, peripheral neuropathy with risk of foot ulcers, amputations, and damaging the nerves of the bladder (Genuth, Alberti, Bennett, Buse, Defronzo, et al., 2003). In addition, kidney failure often leads to death in diabetes (Prakash, Lodha, Singh, Vohra, & Raja, 2007).

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Gestational diabetes mellitus (GDM) is a syndrome often manifests in mid to late pregnancy and characterized by a physiological state of magnified insulin resistance. Furthermore, pregnant women with GDM have many features of the metabolic syndrome (Clark, Qiu, Amerman, Porter, Fineberg, et al., 1997), and about 20 to 50% of women with gestational diabetes have a high risk of developing type 2 diabetes later in life (Kenneth, 2006). It is temporary and fully treatable but, if untreated, may leads to reproductive abnormalities in the offspring, altered fetal growth, congenital abnormalities, central nervous system and skeletal malformations, and spontaneous abortion (Yang, Cummings, O'Connell, & Jangaard, 2006). Castori 2013 reported that diabetes during pregnancy disorganized placental and fetal development. In addition, diabetes mellitus is one of the factors that induced congenital abnormalities of the kidney and urinary tract, during gestation in both human and experimental animal models (Simeoni, Ligi, Buffat, & Boubred, 2011). When normal kidney formation is subjected in both humans and experimental animal models to a high glucose level in utero, abnormal kidney with a low total nephron number may be appeared, either in isolation or as part of multiple malformation syndromes. These changes depend on the degree of maternal hyperglycemia (Tran, Chen, Chenier, Chan, Quaggin, et al., 2008). Also, the maternal hyperglycemia accompanied with neonate future risks such as insulin resistance, type 2 diabetes mellitus, obesity, and metabolic syndrome (Calkins & Devaskar, 2011).

Phytotherapeutic treatment has been used for the treatment of many diseases for a long time, including olive tree (*Olea europaea*), which is highly distributed in the Mediterranean zone (Eidi, Eidi, & Darzi, 2009). The olive tree has antioxidant properties in its oil, fruits, and leaves, which are due to the presence of some antioxidant and phenolic components (Omar, 2010). Furthermore, olive leaves are considered to be the source of a very efficient antioxidants, including oleuropein, 3,4-dihydroxy-phenylethanol, verbascoside, oleuropeinaglycone, and tyrosol (Jemai, Bouaziz, Fki, El Feki, & Sayadi, 2008), and contain the greatest concentration of olive plant polyphenols more than the fruit or fruit oil (de Bock, Derraik, Brennan, Biggs, Morgan, et al., 2013). The olive leaves have a rich history of medicinal uses where a number of biological activities of olive leaves like the antioxidant, hypoglycemic, anti-hypertensive, antimicrobial, and antiatherosclerotic have been reported in various studies (Wang, Geng, Jiang, Gong, Liu, et al., 2008). Recently, the olive leaves have been used in traditional medicine to treat diabetes (Hamden, Allouche, Jouadi, El-Fazaa, Gharbi, et al., 2010). Also, the administration of oleuropein may be helpful in the prevention of diabetic complications associated with oxidative stress (Al-Attar & Alsalmi, 2017; Jemai & Sayadi, 2015).

From the previous literatures, it is clear that the strong combination between diabetes and renal dysfunctions and the diabetes impair the placental function and structure. So, the present work was designed to evaluate the antidiabetic effect of olive leaf extract in the kidney of diabetic pregnant mice and their fetuses.

Methods

Preparation of aqueous olive leaf extract and polyphenol content determination

The olive leaves were purchased from a commercial market. The leaves were thoroughly washed and dried at room temperature. The dried olive leaves (50 g) were powdered and added to 2 L of hot water in a flask. After 6 h, the mixture was slowly boiled for 1 h and then cooled at room temperature. The solution of olive leaves was filtered, and finally, the filtrates were evaporated in an oven at 40 °C to produce dried residues (active principles). Furthermore, these extracts were prepared every 2 weeks and stored in a refrigerator for experimentation (Al-Attar & Abu Zeid, 2013). The polyphenol compounds of olive leaf extract were established by using chromatographic analysis using GC-MS in center lab of Ain Shams University, Faculty of science.

Experimental animals

The present experimental study was carried out on CD-1 mice, with an average body weight of 25–30 g. Virgin females and males were housed separately. All animal experimental protocols were approved by Committee of Scientific Ethics at University of Hail and were carried out in accordance with its guidelines for animal use.

The pregnant experimental animals were divided into four groups: control (GI), diabetic group (GII), olive group (GIII), and diabetic + olive group (GIV).

The hyperglycemia were induced by intraperitoneal injection of a single dose of streptozotocin (STZ, 240 mg/kg body weight, from Sigma chemical Co., St. Louis, Mo) diluted in phosphate-buffered saline pH 7.2 (Oyama, Sugimura, Murase, Uchida, Hayasaka, et al., 2009). After 7 days of injection, the plasma glucose concentration was measured from the tail vein. The mice are defined as diabetic if the blood glucose value exceeds 250 mg/dl. The diabetic female was mated with non-diabetic male overnight. If the vaginal plug appeared in the morning, this day is defined as 0 day of gestation (GD0) and the pregnant females are divided into the diabetic group (GII) and diabetic + olive group (GIV). The pregnant mice of the olive group (GIII) and diabetic + olive group (GIV) were given daily oral dose of 1 ml/100 g of b. wt. of olive leaf

extract equivalent to therapeutic human dose (100 mg/kg) from day 1 to 18 of gestation.

Fetus examination

At day 18 of gestation, the uteri were removed and dissected in normal saline solution and the fetuses were taken out for morphological and histological studies. Living fetuses were distinguished from dead ones by their spontaneous movement. The average body weights of fetuses were recorded. The fetuses were carefully examined externally for any morphological malformations in the limbs, digits, head, eyes, ears, jaws, and palate using a binocular microscope.

