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Original article

Influence of olive leaves extract on hepatorenal injury in streptozotocin diabetic rats

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ABSTRACT

Medicinal plants have always been an important source of new alternative effective compounds for human therapy. Currently, there are many of scientific evidences indicate that the medicinal plants contain a lot of hypoglycemic chemical compounds. The purpose of the present study was to determine the influence of olive leaves extract on hepatorenal injury in diabetic male rats. Experimental diabetes was induced by streptozotocin (STZ). The levels of serum glucose, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transferase, total bilirubin, creatinine, blood urea nitrogen, uric acid and malondialdehyde were significantly increased, while the levels of serum superoxide dismutase, glutathione and catalase were statistically decreased in untreated diabetic rats. Moreover, the histopathological examination showed several alterations in the structure of liver and kidney in untreated diabetic rats. Treatments with low dose and high dose of olive leaves extract in diabetic rats showed remarkable reducing and protecting influences of physiological and histopathological alterations. Moreover, the highly treatment efficiency was noted in diabetic rats treated with high dose followed by low dose of olive leaves extract. Additionally, the results of this study proved that the antioxidant activities of olive leaves extract played a vital role against the hepatorenal injury induced by diabetes. Finally, this study indicates to the importance of the use of olive leaves extract as promising alternative and complementary therapeutic agent against diabetes and its complications.

1. Introduction

Diabetes mellitus (DM) is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism associated with absolute or relative deficiency in insulin secretion and/or action (Akinnuga et al., 2010). Concern regarding this chronic disease is focused on serious DM-related complications which can affect multiple vital organ systems, thereby leading to more severe and irreversible pathological conditions such as nephropathy, retinopathy, vasculopathy, neuropathy and cardiovascular diseases, as well as hepatopathy (Reid, 2006). Longterm hyperglycemia promotes general oxidative stress and increases in the incidence of diabetic nephropathy and liver disease (El-Serag and Everhart, 2002; El-Serag et al., 2004). DM, by most estimates, is now the most common cause of liver disease and liver disease is an important cause of death in diabetic people (de Marco et al., 1999). Diabetic nephropathy is one of the alarming worldwide health problems at present leading to micro vascular (retinopathy, neuropathy and nephropathy) and macro vascular (heart attack, stroke and peripheral vascular disease) complications in many countries of the world (Umar et al., 2010). Diabetic nephropathy is a microvascular diabetic complication that leads to end-stage renal disease. Multiple factors are involved in the onset of diabetic nephropathy; oxidative stress is believed to link these factors (Wolf, 2004; Brownlee, 2005). Free radicals also prompted the development of liver diseases by inducing hepatocyte apoptosis, hepatic inflammatory response and fibrogenesis (Albano, 2006; Novo and Parola, 2008).

Recently, medicinal plants are widely used and experimental studies have shown that many species of medicinal plants with different compounds can be used as hypoglycemic agents. Medicinal plants provide a useful source of oral hypoglycemic compounds for the development of new pharmaceutical leads as well as dietary supplements to existing therapies (Kavishankar et al., 2011). The

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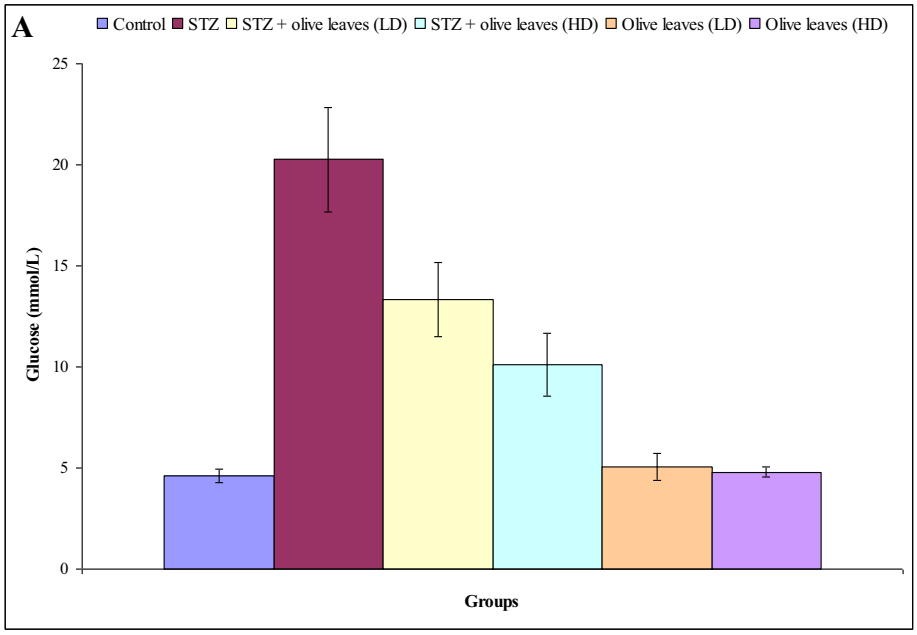
olive tree (*Olea europaea*) has been widely accepted as one of the species with the highest antioxidant activity via its oil, fruits, and leaves. It is well known that the activity of the olive tree byproduct extracts in medicine and food industry is due to the presence of some important antioxidant and phenolic components to prevent oxidative degradations. The olive tree has long been recognized as having antioxidant molecules, such as oleuropein, hydroxytyrosol, oleuropein aglycone, and tyrosol (Jemai et al., 2008a, 2008b). Moreover, experimental studies have shown the ability of olive leaves for the treatment and alleviation of different diseases, and physiological, biochemical and histopathological alterations (Omagari et al., 2010; Grawish et al., 2011; Zari and Al-Attar, 2011; Wainstein et al., 2012; Al-Attar and Abu Zeid, 2013; Al-Attar and Shawush, 2014, 2015; Kumral et al., 2015; Al-Attar et al., 2016, 2017). The present study was aimed to evaluate the influence of olive leaves extract on hepatorenal injury in diabetic male rats.

