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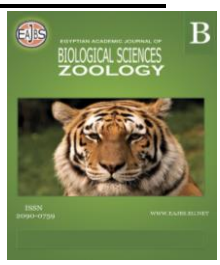

Effect of Ethanolic Olive Leaves Extract on the Renal Cortex of Adult Diabetic Male Albino Rats

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ABSTRACT

Background: renal damage is a common problem in diabetes (D); chronic hyperglycemia is associated with dysfunction in a variety of organs including kidneys. Scientific evidence indicates that medicinal plants comprise a lot of hypoglycemic chemical compounds. ***Aim of the work*:** this study aimed to assess the potential ameliorating effect of ethanolic olive leaves extract (OLE) on streptozotocin (STZ) induced D in the renal cortex of adult male rats. This assessment was achieved by biochemical, histopathological and histochemical studies. ***Materials and Methods:*** thirty adult Wistar male rats were categorized in three separated groups (10 rats in each group); group 1: control group (C), group 2: diabetic rats (D); streptozotocin was injected intraperitoneally at a single dose of 65 mg/kg b. wt. after 15 minutes of a single dose of nicotinamide which was injected intraperitoneally (230 mg/kg b. wt.), group 3: the diabetic rats which were treated with olive leaf extract (D+ OLE) 15 mg/ kg b. wt. by the gastric tube daily, for 30 days. After one month of treatment, creatinine was measured. Furthermore, histopathological and quantitative histological and histochemical studies were performed. ***Results:*** STZ induced a highly significant increase in creatinine level and histopathological changes including desquamation of the epithelial, brush border loss, peritubular infiltration, edematous and hemorrhagic areas, congested vessels and lipofuscin pigments. These pathological findings of the kidney cortex of adult male rats have been reduced by olive leaf extract. ***Conclusion***: use of olive leaf extract should be advised to reduce the hyperglycemic damage to a minimum level.

INTRODUCTION

Diabetes mellitus (D) is one of the human disorders with the most severe metabolic consequences. Death and illness rise in diabetes due to correlated chronic complications such as nephropathy and atherosclerosis marked by hyperglycemia and biochemical changes in lipid and glucose metabolism (Khalil, 2017). Continual hyperglycemia stimulates overall oxidative stress and a rise in the incidence of liver disease and diabetic nephropathy (El-Serag *et. al.*, 2004). In many countries around the world, diabetes nephropathy is a common medical problem, resulting in complications of the microvascular (neuropathy, nephropathy and retinopathy) and macrovascular (cardiac attack, stroke and peripheral vascular diseases) (Umar *et. al.*, 2010).

Hyperglycemia leads to abnormal urea and creatinine release by raising serum urea and nitrogen due to renal damage, such as acute glomerulonephritis, nephrosclerosis and even tubular necrosis. Most animal models have been used for mellitus or antidiabetic testing (Jaramillo-Juarez *et. al.,* 2008). The rat model nicotinamide-streptozotocin is close to diabetes type 2 in people and is used to check medicines and natural products that may minimize diabetic complications (Ahangarpour *et. al.,* 2014). Streptozotocin is a Streptomyces Achromogenes-based antibiotic and promotes diabetes by cutting off the DNA strands that contribute to apoptosis of β cells of diabetic rats' pancreas (Nukatsuka *et. al.,* 1990). Nicotinamide, pyridine-3-carboxamid, is a vitamin B3 derivate with antioxidant potential that protects insulin secretion cells from the cytotoxicity of STZ (Kishore and Kaur, 2017). Diabetes mellitus becomes an actual problem of public health in developing countries, where its spread is increasing steadily, and adequate treatment is often costly or unavailable (Holman *et. al.,* 2008). Substitute strategies to the current modern pharmaco-therapy of diabetes mellitus are crucially needed (Pareek *et. al.,* 2010). Medicinal plants and the potential alternative therapies are active, safer and cheap options when compared to oral hypoglycaemic agents. Antioxidant treatment has been shown to be a necessary therapeutic choice for decreasing tissue damage and averting D complications. For more than 1000 years in the Mediterranean, an olive tree has been planted. The olive oil, foliage and fruit have become established as a folk remedy for diabetes and hypertension (Omar**,** 2010). Different parts of the olive tree have wide pharmacological and biological activities, as antimicrobial, antioxidant, antimalarial, antiviral, antifungal, anticancer, antidiabetic, anti-inflammatory, hypolipidemic, antiatherogenic, cardioprotective, cytoprotective and hepatoprotective activities due to the high flavonoids, polyphenolic, triterpenes and other biologically active components (Manie *et. al.,* 2014& Afify *et. al.,* 2018). Chemically, the active constituents of the olive leaves are oleic acid, squalene and phenolic compounds. Oleuropein and hydroxytyrosol are the main phenolic constituents. Triterpenes and flavonoids, namely and glycosides of luteolin and apigenin, are also found (Bruneton, 1999). Polyphenols especially Oleuropein from olive leaves have important effects on the human body such as its antioxidant capability, hypoglycemic, antihypertensive and hypocholesterolemic factors (Vogel *et. al*., 2014). Another study has shown rutin that oleuropein (up to 6%–9% of dry matter in the leaves) has been correlated with ameliorated glucose metabolism and is liable for the antihyperglycemic effect in diabetic rats (Barbaro *et. al.,* 2014). The oleuropein structure was described by the presence of two hydroxyl groups, which play a vital role in its biologic function. Intake of oleuropein, a phenolic antioxidant, has been noted to decrease the oxidative stress caused by diabetes (El-Kholy *et. al*., 2015). Another study concluded that oleuropein supplementation increased the antioxidant enzyme activities in Cadmium intoxicated mice (Jemai *et. al*., 2020). Activities of antioxidants for various *Olea. europaea* parts were assessed using 2, 2-diphenyl-1-picrylhydrazyl and hydroxyl radicals. These include ethanolic extract of olive fruit and olive leaves. All the extracts have shown excellent antioxidants and are a safe supplement to food (Gonçalves *et. al.,* 2013). Hydroxytyrosol and oleuropein have been shown to be both potential α-glucosidase inhibitors for regulation of post-prandial high glycemia as the antidiabetic properties for various plant extracts by inhibiting carbohydrate-hydrolyzing enzymes (Hadrich *et. al.,* 2016). In the diabetic group treated with OLE (17.8 mg/kg b. wt.), levels of uric acid, creatinine and urea were significantly improved with normal liver function (Afify *et. al.,* 2018). It is evident from the previous literature that diabetes and renal dysfunctions are closely related. So, the present study was planned to assess the antidiabetic effect of olive leaf extract in the kidney cortex of diabetic adult male rats.