Biochemical analysis

At the end of the experimental period, the animals were anesthetized with chloroform, the blood was drawn from the heart and collected in vacuum tube clot activator after that, and the blood samples were centrifuged at 3500 rpm for 10 min in a centrifuge to separate serum samples. Urea and creatinine in the serum were measured using commercial kits from Reflotron and Liquicolor Analyticals. All assessment assays and kits were performed in accordance with the manufacturers' instructions and protocols.

Histological examination

The placenta, fetus's kidney, and kidney of mothers for both control and experimental groups were fixed in 10% formalin for 48 h, after which they were kept in 70% alcohol. The specimens were dehydrated in ascending series of alcohol, 1 h each; cleared in terpineol for 3 days; and embedded in three changes of pure paraffin wax, 1 h each. Serial longitudinal and transverse sections, 5 μm thick, were cut and mounted on clean glass slides. The paraffin sections were stained with Harris' hematoxylin and eosin, cleared in xylol, and mounted in neutral Canada balsam (Bancroft & Gamble, 2008). Sections of placenta and kidney of the different groups were carefully examined, and photomicrographs were made as requested.

Immunohistochemical examination

Five-micron sections of kidneys fixed with Bouin's fixative were immunostained using anti-angiotensin II (Ang II) primary antibody (Labvision, Neomarkers, USA) for 90 min. This was followed by the secondary antibody application using the immunoperoxidase technique (Vectastain ABC kit; Vector Laboratories, Burlingame, CA). Sections were counterstained with hematoxylin.

Statistical analysis

The values were articulated as mean ± SEM. Moreover, the statistical analyses were performed by using Student's t test, and P values < 0.05 were measured a significant values.

Results

Polyphenols in olive leaf extract

The olive leaves are one of main sources of olive polyphenols and contain the highest amount of antioxidant among the all parts of olive tree. There are five groups of phenolic compounds present in olive leaves: oleuropein, flavones, rutin, and substituted phenols (tyrosol, hydroxytyrosol, vanillin, vanillic acid, and caffeic acid). The identification of OLE major polyphenol compounds were achieved by GC-MS showed that the oleuropein (14.25%), mannitol (3.7%), rutin (1.22%), hydroxytyrosol (3.3%), and caffeic acid (0.013%) are among the phenolic derivatives present in the olive leaf extract.

Morphology results

Pregnant mothers

At the present experiment, the average maternal body weights were recorded for control and experimental groups at the beginning of experiment and at the 18th day of pregnancy (Table 1 and Fig. 1). The recorded data indicated that pregnant females of both control and experimental groups showed a steady increase in body weight during the whole gestation period. The mean body weights of STZ group (GII) were significantly less than those of control group (GI) (Table 1 and Fig. 1). At the 18th day of pregnancy, the lowest mean body weight was observed in STZ group (GII) (42.6 ± 8.66) while the highest mean body weight was noticed in control group (GI) (63.55 ± 13.65). No mortalities were recorded either in control or in experimental groups. Moreover, no abortions were recorded among mothers of all groups.

The fetus examination

The mortality rate of 18-day-old fetuses was relatively high (0.4 ± 0.24) in group II as compared with that of both control and other groups (Table 2). Significant increase in the resorption sites was observed also in mothers of group II (1.4 ± 1.1) as compared with those of both control group and the other experimental groups (Table 2).

The fetal size at the 18th day of gestation in STZ group (GII) showed significant decreased compared to all groups (Table 3). However, the treatment of diabetic pregnant mice with the olive leaf extract had a significant ameliorative effect on the body weight and length of fetuses. The mean placental weight in the diabetic group (GII) was (0.058 ± 0.014 g) which was significantly less than control group (GI) (0.224 ± 0.01). However, a slightly significant increase in the weight of placenta was seen in pregnant diabetic group treated with olive leaf extract (GIV) (0.132 ± 0.03) compared with that of GII. The fetal/placental ratio was calculated for all groups and showed decrease value in GII compared with other groups (Figs. 2 and 3).

Table 1 Effects of olive leaf extract and/or STZ administration on the average increase in maternal body weight (g) during pregnancy

	0 day of gestation	18th day of gestation			
		GI	GII	GIII	GIV
Mean body weight (g) Mean ± SD	31.35 ± 1.82	63.55 ± 13.65	42.6 ± 8.66*	55.9 ± 2.86 ns	49.4 ± 4.36 ns

Data are presented as mean (±SD)
*Significantly different from the control group ($P < 0.05$)

Biochemical results

Kidney function was detected by measuring the urea and creatinine levels in serum of pregnant mice in control and experimental groups. A significant increase ($P < 0.05$) was recorded in STZ-treated pregnant mice (GII), and the percentage increase in serum urea and creatinine levels were calculated 87.5 and 58.95%, respectively, when compared to the normal control level (GI) (Table 4). There is no significant ($P > 0.05$) change in the levels of serum urea and creatinine by the administration of olive leaf extract to pregnant mice (GIII) as compared to the normal control level. In post-treatment of STZ-treated group with OLE, serum urea level showed a significant reduction ($P < 0.05$) as compared to diabetic group (GII) and restored to nearly normal when compared to mice in the control group with percentage change 12.3% (Table 4 and Fig. 4a), while the serum creatinine level showed non-significant reduction ($P > 0.05$) as compared to diabetic group (GII) and did not restore to the same level as that of the control group with percentage change 28.6% (Table 4 and Fig. 4b).

Histological results

The placenta

The placenta was divided into labyrinthine zone and decidua basalis zone. The labyrinth forms the major portion of the placental disk; it has very thin fetal capillaries supported by connective tissue and surrounded by trophoblast. The labyrinth constitutes the major site of maternal/fetal exchange. The basal zone is composed of three types of differentiated cells: (1) spongiotrophoblasts, (2) trophoblastic giant cells, and (3) glycogen cells. In the control and OLE groups, there were normal giant, average glycogen cells, and blood vessels (Fig. 5a–c and Fig. 5h, i). However, the STZ group showed decreased area of decidua basalis, excess glycogen cells (Fig. 5d), and labyrinth zone which showed markedly congested blood vessels (Fig. 5e). Some of trophoblastic cells showed pyknotic nuclei and scattered apoptotic cells (Fig. 5f). In the labyrinth area, an irregular dilatation of maternal sinusoids was observed with markedly congested fetal blood vessels. On the other hand, mild degenerative changes were observed in group IV, mild glycogen cells, and markedly congested labyrinth zone (Fig. 5k–l).