2. Material and methods

2.1. Preparation of olive leaves extract

The method of Al-Attar and Abu Zeid (2013) was used to prepare the extract of olive leaves with some modifications. The dried olive leaves (200 g) were powdered and added to 7 L of hot water. After 3 h, the mixture was slowly boiled for 30 min. After boiling



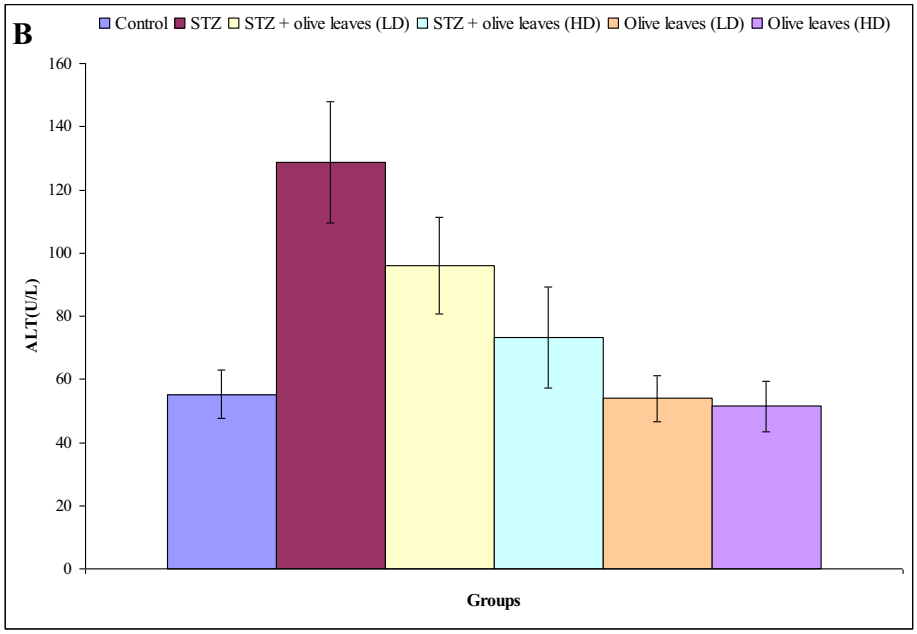
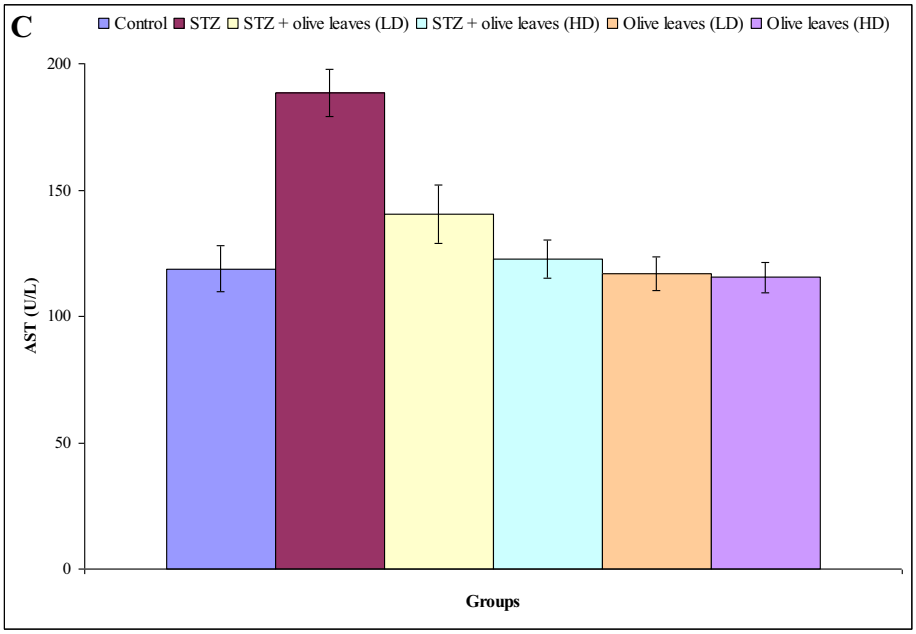
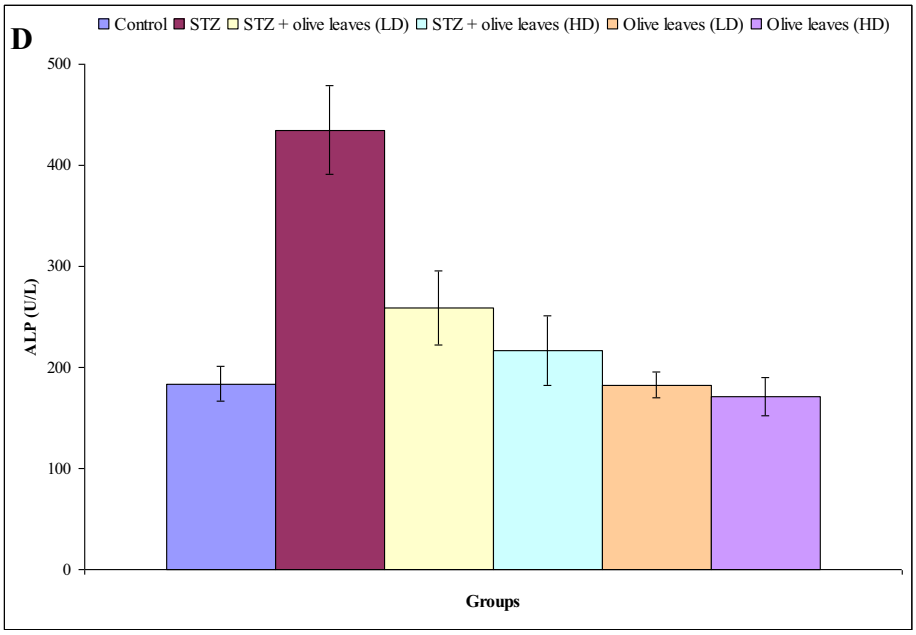


Fig. 1. (A–F) The levels of glucose (A), ALT (B), AST (C), ALP (D), GGT (E) and total bilirubin (F) in serum from control, STZ, STZ plus LD of olive leaves extract, STZ plus HD of olive leaves extract, LD of olive leaves extract and HD of olive leaves extract treated rats.