MATERIALS AND METHODS

Experimental Animals:

Thirty adult Wistar male rats (120-130 gm) were obtained from Nile Pharmaceutical Co., Cairo, Egypt. All rats were placed in metal cages in controlled light cycles (12h: 12h light-dark cycle) at a temperature (25±20C) for a week prior to the experiment. The animals were fed a standard pellet diet and water was provided *ad libtium.* The investigation was performed in accordance with the Guide for the Care and Use of Laboratory Animals.

Preparation of Olive Leaf Extract (*Olea europaea L.*, Family: Oleaceae):

Olive leaves were naturally obtained from the trees of olive, then weighed, dried and ground to a fine powder in an electric mixer. The powdered plant material was extracted in70% ethanol by soxhlet apparatus for 10 hours continuously (Abo-Ghanema and Sadek, 2012). At 90 ° C for 24 h, ethanol was then evaporated. A dry semi-solid extract has been weighed and dissolved in deionized water (100 g/100 ml, wt./vol.) and stored in the refrigerator until use. The extract was administrated daily at a dose of 15mg/kg b. wt. for 30 days by gastric gavages according to the method of Alirezaei *et. al.* (2012). The extract was prepared in the lab of Physiology, Zoology Department, Faculty of Sciences (boy's branch).

Induction of Type-2 Diabetes:

Rats were fasted for 16 h, with free access to drinking water. Streptozotocin (STZ), freshly prepared in a buffer of citrate (pH 4.5), single-dose was intraperitoneally (ip) injected at a dose of 65 mg/kg body weight after 15 minutes of a single-dose of nicotinamide (NA) (230 mg/kg b. wt. dissolved in normal saline) ip. injection (Masiello *et. al.,* 1998). Nicotinamide was used to protect insulin secretion cells from the cytotoxicity of STZ (Kishore and Kaur, 2017). Nicotinamide and STZ were obtained from Sigma Chemicals (St. Louis, MO, USA), Nasr City, Cairo Egypt. After 48 h of NA-STZ injection, diabetes progression was confirmed in blood by assessment of the blood glucose level using a glucometer (blood glucose meter) (Zahkok *et. al.*, 2016). The rats with more than 250 mg/dl fasting blood glucose are known as diabetics. Ultimately, 20rats were treated with NA-STZ for induction of diabetes and all of them were diabetic; and then they were categorized into two groups the first group served as the diabetic group and the second one was used in D+OLE group.

Experimental Design:

Thirty adult male Albino rats (120-130 g) were divided into three groups at the beginning of the experiment (10 in each group) as follows:

Group 1: control group (C), 1 ml of distilled water was given orally every day for a month through gastric intubation.

Group 2: a rat model of diabetes type-2 (D); Nicotinamide (230 mg/kg body weight) was intraperitoneally injected and after 15 minutes Streptozotocin (at a dose of 65 mg/kg body weight) was intraperitoneally injected.

Group 3: diabetic rats treated with OLE **(**D+OLE), OLE (15 mg/kg body weight) was given to diabetic rats orally by gastric intubation daily for a month.

After 30 days the animals of all groups were anesthetized by ether, blood was collected from retro-orbital plexus and left to coagulate, and the serum was separated by centrifugation at 3000rpm for 15 min. for biochemical analysis. The kidney was dissected out quickly, apart from the kidney was prepared for various histological and histochemical studies.

Biochemical Analyses:

Serum creatinine concentrations were determined colorimetrically as described by

Kroll *et. al*. (1987)**.**

Histological and Histochemical Studies:

Small pieces of kidney from the rats were fixed in 10% neutral formalin buffered and Bouin's solution, dehydrated in ascending grades of alcohol, cleared then embedded in paraffin. Paraffin sections of 5 µm thickness were cut and stained with hematoxylin and eosin for general histological structure and Mallory's trichrome stain for detecting the collagen fibers (Suvarna *et. al.*, 2018), mercuric bromophenol blue technique for detecting total proteins (Cook and Warren, 2015), polysaccharides and DNA content were detected respectively by periodic acid Schiff and Feulgen method (Suvarna *et. al.*, 2013) and Congo red technique (Dey, 2018) for demonstrating amyloid-β protein**.**

Quantitative Histological and Histochemical Analysis:

IPWIN 32 image analysis software recorded the optical density of histological and histochemical stained sections in cortex tissue for collagen, total proteins, DNA, polysaccharides and amyloid-β protein.

Statistical Analysis:

Statistical analyzes have been carried out by using Snedecor and Cochran (1980) methods (ANOVA) variance analyses. Statistical Analysis for Social Science, Version 8 was used to process and analyze the data. Student T-test was used to realize significant differences between groups. Data were offered as mean ±SD and statistically significant when P was more than or equal to 0.05 (P≥0.05).

RESULTS

Biochemical Parameter:

The levels of creatinine in the diabetic group showed a significant increase in the mean value which reached 1.02±0.21 as compared to the normal group (0.58±0.17). While the diabetic group treated with OLE (D+OLE) exhibited a non-significant change in these levels (0.58±0.05) in comparison with the control group (Fig.1).