Histology of mother's kidney

The histological structure of the kidney of control pregnant mothers (GI) showed the normal renal corpuscles with glomerulus formed of lobulated tuft of capillaries which separated from the capsule by Bowman's space, normal proximal, and distal tubules (Fig. 6a). The renal tissues of STZ-treated group (GII) (Fig. 6b) showed shrinked degenerated glomeruli and increased in the capsular space around the glomeruli due to diminishing of the glomerular size. Also, the

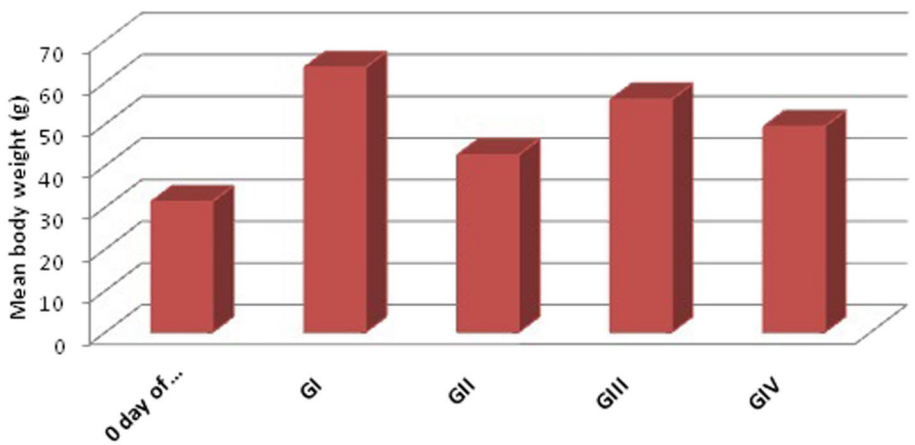

Fig. 1 Histogram showing the maternal body weight for control and treated groups

Table 2 Effects of olive leaf extract and/or STZ administration on the fetal length, fetal weight, placental weight, and the ratio of placental/fetus weight at gestation day 18

Groups	Fetus length (cm)	Fetus weight (g)	Placental weight (g)	Ratio of mean (placental w./fetus w.)%100
GI	2.35 ± 0.111	1.19 ± 0.107	0.224 ± 0.01	18.82
GII	1.44 ± 0.28*	0.454 ± 0.22*	0.058 ± 0.014*	12.77
GIII	2.12 ± 0.22	1.08 ± 0.081	0.208 ± 0.01	18.51
GVI	1.7 ± 0.207*	0.79 ± 0.07*	0.132 ± 0.03*	14.68

Data are presented as mean (±SD)
*Significantly different from the control group ($P < 0.05$)

dilatation in the kidney blood vessels with congestion of hemorrhagic erythrocytes was commonly appeared in (Fig. 6b). In severe degenerated renal tubules, the tubular cells appeared highly vacuolated with pyknotic nuclei (Fig. 6c). Oral administration of olive leaf extract (OLE) showed normal structure of most renal corpuscles and normal proximal and distal tubules (Fig. 6d). The olive leaf extract treatment for diabetic mothers (GIV) showed somewhat variable degrees of amelioration for many histopathological changes induced in renal tissues, where the glomeruli revealed nearly normal appearance (Fig. 6e, f).

Histology of fetuses' kidney

The kidney of fetus is a small bean-shaped differentiated into two regions: an outer cortex and an inner medulla. The renal tissue of 18-day-old control fetuses consists of the uriniferous tubules and stromal tissue. The uriniferous tubule is composed of two principal portions: the nephron and the collecting tubule. The nephron consists of the renal or Malpighian corpuscle, the proximal convoluted tubule, the descending and the ascending limbs of Henle's loop, and the distal convoluted tubule (Fig. 7a). The kidney of STZ-treated group (GII) exhibited some histopathological features including venous congestion, cloudy swelling, hydropic degeneration, and necrosis of the epithelial cells lining numerous tubules (Fig. 7b–d). Some cells showed advanced degenerative features, and their nuclei were pyknotized (Fig. 7d). In addition, some cells were frequently disrupted and lost their nuclei (Fig. 7c). Moreover, numerous hyaline casts were accumulated in the lumens of tubules. Some glomerulus tufts were congested, and their capillaries were packed with blood corpuscles. In contrast, the kidneys of fetus from group of diabetic pregnant mothers treated with the olive leaf extract (GIV) showed signs of tissue repair (Fig. 7f), where some epithelial cells lining some tubules appeared vacuolated and contained either pyknotized or karyolysed nuclei (Fig. 7g). But the lining cells of other tubules displayed normal aspects (Fig. 7h). The kidneys from fetus of mothers treated only with oral administration of olive leaf extract (GIII) revealed somewhat normal tissue characteristics (Fig. 7d).

Immunohistochemistry results

The angiotensin II expression in the kidney tissues was evaluated by immunohistochemistry, and the control sections revealed weakly angiotensin II (Ang II) immunostaining indicated by faint brown color localized in tubular epithelial cells, with no staining observed in the glomeruli (Fig. 8a). However, in STZ-treated group, intense Ang II immunostaining was observed as dark brown color in the glomerular cells, tubular cells, and interstitial cells (Fig. 8b). The administration of olive leaf extract only in GIII showed normal content of Ang II with weakly immune reactivity in the kidney tissue (Fig. 8c). The treatment of diabetic pregnant mice with OLE decreased the Ang II expression in renal tissue to nearly normal content as compared to those observed in the kidney tissues of STZ group alone indicating the ameliorative effects of olive leaf extract (Fig. 8d).