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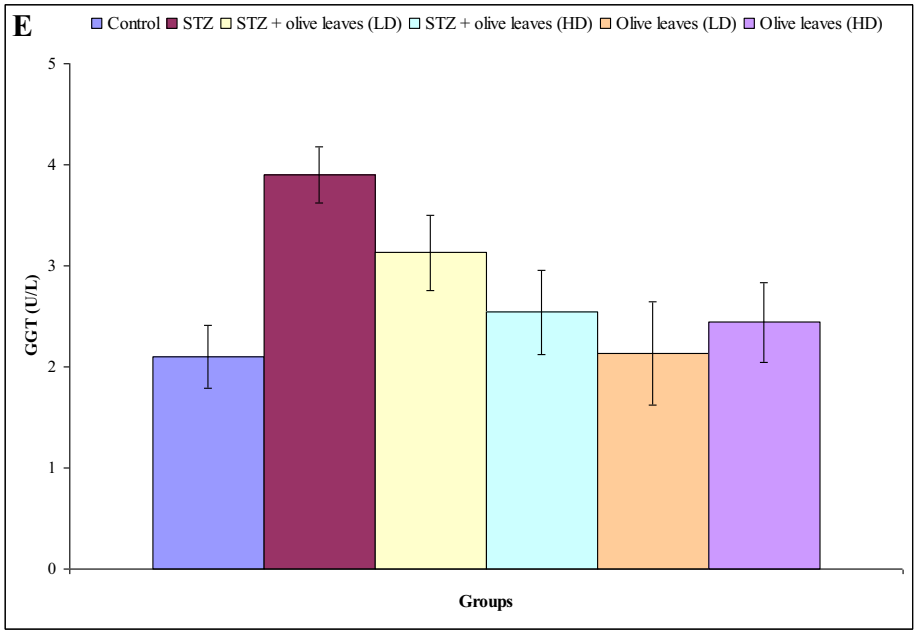
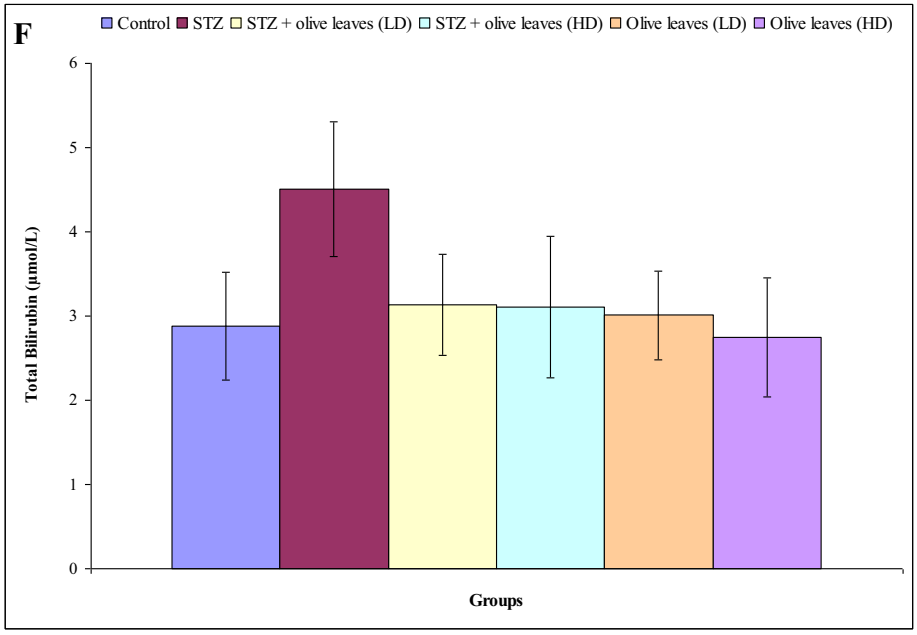
period, the mixture was cooled at room temperature and it was gently subjected to an electric mixer for 20 min. Thereafter the solution of olive leaves was filtered. The filtrate was evaporated in an oven at 40 °C to produce dried residues (active principles). With references to the powdered samples, the yield mean of leaves extract was 20.3%. Moreover, the extract of leaves was prepared every two weeks and stored in a refrigerator for subsequent experiments.

2.2. Animal and experimentation

Sixty Wistar male rats (113.2–183.8 g) used in this study were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. The experimental animals were housed 10 per cage in a room with 65% humidity, 12:12 h light: dark cycle at ambient temperature of 20 ± 1 °C. Standard diet, commercial feed pellets and tap water were freely available. DM was induced using intraperitoneal injection of streptozotocin, STZ, (Sigma- Aldrich Corp, St. Louis, MO, USA) at a single dose of 60 mg/kg body weight dissolved in saline solution. DM was defined using determination of fasting blood glucose levels in rats treated with STZ. The blood glucose levels over than 17 mmol/L were considered as diabetic model rats. The normal (n = 30) and diabetic rats (n = 30) were divided into six experimental groups. The first group was served as normal healthy control, intraperitoneally received saline solution. The second group was diabetic control. The third group was diabetic rats, supplemented orally with olive leaves extract at a low dose (LD) of