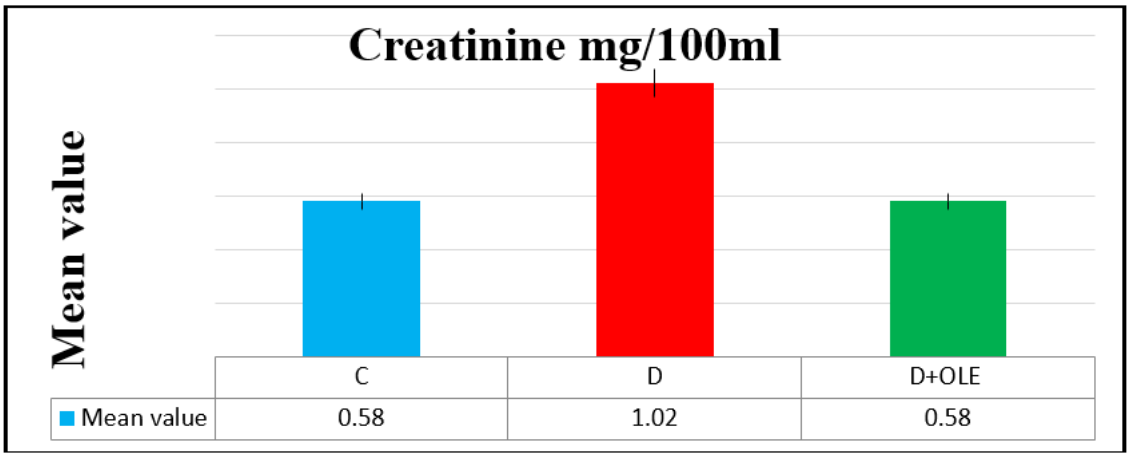

Fig.1. Values of creatinine (mg/100ml) in the studied groups**.**
(C**:** control**,** D: diabetic, D+OLE: diabetic rats treated with olive leaf extract).

Histological Observations:

Histological pattern of the kidney cortex tissue of control rats displayed normal glomeruli, Bowman's space, proximal and distal convoluted tubules (Fig.2). Renal tissue of the diabetic group showed numerous degenerative changes included; thickened walls and atrophy of many glomerular tufts with wide urinary space and some were totally degenerated with exfoliated nuclei in the lumen of some tubules (Fig.3a), in addition to high distortion in most tubules and loss of their normal architecture. Hypercellularity and

congested glomeruli, pyknotic and karyolytic nuclei were also observed in figure 3b with lipofuscin pigments in figure 3c. Congested vessels with thickened walls and intertubular leucocytic infiltration were obvious in figure 3d with pale staining affinity in some convoluted tubules, edematous and hemorrhagic areas. After 30 days of treatment, kidney tissue of D+OLE group showed well-developed kidney architecture, where the glomerulus, proximal and distal convoluted tubules appeared more or less like normal, while some nuclei exfoliated into the lumen of some tubules (Fig.4).

Quantitative Histological and Histochemical Measurements:

A significant increase in the mean value of collagen was observed in the D group which reached 0.47±0.04 (Figs.5b & 10), while a non-significant increase (0.35±0.03) was shown in the D+OLE group (Figs.5c& 10) when compared to the control group (0.34±0.02 in figures.5a & 10). A significant decrease in the mean value of total protein content was noted in the D group which reached 0.50±0.05 (Figs.6b & 10) compared to the control group (0.87±0.12 in figures 6a & 10). While non-significant change (0.85±0.06) was observed in D+OLE group (Figs.6c & 10). PAS +ve materials (Fig.10) in the kidney cortex tissue of D and D+OLE groups induced a significant decrease in the mean value which reached 0.15±0.01 and 0.24±0.02 respectively (Figs.7b&c) compared to the control group (0.26±0.02 in fig.7a). A significant decrease in the mean value of total DNA content was recorded in the groups D and D+OLE which reached 0.12±0.01& 0.21±0.01 respectively (Figs.8b,c&10) compared to the control group (0.26±0.02 in figures 8a&10). A significant increase in the mean value of amyloid-β protein content in the kidney cortex tissue of the D group which reached 0.73±0.09 (Figs.9b&10) compared to the control group (0.29±0.01 in figures.9a&10). While a non-significant increase (0.30±0.04) was observed in D+OLE group when compared to the control group (Figs.9c&10).

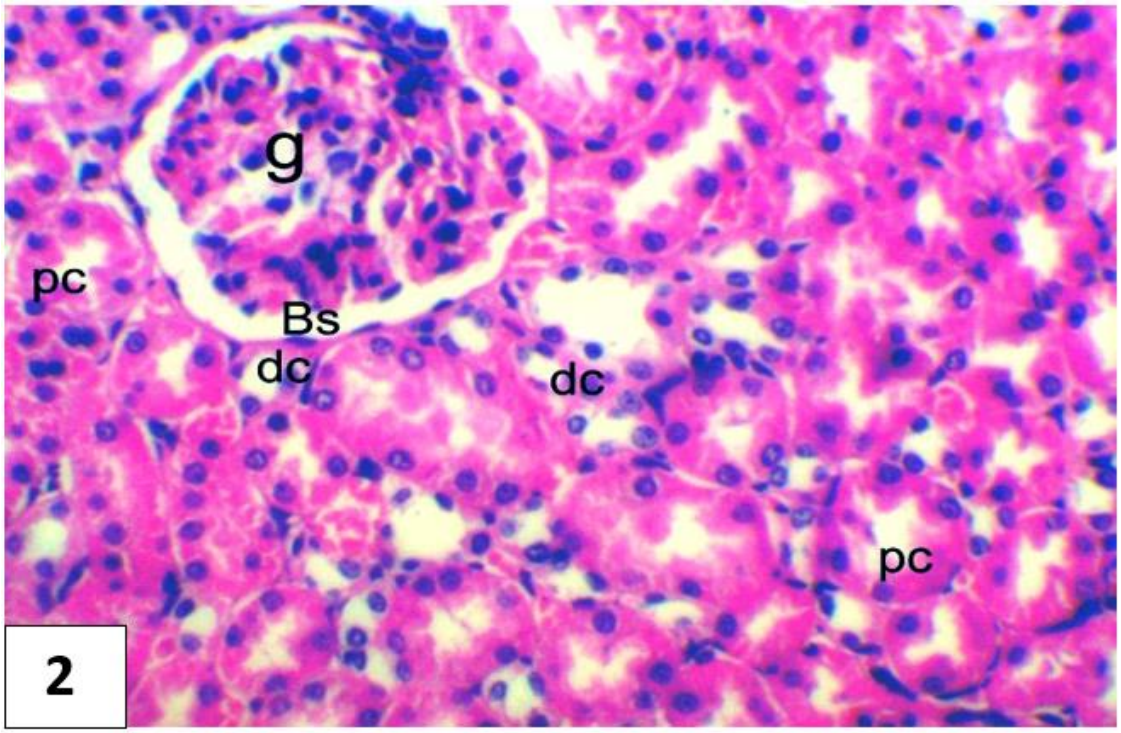

Fig.2. Photomicrograph of the section in renal cortex tissue of a control adult male rats stained with hematoxylin and eosin showing normal glomerulus **(g),** Bowman's space **(Bs),** proximal **(pc)** and distal **(dc)** convoluted tubules. **(Original magnification X400).**