Discussion

In the present study, we estimated the antidiabetic role of olive leaf extract (OLE) against the nephrotoxicity induced by diabetes in pregnant mice and their fetuses.

Table 3 Effects of olive leaf extract and/or STZ administration on the reproductive outcome

Groups	No. of fetuses (mean ± SD)	Alive fetuses (mean ± SD)	Dead fetuses (mean ± SD)	Resorption sits (mean ± SD)
GI	13.2 ± 2.9	13.2 ± 2.9	0.0 ± 0.0	0.0 ± 0.0
GII	9.2 ± 1.6*	8.8 ± 1.9	0.4 ± 0.24	1.4 ± 1.1*
GIII	12.2 ± 2.2	12.2 ± 2.2	0.0 ± 0.0	0.2 ± 0.4
GIV	11.2 ± 1.6	11 ± 1.8	0.2 ± 0.4	0.4 ± 0.5

Data are presented as mean (±SD)
*Significantly different from the control group ($P < 0.05$)

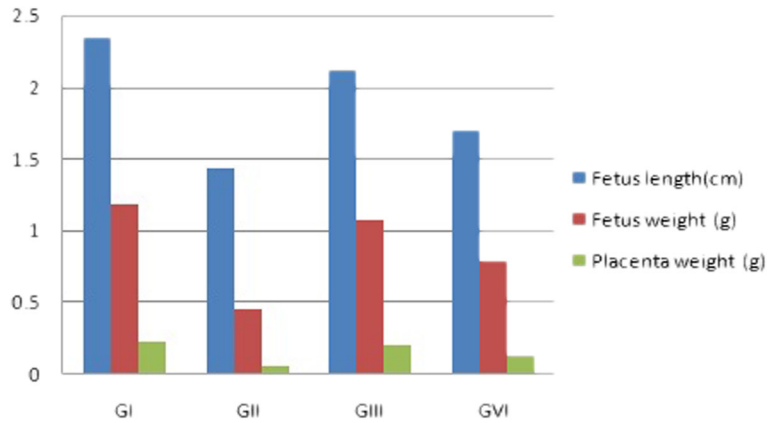

Fig. 2 Histogram showing the fetus weight, fetus length, and placental weight at day 18 of gestation for all experimental groups

Kiss et al. (2009) stated that DM in pregnancy can be divided into gestational diabetes and clinical diabetes. In the present study, we induced clinical diabetes in animal model (mice) with STZ administration (240 mg/kg) during their adult life before pregnancy. This model mimics women with uncontrolled clinical diabetes during pregnancy. In the present investigation, the STZ-induced diabetic pregnant mice showed a significant decrease in maternal body weight gain after 18 days of gestation compared with control group. Similar observations were detected in many experimental studies (Al-Attar & Alsalmi, 2017; Jayaprasad, Sharavanan, & Sivaraj, 2015; Zhang, Feng, Chen, Li, & Shen, 2016; Rudge, Damasceno, Volpato, Almeida, Calderon, et al., 2007; Salahuddin & Jalalpure, 2010). The observed reduced body weights may be due to the metabolic alterations caused by hyperglycemia (Damasceno, Volpato, Calderon, Aguilar, & Rudge, 2004). However, the watery OLE treatment of diabetic group inhibited maternal body weight loss when compared to the STZ-treated group but could not maintain at the same level as that of the control group. This may be due to the improvement of serum glucose levels and metabolism; these results are compatible with those of Al-Azzawie and Alhamdani (2006).

Also, the maternal hyperglycemia has an impact on fetal body size, where the reduced maternal body weight of diabetic mothers could be the reason for reduced body mass of the fetuses in this group compared with the control fetuses. Maternal diabetes constitutes an unfavorable environment for fetal-placental and embryonic development (Rudge, Piculo, Marini, Damasceno, Calderon, et al., 2013). The fetal/placental ratio is an important indicator of fetus later life. In the present study, a decrease in the fetal/placental ratio was observed in diabetic group compared with the control and OLE groups. The placental weight reflects the placental

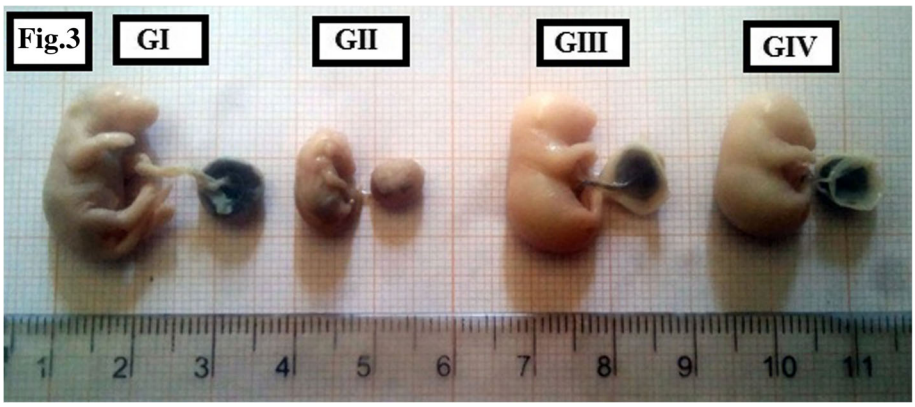

Fig. 3 Photomicrograph of 18-day-old fetuses of different groups showing the effect of diabetes and/or olive leaves extract on fetus/placental growth

Table 4 Effects of olive leaf extract (OLE) and/or STZ administration on some biochemical parameters on pregnant mice serum

Parameters		GI	GII	GII	GIV
Urea (mg/dl)	Mean ± SD %change to con.	39.2 ± 2.4	73.5 ± 11.7* 87.5%	41.8 ± 0.288 06.6%	44.06 ± 0.66 12.3. %
Creatinine (mg/dl)	Mean ± SD %change to con.	0.346 ± 0.045	0.550 ± 0.028* 58.95%	0.4 ± 0.07 15.6%	0.455 ± 0.06 28.6%

Data are presented as mean (±SD)
%change to con % change to control level
*Significantly different from the control group ($P < 0.05$)

structure and function which, in turn, has a relation to the fetus weight (Sanin, López, Olivares, Terrazas, Silva, et al., 2001).