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200 mg/kg body weight/day. The fourth group was diabetic rats, supplemented orally with olive leaves extract at a high dose (HD) of 400 mg/kg body weight/day. The fifth group was non diabetic rats, intraperitoneally received saline solution and treated with olive leaves extract at the same dose given to the third group. The sixth group was non diabetic rats, intraperitoneally received saline solution and supplemented with olive leaves extract at the same dose given to the fourth group. After eight weeks, all rats were fasted for 12 h, water was not restricted. Blood samples were taken from orbital venous plexus under total anesthesia with diethyl ether. Blood samples were for separating the serum for analyzing the biochemical parameters. The level of serum glucose was measured according to the method of Trinder (1969). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured using the method of Reitman and Frankel (1957). Serum alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and total bilirubin were estimated using the methods of MacComb and Bowers (1972), Szasz (1969) and Doumas et al. (1973) respectively. The methods of Larsen (1971), Patton and Crouch (1977), and (Young, 1990) were used to evaluate the levels of creatinine, blood urea nitrogen (BUN) and uric acid respectively. The methods of Beutler et al. (1963), Nishikimi et al. (1972), Ohkawa et al. (1979) and Aebi (1984) were used to measure the levels of serum glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA) and catalase (CAT) respectively. After blood sampling, rats were dissected and liver and kidney tis-

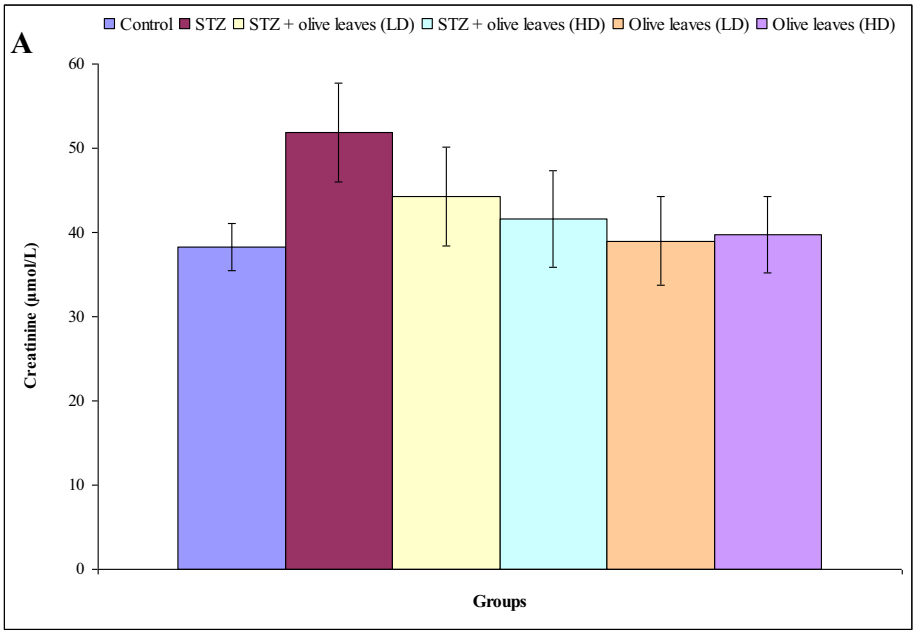
sues were collected from each group for histopathological examinations. The collected tissues were fixed in 10% buffered formaldehyde, sectioned and stained with hematoxylin and eosin. The resulting slides were observed under light microscope (Olympus BX61- USA) connected to motorized controller unit (Olympus bx-ucb- USA) and photographed by a camera (Olympus DP72- USA).

2.3. Statistical analysis

All results were expressed as mean ± standard deviation (S.D.). One-way analysis of variance (ANOVA) was used to evaluate differences among experimental groups. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for windows, version 22.0). The results were considered statistically significant when $P < 0.05$.

3. Results

The levels of serum glucose, ALT, AST, ALP, GGT and total bilirubin in all experimental groups are presented in Fig. 1A–F. The levels of serum glucose were significantly increased in diabetic rats of group 2 (340.0%), diabetic rats of group 3 treated with LD of olive leaves extract (190.2%) and diabetic rats of group 4 treated with HD of olive leaves extract (119.8%) compared with normal control rats of group 1. The levels of serum ALT (133.0%), AST (58.5%), ALP (137.9%), GGT (85.7%) and total bilirubin (56.6%) were statistically



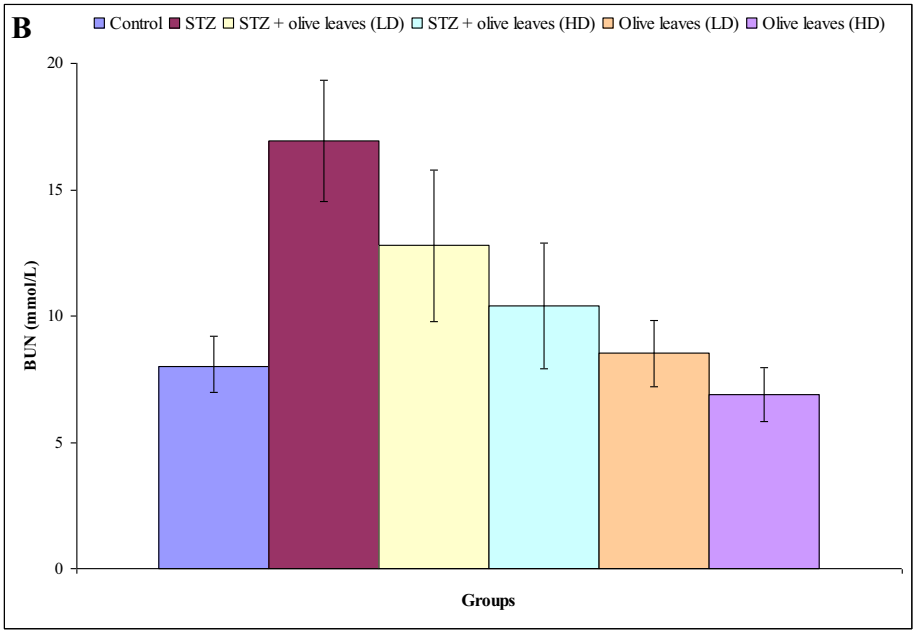


Fig. 2. (A–C) The levels of creatinine (A), BUN (B) and uric acid (C) in serum from control, STZ, STZ plus LD of olive leaves extract, STZ plus HD of olive leaves extract, LD of olive leaves extract and HD of olive leaves extract treated rats.