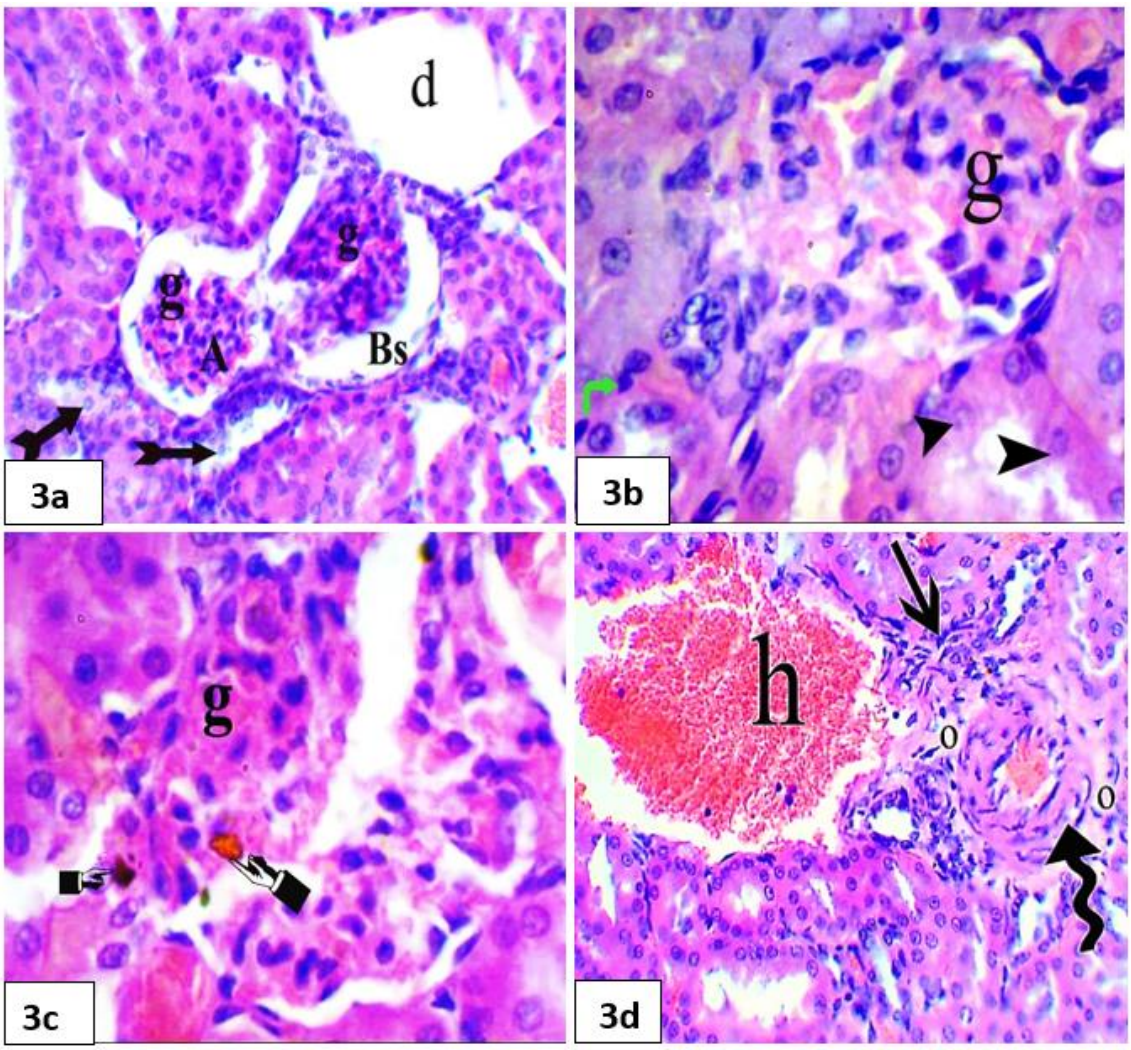

Fig.3(a-d). Photomicrographs of sections in renal cortex tissue of diabetic adult male rats stained with hematoxylin and eosin **s**howing:
3a: the glomerular tuft **(g)** with thickened wall, atrophy **(A)** with wide urinary space **(Bs)** or totally degenerated **(d)** and some exfoliated nuclei in the lumen of some tubules **(tailed arrows)**.
;3b: hypercellularity and congested glomerulus **(g)**, pyknotic **(curved arrow)** and karyolitic **(arrows heads)** nuclei.
;3c: lipofuscin pigments **(hands)** in glomerulus and convoluted tubule.
;3d: hemorrhagic area **(h)**, congested vessels with a thickened wall (**corrugated arrows**), intertubular leucocytic infiltration **(arrow)** and edematous areas **(o).**
(Original magnification a,d X 400 &b,c X 800).