However, gestational diabetes cause greater insulin resistance (Setji, Brown, & Feinglos, 2005), which leads to increase levels of glucose transported across the placenta to the fetus and cause fetal hyperglycemia (Lampl & Jeanty, 2004). The reduced body weight fetuses in STZ-induced diabetic group may be due to fetal hypoinsulinemia which causes the growth restriction in fetuses (Aerts & Van Assche, 2006). The altered maternal-fetal metabolic fuel relationship resulting from diabetes in pregnancy modulates fetal growth where the mechanisms are a brutal destruction of the mother and fetus' pancreas and intrauterine growth restriction is due to lack of insulin (Schaffer & Mozaffari, 1999). The diabetes in pregnant rats lead to variable changes in fetus body weight ranging from developing microsomia (Eid, Shoman, & Abu Elnaga, 2014), to no change in the fetal or newborn body weight (Gerber, Holemans, Brien-Coker, Mallet, van Breed, et al., 2000), or to macrosomia of fetuses (Merzouk, Madani, Sari, Prost, Bouchenak, et al., 2000). Furthermore, severely diabetic mothers are insulin deficient and hyperglycemic with low body weight and give birth to microsomic and malformed fetuses (Vambergue & Fajardy, 2011). There is evidence that the intrauterine hyperglycemia stimulates the fetal endocrine pancreas to hyperinsulinemia and accelerated anabolism, resulting in fetal and neonatal macrosomia. In contrast, our results and other results also did not obtain macrosomic fetuses when studying the effect of hyperglycemia on offspring. Kervran et al. (1978) concluded that the differences between the human's clinical findings and the experimental results using rats are due to the short pregnancy time in the rat and differences in the percentages of adipose tissue in rat fetuses (1%) and human offspring (16%) and the greater weight gain in the human species. The present study indicates the ameliorative effect of OLE treatment on body weight gain of fetuses of STZ-diabetic mothers; this is in agreement with those of Eid, Shoman, and Abu Elnaga (2014). Saad et al. (2016) concluded that maternal diabetes increase the oxidative stress in placenta, which activates apoptosis, as well as disorganized the endothelial and vascular placental functions. The present study showed a statistically significant increase in placental weight after treatment of diabetic pregnant mice by OLE (GIV) which leads to an increase in placenta/fetal ratio. Moreover, Omar (2010) reported that olive leaf extract has significant role in controlling blood glucose level and reducing high blood pressure in diabetic animals and may improve the development processes of fetuses inside the uteri. According to El-Nabarawy (2014), the

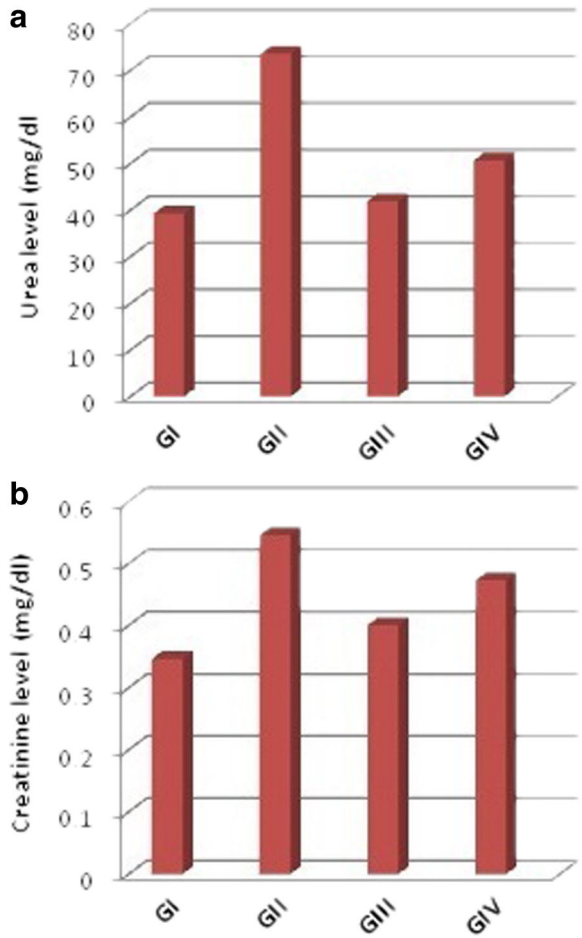

Fig. 4 Effects of olive leaf extract (OLE) and/or STZ administration on urea level (**a**) and creatinine level (**b**) in pregnant mice serum

