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Effect of *Olea europaea* leaves extract on streptozotocin induced diabetes in male albino rats



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ABSTRACT

The present study was aimed to evaluate the effect of olive (*Olea europaea*) leaves extract on streptozotocin (STZ)-induced diabetic male rats. The experimental rats were divided into six groups. Rats of the first group were served as normal controls. Rats of the second group were diabetic control. The third and fourth groups were diabetic rats, treated with olive leaves extract at low and high doses respectively. The fifth and sixth groups were non diabetic rats, subjected to olive leaves extract at the same doses given to the third and fourth groups respectively. The minimum of body weigh gain was noted in diabetic rats of the second group. The levels of serum glucose, insulin, total protein, albumin, triglycerides, cholesterol, low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), creatine kinase (CK), lactate dehydrogenase (LDH) and malondialdehyde (MDA) were significantly increased, while the levels of high density lipoprotein cholesterol (HDL-C), superoxide dismutase, (SOD) glutathione (GSH) and catalase (CAT) were statistically decreased in diabetic rats of the second group. The levels of liver insulin receptor substrate 1 (IRS1) and insulin receptor A (IRA) were significantly declined in diabetic rats of the second group. The diabetic pancreatic sections from diabetic rats of the second group showed several histopathological changes. Administration of low and high doses of olive leaves extract improved the observed physiological, molecular and histopathological alterations. Collectively, the obtained results confirmed that the protective effects of olive leaves extract are attributed to the antioxidant activities of olive leaves extract and its active constituents.

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1. Introduction

Diabetes mellitus (DM) is defined as a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, protein and lipid metabolism resulting from defects in insulin secretion, insulin action, or both. The clinical diagnosis of diabetes is often indicated by the presence of symptoms such as polyuria, polydipsia, and unexplained weight loss, and is confirmed by measurement of abnormal hyperglycemia (WHO and IDF, 2006). The World Health Organization (WHO) estimates that over 300 million people worldwide will have DM by the year 2025 (Park et al., 2011). Glycemic management in type 2 DM

has become increasingly complex and, to some extent, controversial, with a widening array of pharmacological agents now available, mounting concerns about their potential adverse effects and new uncertainties regarding the benefits of intensive glycemic control on macrovascular complications. Many clinicians are therefore perplexed as to the optimal strategies for their patients (Bolen et al., 2007; Matthews and Tsapas, 2008; Greenfield et al., 2009; Skyler et al., 2009; Bergenstal et al., 2010; Blonde, 2010; Nolan, 2010; Nyenwe et al., 2011; Yudkin et al., 2011).

Experimentally, DM can be induced by selective destruction of the insulin-producing β -cells of the pancreas with a single, rapid injection of streptozotocin (STZ), a glucose moiety with a very reactive nitrosourea group from the mould *Streptomyces griseus*. STZ has been used as diabetogenic factor in experimental animals (Al-Attar and Zari, 2007; Zari and Al-Attar, 2007; Al-Attar and Zari, 2010; Al-Attar, 2010a,b; Shirali et al., 2013; Hsu et al., 2015; Emordi et al., 2016).

In modern medicine, there is no satisfactory effective therapy to cure DM. The management of DM by insulin therapy has several drawbacks like insulin resistance (Wild et al., 2004) and in chronic treatment cause anorexia nervosa, brain atrophy and fatty liver

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(Tobias et al., 2001). Medicinal plants have always been an important source for finding new remedies for human health problems. Traditionally, numerous herbs have been recommended for treatment of DM. Additionally, antidiabetic effects of so many plants have been reported by many researchers (Ghorbani, 2013). The olive tree (*Olea europaea* L.), family: Oleaceae and in particular, its leaves have been used for the treatment of different diseases. Olive leaves have been widely used in traditional remedies in European and Mediterranean countries (Abd El-Rahman, 2016). Experimental animal studies on different total olive leaves extract or their constituents have demonstrated hypotensive (Scheffler et al., 2008), antiarrhythmic (Somova et al., 2004), anti-atherosclerotic (Wang et al., 2008), hypoglycemic (Jemai et al., 2009; Wainstein et al., 2012) and vasodilator effects (Zarzuolo et al., 1991). Antimicrobial (Lee and Lee, 2010), antiviral (Micol et al., 2005), anti-tumor (Grawish et al., 2011) and anti-inflammatory activity (Süntar et al., 2010) were also reported. Therefore, the present study was carried out to investigate the protective potential of olive leaves extract on experimental diabetes induced by STZ in male rats.