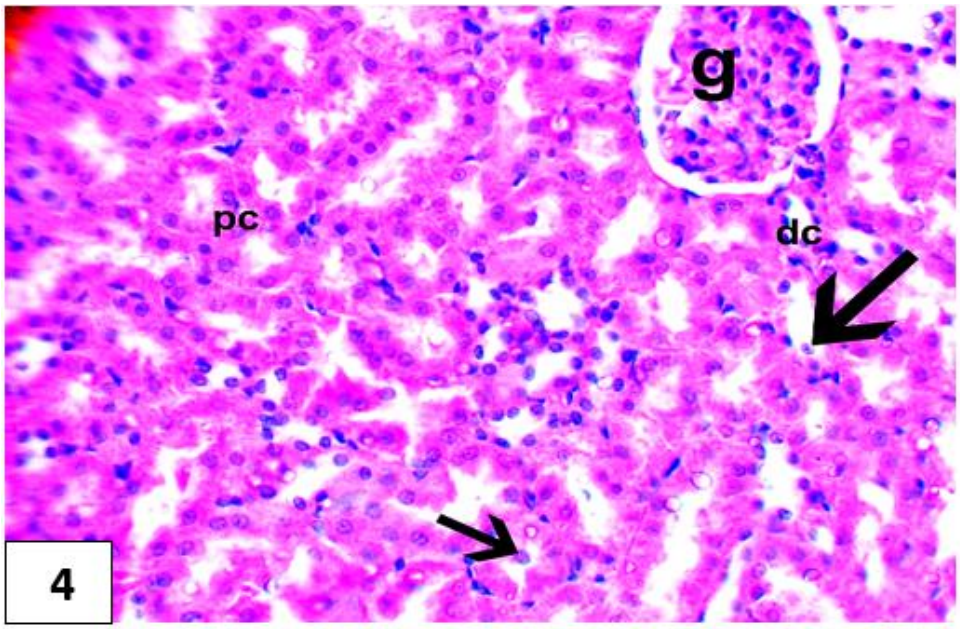

Fig.4. Photomicrograph of the section in renal cortex tissue of D+OLE group stained with hematoxylin and eosin, showing well-developed kidney architecture, the glomerulus **(g),** proximal **(pc)** and distal **(ds)** convoluted tubules appeared more or less like normal while some nuclei exfoliated into the lumen of some tubules **(arrows). (Original magnification, X 400).**

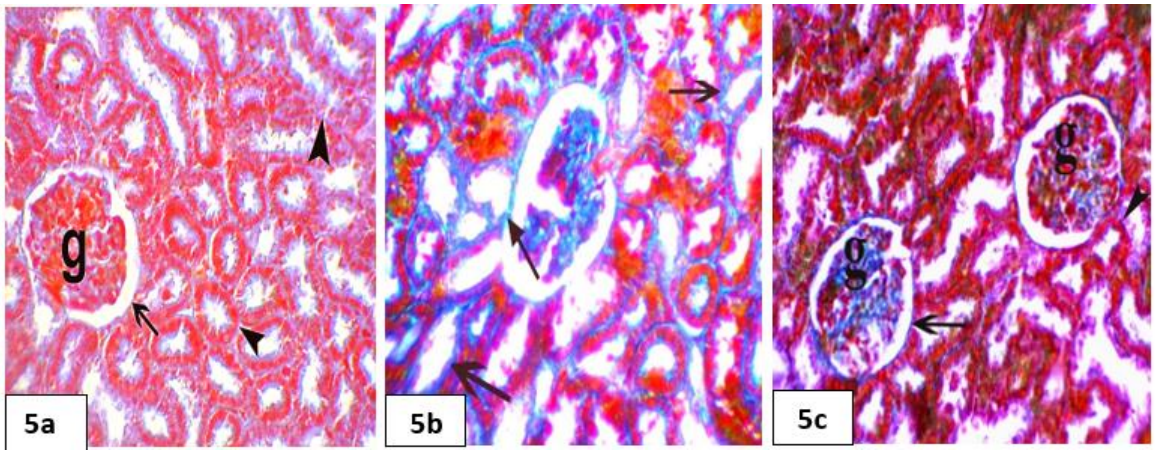

Fig.5 (a-c). Photomicrographs of sections in renal cortex tissue of adult male rats stained with Mallory's trichrome stain showing: **(a)** the control group with thin collagen fibres in the glomerulus **(g)**, bowman's capsules **(arrow)** and basement membrane of convoluted tubules **(arrows heads)**; **(b)** diabetic group with dense stain affinity of collagen fibres **(arrows); (c)** D+OLE group with thin collagen fibres in glomeruli **(g)**, bowman's capsules **(arrow)** and basement membrane of convoluted tubules **(arrowhead)**. **(Original magnification X 400).**

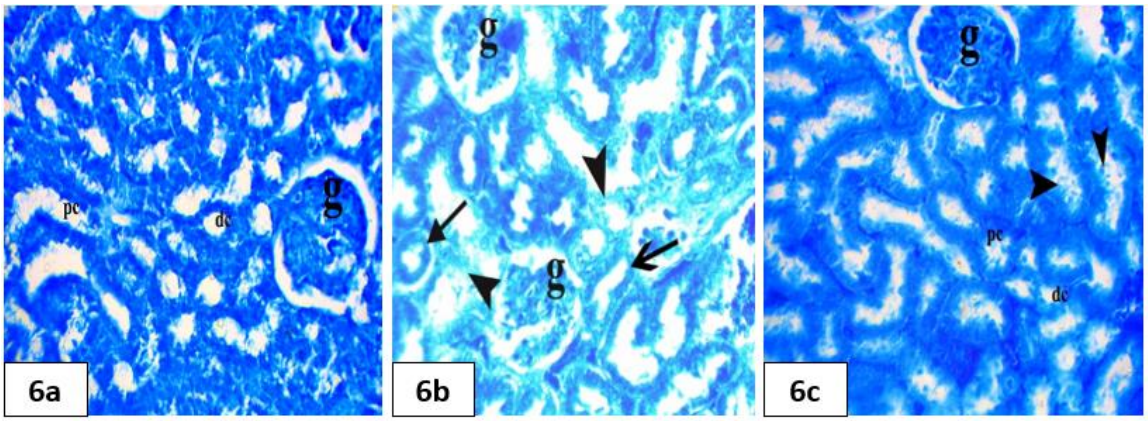

Fig.6 (a-c). Photomicrographs of sections in renal cortex of the adult male rats stained with mercuric bromophenol blue showing: **(a)** the control group with deeply stained glomerulus **(g)**, and faintly stained brush borders cells of the distal **(dc)** and proximal **(pc)** convoluted tubules; **(b)** diabetic group with decreased staining affinity of total protein in the glomeruli **(g)** and walls of the convoluted tubules **(arrows)** and negatively stained degenerated areas **(arrows heads)**; **(c)** D+OLE group with deeply stained glomerulus **(g)** and less stained brush borders cells of the distal **(dc)** and proximal **(pc)** convoluted tubules **(arrowhead)**. **(Original magnification X 400).**