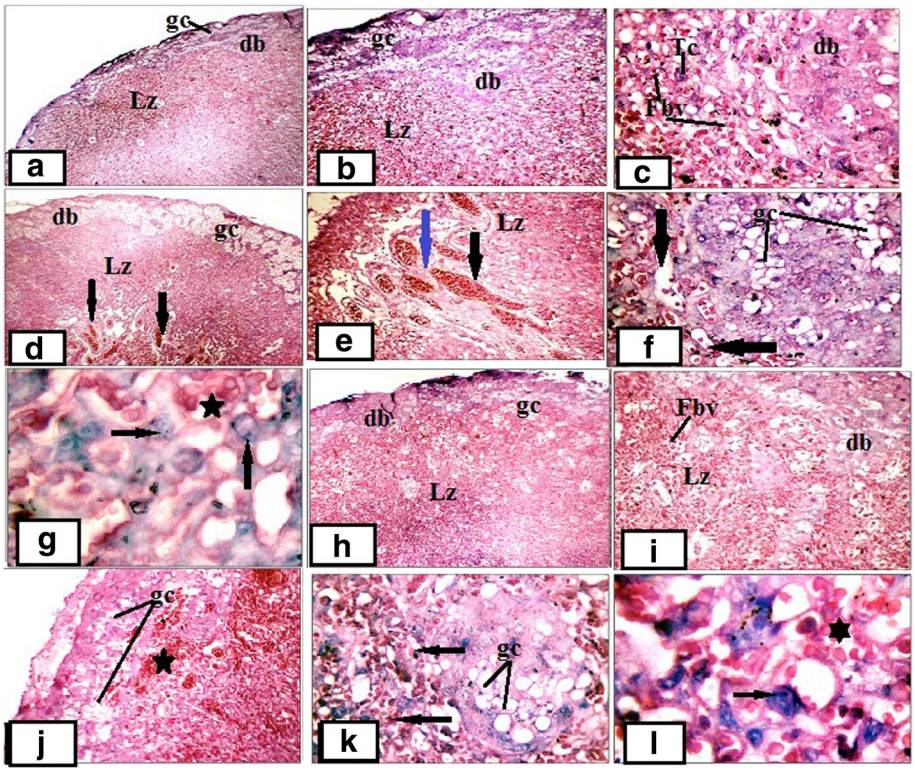

Fig. 5 Photomicrographs from placenta collected from mice of control and treated groups stained with H&E stain. The control placenta showing average decidua basalis (db), labyrinth zone (Lz), and glycogen cells (gc). The area of labyrinth zone showing fetal blood vessels (Fbv) and trophoblastic cells (Tc) (**a**–**c**) (H&E ×4, ×10, and ×40 respectively). **d** G II showing decreased area of decidua basalis (db), excess glycogen cells (gc) (H&E ×4) and labyrinth zone showing markedly congested blood vessels (arrows) with excess fibrin deposition (blue arrow) (**e**) (H&E ×10). **f** G II showing excess glycogen cells (gc) and dilated fetal blood vessels (arrows) (H&E ×40). The high power showing markedly congested fetal blood vessels (star) and some of trophoblastic cells showing pyknotic nuclei (arrows) (**g**) (H&E ×100). The placenta of GIII showing average decidua basalis (db), labyrinth zone (Lz), and glycogen cells (gc). The area of labyrinth zone showing fetal blood vessels (Fbv) (**h**, **i**) (H&E ×4 and ×10 respectively). The placenta of GIV (**j**) showing decidua basalis (db) with mild glycogen cells (gc) and markedly congested labyrinth zone (star) (H&E ×10). The area of labyrinth zone with some apoptotic cells (black arrows) (**k**) (H&E ×40), the higher power showing mild congested fetal blood vessels in labyrinth zone (star), with trophoblastic giant cell (arrow) (**l**) (H&E ×100)

olive leaves water extract improved the oxidative and DNA damage in both embryos and placentas of the diabetic mothers.

Removal of metabolic wastes such as urea and creatinine in the kidneys maintains optimum chemical composition of body fluids where serum levels of urea and creatinine are useful tools in diagnosis as they pick any disturbances to the renal system. However, the obtained results in this study revealed that STZ induced significant increases in the level of urea and creatinine which indicate kidney dysfunction in diabetic group. The present elevations of these parameters are generally in accordance with the findings of several studies in experimental diabetes animals (Salahuddin, Jalalpure, & Gadge, 2010). However, the increased urea and creatinine production in diabetic animals may be due to accelerated catabolism of both liver and plasma proteins and the body failure to excrete the proteins metabolic products (Hassan, El-Agmy, Gaur, Fernando, Raj, et al., 2009). On the other hand, the treatment of pregnant mice subjected to STZ exposure with OLE showed reduction in the serum urea and creatinine levels compared to diabetic group. Our results are in agreement with those of Eid et al. (2014) who reported that the reduction of serum urea and creatinine levels may be due to regeneration of kidney glomeruli that improved the kidney filtration process. Thus, this nephroprotective function could be mediated via antioxidant and/or free radical scavenging activities as they possess high concentration of flavonoids (Helal, El-Wahab, El Refaey, & Mohammad, 2013). In addition, the oral administration of olive fruit and leaf extracts decreased the levels of serum creatinine, urea, and uric acid in STZ diabetic male rats (Laaboudi, Ghanam, Ghoumari, Sounni, Merzouki, et al., 2016).

Furthermore, the obvious biochemical results may also be due to the damage occurred in the kidney which is confirmed by the marked histopathological alternations