2. Material and methods

2.1. Experimental animals

The experiments were done using male albino rats of Wistar strain, weighing 113.2–183.8 g. The experimental animals were maintained in controlled temperature (20 ± 1 °C), humidity (65%) and a 12-h dark-light cycle, with balanced food and free access to water. Rats were acclimatized to the laboratory conditions for one week prior to the initiation of experimental treatments. The protocol for these experiments was approved by the Committee on Experimental Animal Ethics of King Abdulaziz University.

2.2. Extraction of *O. europaea* leaves

Fresh leaves of olive (*O. europaea*) were directly collected from the olive tree plantation farms from Aljouf region of Saudi Arabia. The leaves were scientifically defined by the herbarium of Biological Sciences Department, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. The leaves were thoroughly washed and dried at room temperature. The fine quality of dried leaves was kept in dry plastic container until use for extract processes. The method of Al-Attar and Abu Zeid (2013) was used to prepare the extract with some modifications. The aqueous extract of leaves was prepared every two weeks. 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 period, the mixture was cooled at room temperature and it was gently subjected to an electric mixer for 20 min. Thereafter the solutions of olive leaves were filtered. Finally, the filtrates were evaporated in an oven at 40 °C to produce dried residues (active principles). With references to the powdered samples, the yield means of leaves extract were 20.3%. Additionally, the extract was stored in a refrigerator for subsequent experiments.

2.3. Induction of diabetes

Experimental DM was induced in overnight fasted rats by intraperitoneal injection of STZ (Sigma- Aldrich Corp, St. Louis, MO, USA) at a single dose of 60 mg/kg body weight dissolved in saline solution. After injection, the rats had free access to food and water. DM was allowed to develop and stabilize in these STZ-treated rats over a period of four days. DM was defined in these

rats using determination of fasting blood glucose levels. The blood glucose levels over than 17 mmol/L were considered as diabetic model rats.

2.4. Experimental treatments

A total of 60 rats including the normal ($n = 30$) and diabetic rats ($n = 30$) were randomly divided into six experimental groups, each group consisting of 10 rats. Rats of the first group were normal healthy control, intraperitoneally received saline solution. Rats of the second group were diabetic control. The third group was diabetic rats, administered orally with olive leaves extract at a low dose (LD) of 200 mg/kg body weight/day. The fourth group was diabetic rats, administered 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. The duration of these experimental treatments was eight weeks.

2.5. Body weight changes

Rats body weights were evaluated at the start of the experimental duration and after eight weeks using a digital balance. Body weights were measured at the same time during the morning (Al-Attar and Zari, 2010). The experimental animals were observed for signs of abnormalities throughout the period of study.

2.6. Blood sampling

At the end of eight weeks, the experimental animals were fasted for 12 h, water was not restricted, and then blood samples were drawn from diethyl ether anaesthetized rats via orbital venous plexus. Blood specimens were centrifuged at 2500 rpm for 15 min, and the clear samples of blood serum were separated and stored at -80 °C. Serum glucose was measured according to the method of Trinder (1969). Serum insulin was determined using BIOSOURCE INS-ELISA kit according to the method of Judzewitsch et al. (1982). The levels of serum total protein and albumin were estimated using the methods of Peters (1968) and Dumas et al. (1971) respectively. Serum triglyceride, cholesterol, high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were evaluated according to the methods of Fossati and Prinicip (1982), Richmond (1973), Warnick et al. (1983) and Friedewald et al. (1972) respectively. Level of serum very low density lipoprotein cholesterol (VLDL-C) was estimated using the following equation:

$$\text{VLDL-C} = \text{Triglycerides}/2.175$$

The method of Horder et al. (1991) was used to evaluate the level of serum creatine kinase (CK). Serum lactate dehydrogenase (LDH) was estimated according to the method of Weishaar (1975). 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.