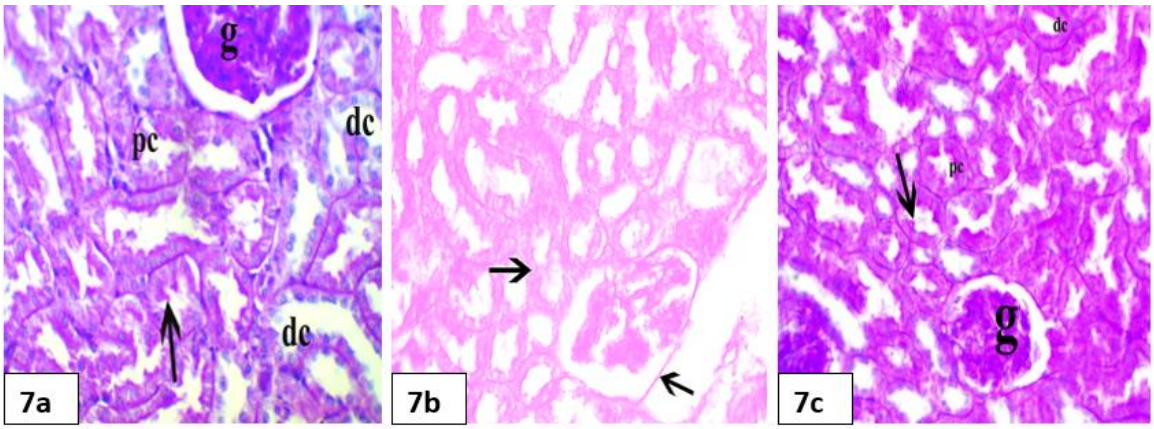

Fig.7 (a-c). Photomicrographs of sections in renal cortex of the adult male rats stained with periodic acid Schiff technique showing: **(a)** control group with deeply stained glomerulus **(g)**, Bowman's capsules, distal convoluted tubules **(dc)** and brush borders **(arrow)** of proximal convoluted tubules **(pc); (b)** diabetic group with faintly stained PAS +ve materials **(arrows); (c)** D+OLE group with deeply stained PAS +ve materials in glomerulus **(g)**, Bowman's capsules, distal convoluted tubules **(dc)** and brush borders **(arrow)** of proximal convoluted tubules **(pc). (Original magnification X 400)**.

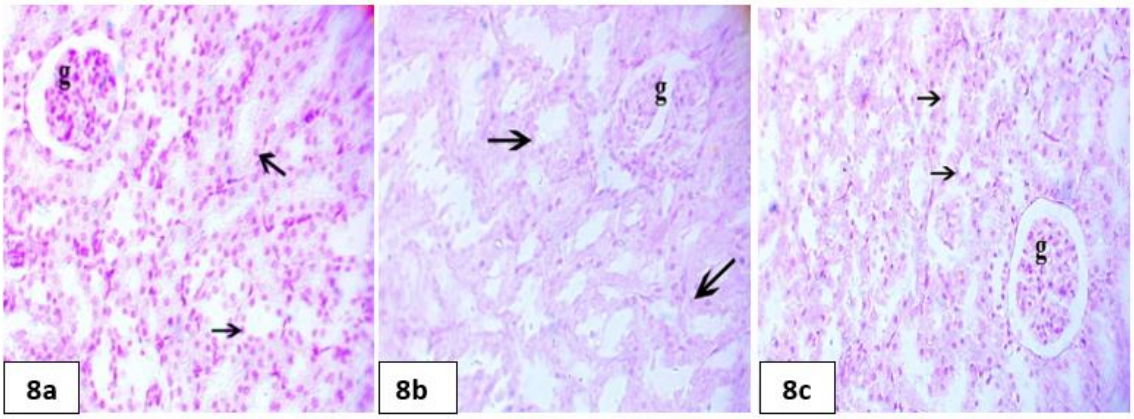

Fig.8 (a-c). Photomicrographs of sections in renal cortex of the adult male rats stained with Feulgen reaction showing: **(a)** the control group with moderately stained DNA in glomerular capillaries **(g)** and convoluted tubules **(arrows)**; **(b)** diabetic group with decreased total DNA content and faintly staining affinity in the glomerulus and convoluted tubules **(arrows); (c)** D+OLE group with moderately stained DNA in glomerulus **(g)** and convoluted tubules **(arrows). (Original magnification X 400).**

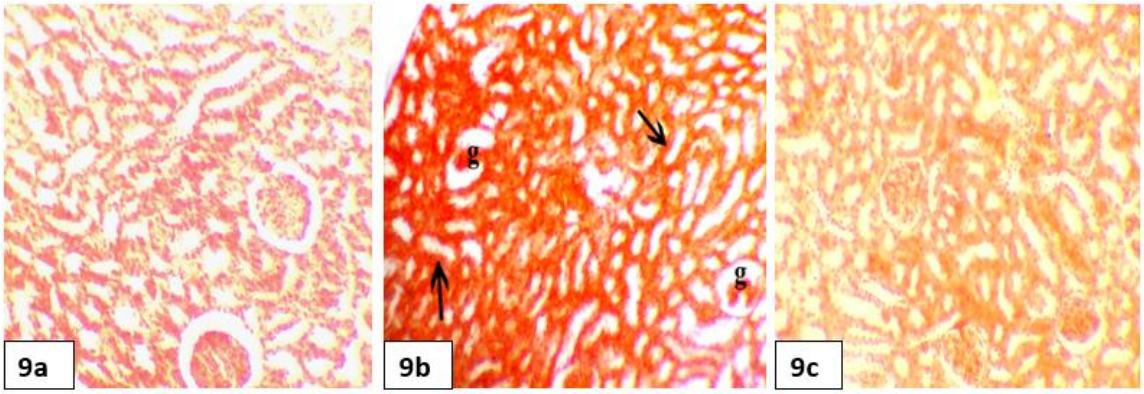

Fig.9 (a-c). Photomicrographs of sections in renal cortex of the adult male rats stained with Congo red showing: **(a)** the control group with faintly stained amyloid-β protein; **(b)** diabetic group with condensed clumps of amyloid-β protein in the glomerular capillaries **(g)** and most convoluted tubules **(arrows); (c)** D+OLE group with faintly stained amyloid-β protein. **(Original magnification X200)**