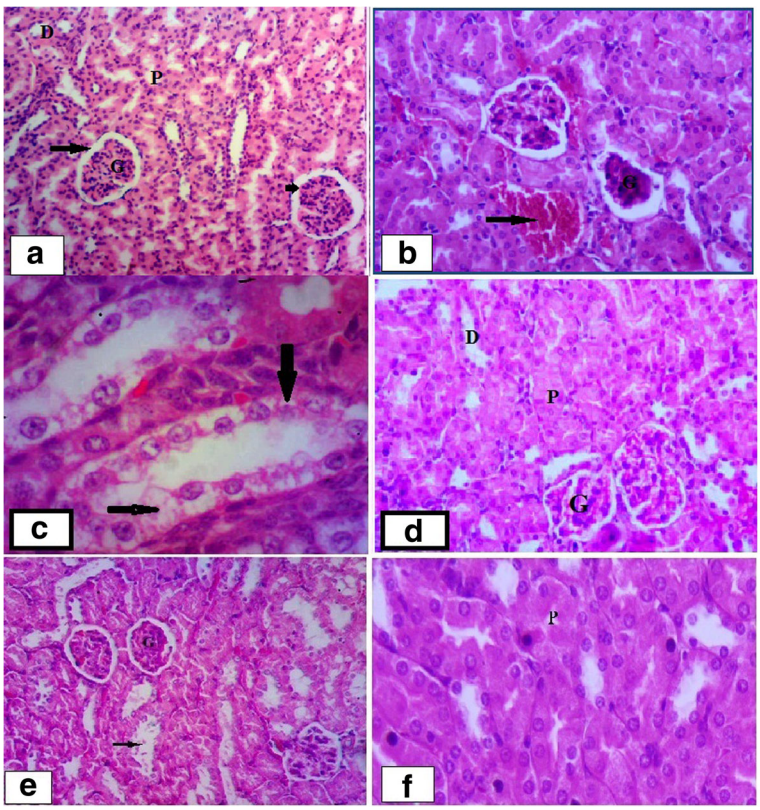

Fig. 6 Photomicrographs from the kidney tissues collected from mice of control and treated groups stained with H&E stain. The kidney of control group (GI) showing kidney cortex with Malpighian corpuscle where the parietal layer of Bowman's capsule (arrow) and the glomerulus (G) is formed of lobulated tuft of capillaries being separated from the capsule by Bowman's space (short arrow). Normal proximal (P) and distal tubules (D) are also seen (**a**) (×10). The renal tissue of diabetic group (GII) (**b**, **c**) showing highly shrinked glomeruli (G) with wide renal space and dilatation in the blood vessels with erythrocytic congestion (arrow) (**b**) (×10). **c** High magnification (×40) showed renal tubules with necrotic nuclei (arrows). Section of the kidney of (GIII) showing normal architecture (**d**). **e**, **f** GIV showing obvious return to nearly normal renal appearance with nearly normal glomeruli (G) and renal tubules (×10)

in kidney of diabetic pregnant mice observed during the present study. However, the histopathological examination of the kidney sections in STZ-treated group revealed inflammatory cells, degenerated and shrinked glomeruli, severe degenerated renal tubules with vacuolated tubular cells and pyknotic nuclei, mesangial expansion, and glomerular hypertrophy. Our present results were in accordance with the results of (Eid et al., 2014; Hebi, Farid, Ajebli, & Eddouks, 2017; Sugumar, Doss, & Maddisetty, 2016). The degeneration of renal tubules detected in present study might be due to oxidative stress caused by hyperglycemia which induces oxidative stress by alteration of the balance between reactive oxygen species (ROS) production and antioxidant defense (Baynes, 1991). Furthermore, the free radicals are involved in the pathogenesis of diabetes and the development of diabetic complications (Bindhumol, Chitra, & Mathur, 2003). Nevertheless, the treatment of STZ-treated mice with OLE showed somewhat variable degrees of amelioration of the histopathological changes in the kidney tissues; this result is in agreement with those of Tavafi, Ahmadvand, and Toolabi (2012) who reported that olive leaf extracts have powerful antioxidant characteristic and are able to remove ROS and reinforce antioxidant system of the kidney. In the same perspective, histological examination of fetal kidney tissue in STZ-induced diabetic mother mice showed some histopathological features including venous congestion, cloudy swelling, hydropic degeneration, and necrosis of the epithelial cells lining numerous tubules; this finding is in agreement with those of Eid et al. (2014). In addition, Zhang et al. (2007) suggested that a high-glucose milieu in uteri retards renal morphogenesis by inducing a significantly higher number of apoptotic podocytes in the developing glomeruli. The usage of olive leaf extract in the present study showed signs of tissue repair with little degenerative cells in some renal tubules of the kidney in fetuses of diabetic mothers; this is in accordance with Eid et al. (2014). Also, Al-Attar and Alsalmi (2017) and Tavafi

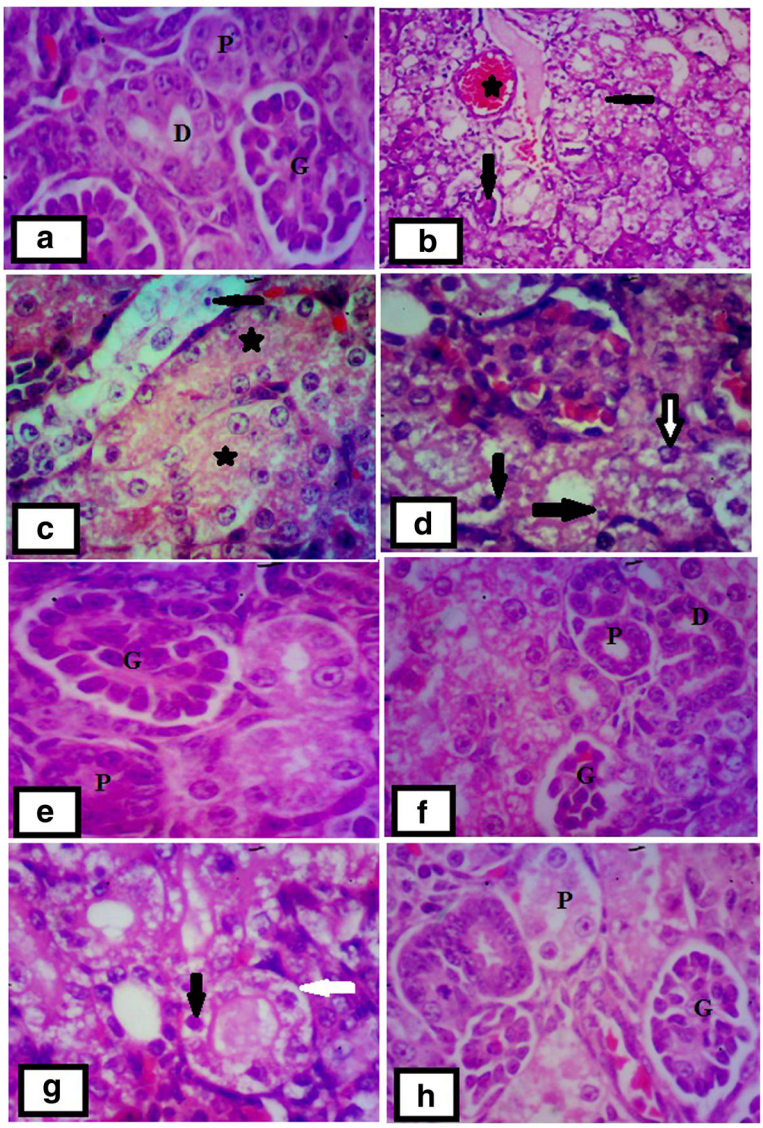

Fig. 7 Photomicrographs from the kidney tissues of 18-day-old fetuses in control and treated groups stained with H&E stain. The kidney tissue of control (GI) showing Malpighian corpuscles (G), the proximal (P), and distal convoluted tubules (D) (**a**) (×40). For the kidney of diabetic group (GII) showing venous congestion (asterisk), shrinkage glomerulus (thick arrow), hydropic degeneration (arrow) (**b**) (×20) and necrosis of the epithelial cells lining numerous tubules (black and white arrows) (**c**, **d**) (×40), in addition of hyaline casts (stars) (**c**) (×40). The kidney section from GIII revealed somewhat normal tissue characteristics (**e**) (×40). GIV showing signs of tissue repair (**f–h**) (×40) but some renal tubules have degenerative cells (white and black arrows) some blood congestion

et al. (2012) suggested the potential therapeutic use of OLE as a new nephroprotective agent against acute kidney failure.