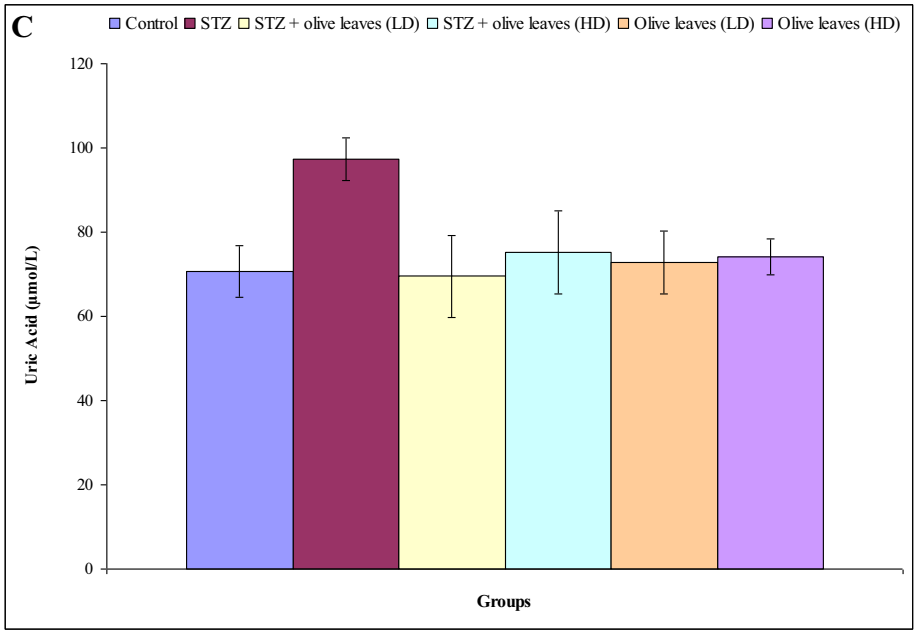


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Table 1
The levels of serum SOD, GSH, MDA and CAT of control, STZ, STZ plus LD of olive leaves extract, STZ plus HD of olive leaves extract, LD of olive leaves extract and HD of olive leaves extract treated rats. Percentage changes are included in parentheses.

Treatments	Parameters			
	SOD (U/ml)	GSH (µmol/ml)	MDA (nmol/ml)	CAT (U/ml)
Control	87.83 ± 2.51	21.07 ± 1.10	14.53 ± 1.31	2.29 ± 0.18
STZ	41.69 ± 2.89 (−52.5)	7.99 ± 0.91 (−62.1)	41.20 ± 8.41 (+183.6)	1.01 ± 0.11 (−55.9)
STZ + olive leaves extract (LD)	55.05 ± 5.33 (−37.3)	12.68 ± 1.50 (−39.8)	30.42 ± 4.38 (+109.4)	1.53 ± 0.20 (−33.2)
STZ + olive leaves extract (HD)	63.93 ± 6.00 (−27.2)	17.63 ± 1.87 (−16.3)	23.02 ± 5.25 (+58.4)	1.58 ± 0.28 (−31.0)
Olive leaves extract (LD)	87.65 ± 2.92 (−0.2)	20.79 ± 2.38 (−1.3)	15.68 ± 2.20 (+7.9)	2.30 ± 0.29 (+0.4)
Olive leaves extract (HD)	88.38 ± 2.41 (+0.6)	21.04 ± 1.71 (−0.1)	14.46 ± 1.69 (−0.5)	2.13 ± 0.43 (−7.0)

evoked in diabetic rats of group 2. The levels of serum ALT (73.8%), AST (18.3%), ALP (41.3%) GGT (49.1%) and were statistically evoked in diabetic rats of group 3, while the level of serum total bilirubin was unchanged compared with normal control rats of group 1. In diabetic rats of group 4, the levels of ALT (32.6%) and ALP (18.3%) were significantly enhanced, while the levels of AST, GGT and total bilirubin were statistically unchanged compared with normal control rats of group 1. Additionally, insignificant changes were noted in the levels of serum glucose, ALT, AST, ALP, GGT, and total bilirubin in normal rats treated with LD (group 5) and HD (group 6) of olive leaves extract.

Measured values for serum creatinine, BUN and uric acid in all groups are given in Fig. 2A–C. Statistically increases in the levels of creatinine (35.6%), BUN (112.2%) and uric acid (37.7%) were detected in diabetic rats of group 2. The levels of serum creatinine (15.7%) and BUN (60.2%) were significantly raised in diabetic rats of group 3, while the level of uric acid was unchanged compared with normal control rats of group 1. Serum BUN (30.3%) was significantly increased in diabetic rats of group 4, while the levels of creatinine and uric acid were unchanged compared with normal control rats of group 1. In comparison with normal control rats of group 1, normal rats supplemented with LD (group 5) and HD (group 6) of olive leaves extract showed insignificant alterations in the level of serum creatinine, BUN and uric acid.