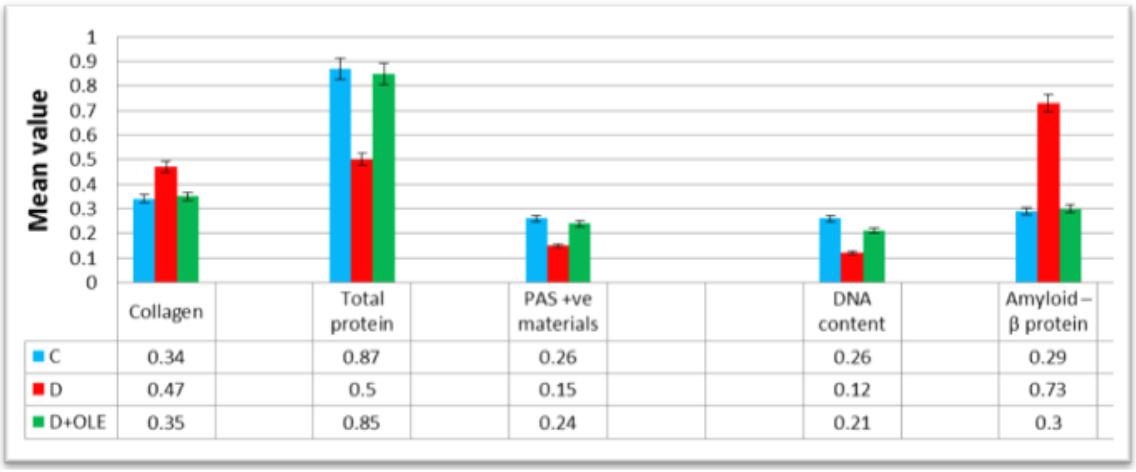

Fig. 10. The optical density of the collagen, total protein, PAS +ve materials, DNA content and amyloid-β protein in the studied groups
(C: control, D: diabetic, D+OLE: diabetic rats treated with olive leaf extract)

DISCUSSION

In the present study, the renal ameliorative effect of ethanolic olive leaves extract on nicotinamide-streptozotocin-induced diabetic rats was performed. Diabetes is related to multiple tissue damage (Jia *et. al.,* 2019). Renal pathologies are the most serious diabetes-related diseases which need new treatment options (Sadi *et. al.,* 2019). Olive leaf extract antioxidant activity has led to inhibition of physiological, molecular and histopathological changes in diabetic rats (Al-Attar and Alsalmi, 2019a). In diabetic rats, Berköz *et. al.* (2021) observed a remarkable improvement in serum insulin level after OLE treatment. This research has shown that diabetic rats display a substantial decrease in renal function, which is confirmed by a significant increase in serum creatinine levels. Creatinine is a chemical waste product in the blood that is filtered through the kidneys and excluded in the urine. The chemical waste is a byproduct of natural muscle contractions. Kidney damage or disease makes waste can't be correctly filtered and creatinine levels in the blood rise. The diabetic rats explained an elevation in the level of creatinine and urea (Salahuddin and Katary, 2017). The increase of creatinine, urea and uric acid in the diabetic group was due to progressive renal damage (Abdel Aziz *et. al.,* 2017). In the present study, a non-significant change in the creatinine level was recorded in the diabetic group treated with OLE. In STZ diabetic male rat's creatinine, uric acid and urea levels were decreased with OLE administering (Laaboudi *et. al.,* 2016). The administration of OLE can inhibit serious changes of renal haematobiochemical indicators and disturbances of its histological structure (Al-Attar *et. al.,* 2017). The olive leaf extract improved gentamicin nephrotoxicity throughout the antioxidant activity by increasing renal glutathione content and renal antioxidant enzymes activity (Tavafi *et. al.,* 2012). The kidney functions were attenuated by olive leaf powder and the serum glucose was decreased in diabetic rats (Abdelkarem *et. al.,* 2021).

Histopathological examination of the kidney cortex of the diabetic group in the present study showed many deleterious changes. These changes included congested and atrophied glomeruli with thickened walls of glomerular tuft, and some glomerular tufts were totally degenerated. Intertubular leucocytic infiltration, edematous and hemorrhagic areas were also obvious as well as congested vessels with a thickened wall. Pyknotic and karyolytic nuclei were also observed with exfoliated nuclei in the lumens of some tubules, in addition to the presence of lipofuscin pigments. These histopathological findings come in agreement with the results of Nassar *et. al*. (2019), who found that experimental animals injected with STZ resulted in a variety of injuries including, damaged, lobulated glomeruli, necrotic proximal convoluted tubules and severely-damaged distal convoluted tubules in macula densa and several necrotic foci in the renal tissue which may an indication to the oxidative stress of hyperglycemia. Histopathological analysis of kidney cortex from non-treated diabetic rats (induced by STZ 60 mg/ kg injection) showed mild tubular degeneration and necrosis characterized by eosinophilic cytoplasm with pyknotic nuclei, mild tubular cytoplasmic vacuolations, degenerative and necrotic changes in the glomerular epithelium, multifocal mononuclear cell infiltration in the interstitial tissue, congestion and diffused glomerular with interstitial hemorrhages. Furthermore, cortical tubular and glomerular hypertrophy and an apparent rise in the thickness of Bowman’s capsules were observed in the study of Goli *et. al*. (2019). The storage of Lipofuscin pigments in the renal tubules cells of rat diabetic nephropathy was a sign of cell injury (Pourghasem *et. al*., 2000) and may be due to lysosomes dysfunction (Forbes *et. al*., 2003). In the present study, oral treatment with OLE to the diabetic group ameliorated diabetic renal injury. This result is in agreement with Khattab *et. al*. (2020), who found that in D rats treated with OLE the renal section

showed a near to normal appearance. The improvement of renal tissue could be explained by the antioxidant components in OLE, which reduce and prevent the complications related to oxidative stress, vascular health, inflammation and endothelial function in D (Ahmadvand *et. al*., 2017). Hashmi *et. al*. (2015) suggested that the anti-diabetic effect of OLE might be due to the ability of oleuropein and hydroxytyrosol to prevent oxidative stress which is commonly correlated with pathological diabetes complications**.** OLE showed a lower inflammatory reaction in the kidney tissue that might be pointed to OLE's anti-inflammatory effects (Chebbi *et. al*., 2011)**.** The kidney of rats treated with olive after exposure to oxidative stress showed normal renal glomeruli and tubules with slight congestion (Morgana *et. al*., 2014). Furthermore, diabetic rats treated with aqueous OLE, showed approximate improvement of histological changes (Mehanna *et. al*., 2016). Antioxidant activities of the olive leaf extracts played a fundamental role against the renal damage induced by diabetes (Al-Attar and Alsalmi, 2019b). These studies were confirmed by the study of Ağgül *et. al*. (2021) who reported that the ethanolic OLE has an antidiabetic effect and reinforces antioxidant activities.