The excessive renin-angiotensin system (RAS) activation is known to be one of the main reasons for myocardial injury, stress, cardiovascular disease, and end-stage kidney disease (Santos, Ferreira, & Simões e Silva, 2008). The Ang II is the most important effectors of RAS and has an important role in controlling the glomerular filtration rate by influencing the contractility of glomerular arteries in the renal tubule, thus regulating glomerular capillary internal pressure (Mizuiri, Hemmi, Arita, Ohashi, Tanaka, et al., 2008). The present results showed that STZ-treated group increased Ang II content. These results were in accordance with those of Ma, Xin, Jiang, Wang, and Zhang (2014) who suggested the important role of Ang II in causing renal injury in STZ-rat models. However, excessive Ang II expression may cause changes to the filtration rate of the mesangial cells of the renal glomerulus by stimulating the

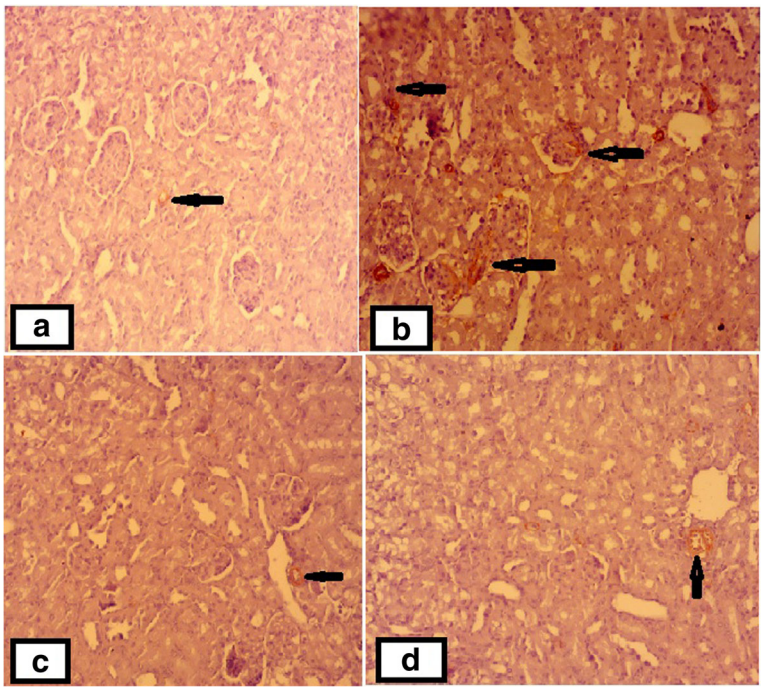

Fig. 8 Photomicrographs of immunohistochemistry expression of Ang II from the kidney tissues (×10). Low level of Ang II observed in the control group mainly in the tubular cells (arrow) (**a**). Ang II expression was elevated in glomerulus, tubular cells, interstitial cells, and blood vessels (arrows) in group II (**b**). **c**, **d** For GIII and GIV respectively revealed normal Ang II content in renal tissue

contraction and the proliferation of mesangial cells, thus leading to glomerular injury and loss of long-term renal function (Ye, Wysocki, William, Soler, Cokic, et al., 2006). Moreover, Ang II plays a significant role in renal fibrosis in immunoglobulin A nephropathy patients (Wen-lun, Yun-yun, Yu, & Hui-deng, 2016). However, the potential of olive leaf treatments in reducing diabetic complications, the possible contribution of the Ang II, as a central target for the anti-diabetic potential of olive, where the treatment of diabetic group with OLE in the present study showed decrease in Ang II content in the kidney tissue, could be attributed to the oleuropein which is the key hypotensive component of OLE due to L-type Ca^{2+} channel antagonistic effects (Scheffler, Rauwald, Kampa, Mann, Mohr, et al., 2008). In addition, verbascoside has been demonstrated to inhibit angiotensin-converting enzyme in vitro (Kang, Lee, Kim, Lee, & Lee, 2003) as has oleacein (Hansen, Adsersen, Christensen, Jensen, Nyman, et al., 1996).

Conclusions

In conclusion, the oral administration of olive leaf extract on STZ-induced diabetic pregnant mice has numerous ameliorative effects in weight gain of diabetic pregnant mice and their fetuses and in maintaining of the histological integrity of the renal and placental tissues by reducing the degenerative changes and reduction of serum urea and creatinine levels.

Acknowledgements

We would like to thank Hail University for supporting this work.

Funding

This research has been funded by Hail University and Scientific Research Deanship as a project no. (SCBI16).

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article and any explanations are available upon request from the corresponding author.

Authors' contributions

HM suggested the study, participated in its design and coordination, and carried out the embryological studies and immunohistochemical studies with the statistical analysis. HO carried out the biochemical analysis for kidney function, participated in the design of the study, and performed the statistical analysis. MI and NE participated in the design of the study and carried out the histological studies. All authors participated in the sequence alignment, drafted the manuscript, and read and approved the final manuscript.

Ethics approval

All animal experimental protocols were approved by the Committee of Scientific Ethics at the University of Hail and were carried out in accordance with its guidelines for animal use.

Consent for publication

Not applicable

Competing interests
The authors declare that they have no competing interests.

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Received: 28 July 2017 Accepted: 5 January 2018
Published online: 19 January 2018

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