Table 1 represents the levels of serum SOD, GSH, MDA and CAT in all groups. The levels of serum SOD (52.5%), GSH (62.1%) and CAT (55.9%) were significantly diminished in diabetic rats of group 2 compared with normal control rats of group 1. Likewise, the levels of serum SOD (37.3% and 27.2%), GSH (39.8% and 16.3%) and CAT (33.2% and 31.0%) were significantly declined in diabetic rats treated with LD and HD of olive leaves extract respectively compared with normal control rats of group 1. Noticeably increases of serum MDA in diabetic rats of group 2 (183.6%), diabetic rats exposed to LD of olive leaves extract (109.4%) and diabetic rats treated with HD of olive leaves extract (58.4%) compared with normal control rats of group 1. In comparison with normal control rats of group 1, there were no significant alterations in the levels of serum SOD, GSH, MDA and CAT in normal rats treated with LD (group 5) and HD (group 6) of olive leaves extract

Histopathological examination indicated a normal structure of the liver in the normal control rats (Fig. 3A) as well as the diabetic rats treated with LD of olive leaves extract (Fig. 3C), the diabetic rats treated with HD of olive leaves extract (Fig. 3D), the normal rats treated with LD (Fig. 3E) and HD (Fig. 3F) of olive leaves extract. Liver structure of diabetic rats of group 3 showed several changes including disarrangement of hepatic strands, rupture in liver cells (hepatocytes), mild hepatocellular necrosis, dilation and congestion of blood vessels with mild hemorrhage, dense

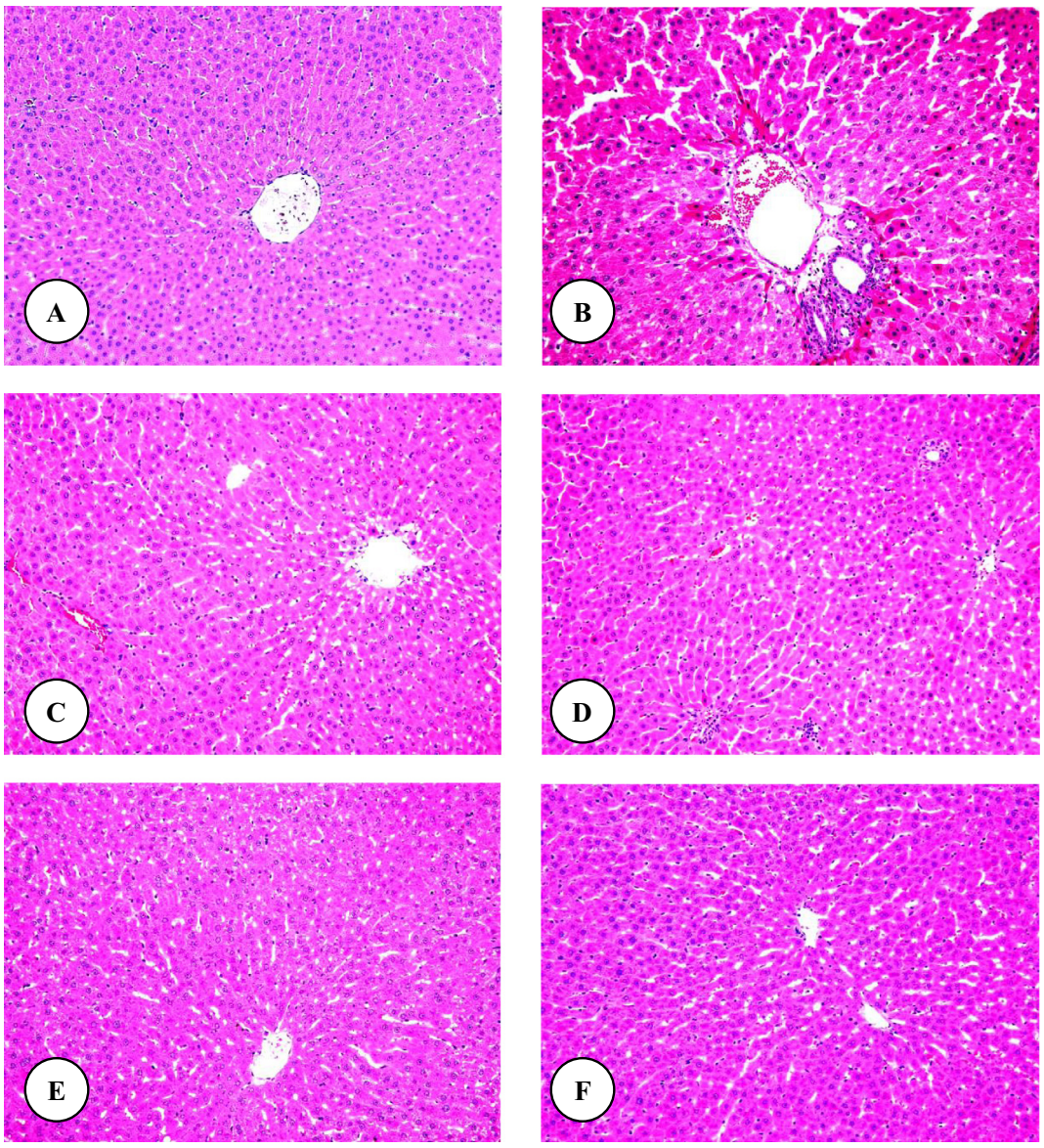

Fig. 3. (A–F) Photomicrographs of liver sections in each group. (A) Control, (B) STZ, (C) STZ plus LD of olive leaves extract, (D) STZ plus HD of olive leaves extract, (E) LD of olive leaves extract and (F) HD of olive leaves extract (×200) treated rats. Original magnification ×200.

lymphocytic infiltration around the central vein and dark stained hepatocytic nuclei indicating cell pyknosis (Fig. 3B). Histopathological examinations of kidney or renal sections from all groups are represented in Fig. 4A–H. Areas of renal cortex containing renal corpuscles and associated tubules were showed more pronounced changes in treated rats compared with normal control. Therefore, these areas were selected for histological examination with the light microscope. The normal renal corpuscle consists of a tuft of capillaries, the glomerulus, surrounded by a double walled epithelial capsule called Bowman's capsule. Between the two layers of the capsule is the urinary or Bowman's space (Fig. 4A). In comparison with normal control rats, the renal sections from diabetic rats treated with LD of olive leaves extract (Fig. 4E) and HD of olive leaves extract (Fig. 4F) showed normal structures. Moreover, no detectable histological differences are observed by the light microscope between renal sections of normal control and normal rats treated with LD (Fig. 4G) and HD (Fig. 4H) of olive leaves extract. In diabetic rats of group 2 there were pronounced alterations in the structure of renal corpuscles including hemorrhage, shrinkage, and a highly degeneration and necrosis of glomeruli and Bowman's capsules (Fig. 4B–D).