In this study, significantly increased collagen fibres were detected in the renal cortex tissue of the diabetic group. Increased collagen deposition could be attributed to oxidative stress that enhances the expression of collagen biosynthesis genes (Guler *et. al*., 2009)**.** Likewise, Abd Rabou and Eid (2017) recorded highly increased collagen fibres in the kidney cortex of diabetic rats and their fetuses. Collagen fibres in tests of the diabetic young rats were also significantly increased (Mohamed *et. al*., 2017). However, in the present study oral treatment with OLE to rats of the diabetic group showed non-significant increased collagen fibres in the kidney cortex tissue. The collagen fibers of diabetic rats received normal distribution in the kidney cortex treated with olive leaves extract at dose 1 ml/100gm. b. wt. (Eid *et. al*., 2014). In the present findings, the mean value of total protein was significantly decreased in the renal cortex of diabetic rats. The nephritic syndrome led to proteinuria, which caused a significant loss of protein molecules in the urine due to reabsorption activity disturbance and inhibition of the content of protein in the tissue (Curran, 2000). The decrease in protein was indicated by the interruption of lysosomal membranes under the effects of different toxicants, which led to the liberation of their hydrolytic enzymes in the cytoplasm. In addition, hydrolytic enzymes can cause lysis and dissolution of the target material inside the cytoplasm (Abdel-Meguid *et. al*., 2010). High protein catabolism (a breakdown of the protein for obtaining energy when there are no carbohydrates) will reduce protein synthesis in diabetic individuals (Jia *et. al*., 2010). In this study, a non-significant decrease was realized in the mean value of total protein in the renal cortex tissue of D+OLE group compared to the control group. The improvement in protein content in OLE +D may be due to oleuropein, which stimulates endothelium formation in addition to mRNA and protein synthesis (Carluccio *et. al*., 2003). It may also be due to the increased amount of ribosomes in the rough endoplasmic reticulum in cells, which reflects their ability to promote protein synthesis (Tunez *et. al*., 2003)**.** This improvement in protein content may be caused by antioxidant properties of olive oil-derived phenolic compounds (oleuropein and hydroxytyrosol) which are associated with lipid peroxidation inhibition and free radical scavenger activities (Tuck *et. al*., 2001). Oleuropein has been reported to prevent protein, lipid, or DNA from oxidative damage (Fatani *et. al*., 2015).

The present study revealed a significant decrease of polysaccharides in the renal cortex tissue of D and D +OLE groups when compared to the control group, but there is an improvement in the mean value of polysaccharides in D+OLE group. These changes in polysaccharides may be due to the impairment of synthesis and action of

glycogenolytic and glycogenic enzymes, and this may be due to reduction or inhibition synthesis of glycogen or an increase in its breakdown in the organs of the animals as a whole. This glycogen breakdown in the diabetics would be the initiator for more polysaccharides released to the blood, hyperglycemia increased, and species were further harmed (Nico *et. al*., 2013). The improvement in the mean value of polysaccharides in D+OLE group may be due to oleuropein in the olive leaves extract which led to hypoglycemic effects because it enhances glucose-done insulin release and delays the absorption of carbohydrate from the gut, resulting in minimizing plasma-glucose levels (Wainstein *et. al*., 2012). Olive leaf extract is used as an active agent against acute kidney insufficiency. The mucopolysaccharides content was more or less close to the control level in flavonoid-treated diabetic rats (Helal *et. al*., 2014).

In the present study, the mean value of DNA materials was significantly decreased in the diabetic group. While improvement in total DNA content in the kidney cortex of D+OLE group was observed. Antioxidants inhibit oxidative damage to biologically vital molecules such as proteins, DNA and lipids and therefore diminish the risk of several chronic diseases (Myung *et. al*., 2013). The extract of olive leaves was more effective to reduce the damage to renal cells caused by free radicals due to streptozotocin (Eid *et. al*., 2014). In addition, it has been demonstrated that supplemented oleuropein successfully prevented H_2O_2-induced lipid peroxidation, protein oxidation and DNA damage (Carluccio *et. al*., 2003). Diabetes may induce oxidative stress and DNA destruction in the embryo and placenta, and this can be improved by the administration extract of olive leaves by using alkaline comet assay (El-Nabarawy, 2014). Saber *et. al*. (2015) also found that, the toxic effects of K2Cr2O7 (injuries to renal function, DNA destruction and histopathological changes in the kidney of rats) was improved by extra virgin olive oil administration, due to its antioxidant and free radical scavenging properties. In diabetic rats treated with OLE a non-significant change was observed in the mean optical density of DNA sperms abnormalities (Mousa, 2016). The current study recorded a significant increase in the amyloid-β protein content in the kidney cortex of the diabetic group. Oxidative damage is related to neuropathological lesions, by accumulating amyloid-β peptides and hyperphosphorylated protein (Goldsbury *et. al*., 2008). The present finding showed a non-significant increase in the mean value of amyloid-β protein content in D+OLE group in the renal cortex when compared to the control group. This result confirms the reduction of amyloid-β in the kidney cortex of D+OLE group when compared to D group. The improvement in antioxidant compounds may also have a role in reducing amyloid-β induce toxicity (Massaad, 2011). OLE nutritional complement can inhibit or delay the production of Alzheimer's disease and may decrease the severity of its indications such amyloid-β deposition (Grossi *et. al*., 2013). Mice nourishing with the extra-virgin olive oil-enriched diet for 3 months, beginning at an age after amyloid-β accumulation starts, displayed improved blood-brain barrier clearance and a significant decrease in amyloid-β levels (Qosaa *et. al*., 2015).

CONCLUSION

According to previous observations, it could be concluded that the treatment with olive leaf extract on STZ-induced diabetic adult male rats had diverse ameliorative effects in preserving the histological integrity of the renal cortex tissues by decreasing the deleterious changes and decrement of serum creatinine level.

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