4. Discussion

The incidence and prevalence of DM have continuously been increased over the last 20 years. Meanwhile an estimated number of 387 million people worldwide suffer from DM (Aziz et al., 2015). Currently, beside insulin, the most widely used medication for DM is oral hypoglycemic drugs. Furthermore, clinical uses of the current drugs are accompanied by unpleasant side effects such as severe hypoglycemia, lactic acidosis, peripheral edema and abdominal discomfort (Lorenzati et al., 2010). Modern therapies are far too costly and also they are beyond the reach of tribal people to be practiced for the majority of DM refers; so the ethnopharmacological use of herbal remedies for the treatment of DM is an area of study, which ripe with potential as a starting point in the development of alternative, inexpensive therapies (Rajendran and Manian,

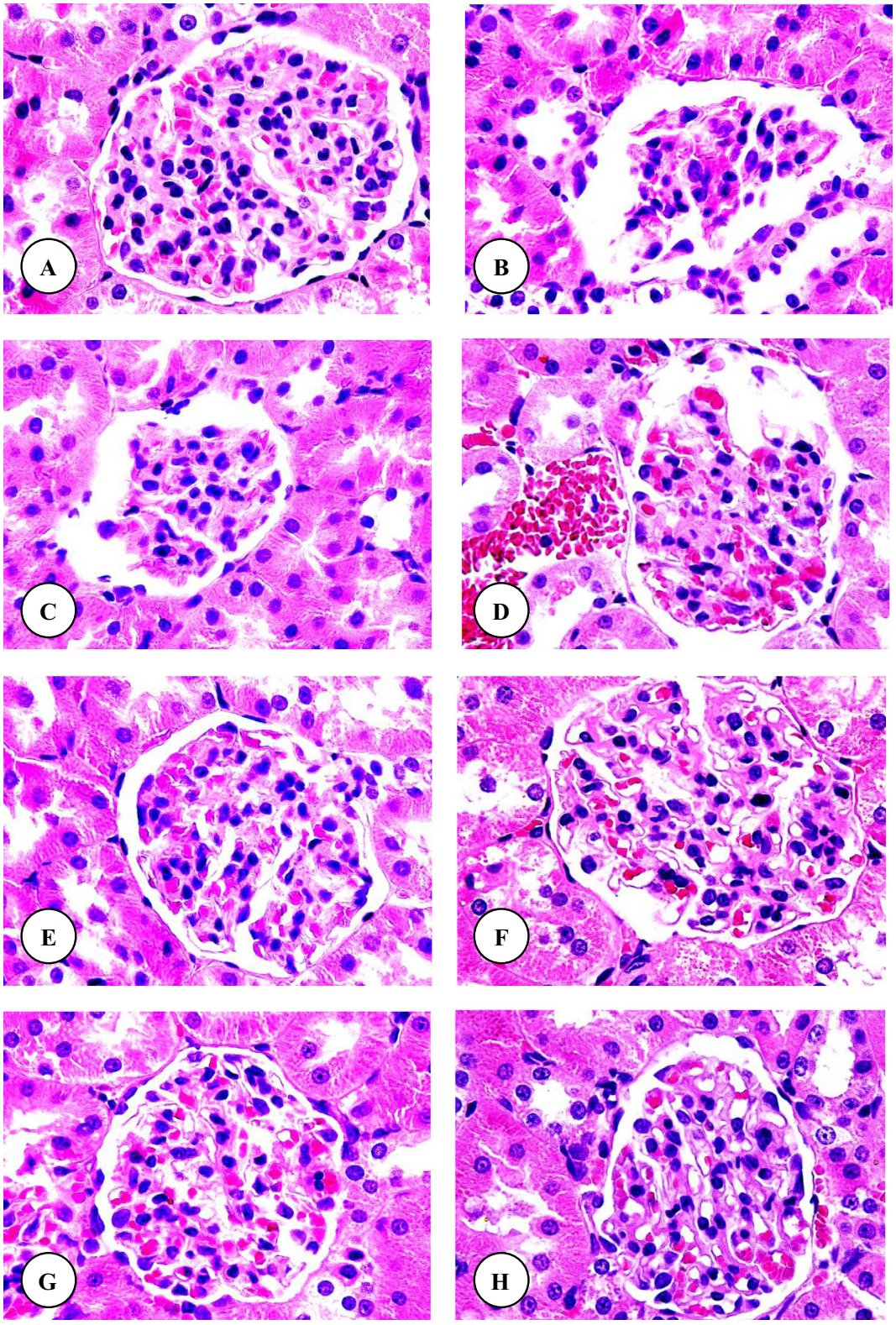

Fig. 4. (A–H) Photomicrographs of renal corpuscle in each group. (A) Control, (B, C and D) STZ, (E) STZ plus LD of olive leaves extract, (F) STZ plus HD of olive leaves extract, (G) LD of olive leaves extract and (H) HD of olive leaves extract treated rats. Original magnification ×1000.

2011). Therefore, the search for new antidiabetic agents with more effectiveness and less side effects has been continued. In the present study, untreated diabetic rats showed highly significant increases in the levels of serum glucose, ALT, AST, ALP, GGT, total bilirubin, creatinine, BUN, uric acid and MDA, while the levels of SOD, GSH and CAT were significantly decreased. Moreover, the histopathological examination of liver and kidney showed several changes. These findings are generally in agreement with previous experimental diabetes studies (Mohamed et al., 2009; Al-Attar and Zari, 2010; Abolfathi et al., 2012; Al-Musa and AL-Hashem, 2014; Nwaehujor et al., 2015; Roy et al., 2015; Hu et al., 2016; Sugumara et al., 2016; Hebi et al., 2017; Kou et al., in press).

The observed increase in the levels of ALT, AST, ALP, GGT and total bilirubin are the major diagnostic symptoms of hepatic damage and diseases (Hukkeri et al., 2002; Chatterjea and Shinde, 2005; Porchezhian and Ansari, 2005; Malarvizhi and Srinivasan, 2015). Moreover, serum or plasma enzyme levels have been used as markers for monitoring chemically induced tissue damages (Lin and Wang, 1986; Ngaha et al., 1989; Obi et al., 2001). The present liver injury was confirmed by the measurement of liver markers in serum associated with the histopathological changes of liver structure in untreated diabetic rats.

The present study demonstrates that untreated diabetic rats display a pronounced impairment in renal function which is confirmed by the enhancement of serum levels of creatinine, BUN and uric acid, and histopathological changes. BUN is a byproduct from protein breakdown. About 90% of urea produced is excreted through the kidney (Walmsley et al., 2010). Meanwhile, the creatinine is a waste product from a muscle creatinine, which is used during muscle contraction. Creatinine is commonly measured as an index of glomerular function (Treasure, 2003). BUN level can be increased by many other factors such as dehydration, antidiuretic drugs and diet, while creatinine is more specific to the kidney, since kidney damage is the only significant factor that increases the serum creatinine level (Cheesbrough, 1998). Additionally, creatinine is excreted exclusively through the kidney. Therefore, damage to the kidney will make the kidney inefficient to excrete both urea and creatinine and causes their accumulation in the blood. Therefore, the high level of blood urea and creatinine will indicate kidney damage (Dollah et al., 2012).

In the present study, the levels of serum SOD, GSH and CAT were significantly increased, while the level of MDA was decreased in STZ treated rats. These findings clearly showed that STZ induced oxidative stress in experimental diabetic rats. Diabetic complications are linked to hyperglycemia-induced oxidative stress which eventually overcomes the endogenous antioxidant defense system through glucose autoxidation, induction of nonenzymatic glycosylation of various macromolecules, and generation of reactive oxygen species (ROS) (Ademiluyi and Oboh, 2012). The human body possesses several enzymes associated with antioxidant defense and repair mechanisms against oxidative stress (Gul et al., 2013). Abundant clinical evidence demonstrated that the diabetes correlated closely with oxidative stress, resulting in an increased ROS production or a reduction in the antioxidant defense system (Susztak et al., 2006). The hyperglycemic in STZ-treated animals leads to the formation of hydrogen peroxide, which subsequently generates free radicals such as O^{2-} and OH^{-}. These reactive compounds can cause peroxidation of lipids, resulting in the formation of hydroperoxy fatty acids and endoperoxides (Pushparaj et al., 2000). However, there are many evidences demonstrated that the levels of these biochemical parameters were differed and changed in diabetic rats compared with non diabetic rats (Miao et al., 2015; Nwaehujor et al., 2015; Roy et al., 2015; Obi et al., 2016; Sheweita et al., 2016; Zhu et al., 2016).

From the present study, It is obviously that the treatment of diabetic rats with LD and HD of olive leaves extract attenuated the highly increases of serum glucose, ALT, AST, ALP, GGT, total bilirubin, creatinine, BUN and uric acid. Furthermore, the histopathological examination showed that the treatment of diabetic rats with LD and HD of olive leaves extract protected the hepatic and renal structures. Administration of LD and HD of olive leaves extract led to reduce the severe alterations of serum SOD, GSH, MDA and CAT in diabetic rats. These findings proved that LD and HD of olive leaves extract play a protective role against hepatorenal injury in diabetic rats. Moreover, the highly treatment efficiency was observed in diabetic rats treated with HD followed by LD of olive leaves extract. Al-Janabi et al. (2013) showed that the use of olive leaves extract improved the levels of blood glucose, albumin, total protein and creatinine of diabetic rats induced by STZ. Furthermore, the administration of olive leaves extract to diabetic rats caused a modulation in the regeneration of β-cells of pancreas, compared with diabetic rats. Laaboudi et al. (2016) demonstrated that the oral administration of olive fruits and leaves extracts decreased the levels of serum glucose, total protein, total cholesterol, triglycerides, LDL-C, ALT, AST, creatinine, urea and uric acid, and increased the level of HDL-C in STZ diabetic male rats. Sakr et al. (2016) showed that injection of STZ provoked a significant increase in serum glucose, ALT, AST, total cholesterol, triglycerides, LDL-C, VLDL-C, while the level of serum HDL-C was significantly decreased in male rats. Moreover, serum MDA was increased and the antioxidant enzymes SOD and CAT decreased. Diabetic rats showed many histopathological alterations in the structure of pancreas and liver. When diabetic rats treated with olive leaves extract, an improvement was observed in the biochemical parameters and histology of pancreas and liver. They concluded that the ameliorative effect of olive leave extracts against toxicity of diabetes in rats may be attributed to the presence of its phenolic compounds. Most phenolic and flavonoids compounds were described as having antioxidative action in living systems, as they act as scavengers of free radicals (Rice-Evans et al., 1997). Polyphenols of olive leaves, especially oleuropein, have interesting effects on the human body such as antioxidant capability, antihypertensive, hypoglycemic, hypocholesterolemic factors (Vogel et al., 2014). Based on above mentioned observations, it can be concluded that the olive leaves extract supplementation is beneficial in lowering the level of blood glucose and associated hematobiochemical parameters, and histopathological alterations in experimental diabetic rats. The hepatorenal protective role of LD and HD of olive leaves extract attributed to its polyphenolic components which act as antioxidant factors.

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