2.7. Molecular determinations

Thirty milligram of the liver tissues were taken as early as possible before rats death and were immediately kept in liquid nitrogen until be used for determination of insulin receptor substrate 1

(IRS1) and insulin receptor A (IRA) gene expressions. Liver IRS1 and IRA gene expressions were quantified using real time PCR. Total RNA was extracted from the tissue samples using the RNeasy Mini Kit Qiagen (Germantown, USA, Catalog no. 74104). After testing different RNA concentrations and assessed their quality control, 0.5 µg of total RNA was reverse transcribed into first strand cDNA using tow Step RT-PCR kit (Qiagen Long Range 2 Step RT-PCR Kit) (Germantown, USA, Catalog No. 205920). Five µL of cDNA was mixed with 12.5 µL of 2x SYBR[®] Green PCR mix with ROX from BioRad and 10 pmol/µL of each forward and reverse primer for the measured genes. The house keeping gene β -actin was used as a constitutive control for normalization. Primers were designed using primer3 software (<http://bioinfo.ut.ee/primer3/>) as per the published rat gene sequences and as previously published (Ali et al., 2014).

IR-A, F 5'TTCATTCAGGAAGACCTTCGA3\
R 5'AGGCCAGAGATGACAAGTGAC 3\
IRS-1 F 5'ATGGCCCTGTGGATGCGCTT3\
R 5'TAGTTGCAGTAGTTCTCCAGCT3\
and β -actin F 5'TCACTATCGCAATGTGCGG3\
R 5'GCTCAGGAGGCAATGATG 3'.

All primaries were provided by Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). PCR reactions were carried out in ABI PRISM 7300 (Applied Biosystems, USA). The RNA concentration in each sample was determined from the threshold cycle (Ct) values. The mRNA expression levels were calculated relative to β -actin gene's mRNA levels using $2^{-\Delta\Delta CT}$ method.

2.8. Histopathological examination

For histopathological examinations, pancreatic tissues were quickly isolated from each experimental group. Pancreatic tissues were preserved in 10% buffered formaldehyde. Preserved tissues were routinely processed, then embedded in paraffin, and cut into 4 µm thick sections; they were mounted on slides for hematoxylin and eosin staining. All sections were examined using light microscope (Olympus BX61-USA) connected to motorized controller unit (Olympus bx-ucb-USA) and photographed by a camera (Olympus DP72-USA).

2.9. Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for windows, version 22.0). Each value is expressed as mean \pm standard deviation (S.D.). One-way analysis of variance (ANOVA) was used to evaluate differences among experimental groups. The results were considered statistically significant if the *P*-values were less than 0.05.

3. Results

The body weights in all diabetic and non-diabetic groups are represented in Fig. 1. The percentage change of body weight gain in normal control rats was 141.9% after eight weeks. Likewise, the changes of body weight gain were 159.2% and 135.1% in LD (group 5) and HD (group 6) of olive leaves treated normal rats respectively. The percentage change of body weight was 42.1% in diabetic rats treated with normal diet (group 2). Significant decrease in the values of body weight gain was observed in diabetic rats fed with normal diet. Similar observations were noted in diabetic rats supplemented with LD (group 3) and HD (group 4) of olive leaves extract. The changes of body weight gain were 75.9% and 74.6% in diabetic rats treated with LD and HD of olive leaves extract respectively. Supplementation with the olive leaves extract in diabetic rats showed remarkable lowering effect on the percentage changes of body weight compared with diabetic rats fed with normal diet (group 2).

Fig. 2A–D demonstrates the levels of serum glucose, insulin, total protein and albumin in all experimental groups. Remarkable elevations in the levels of serum glucose (340.0%), total protein (13.4%) and albumin (23.4%) were noted in diabetic rats (group 2) compared with normal control rats. The level of serum insulin was declined (53.1%) in group 2. Serum glucose levels were statistically increased in diabetic rats supplemented with LD (190.2%) and HD (119.8%) of olive leaves extract. Insignificant changes were noted in the levels of serum total protein and albumin in diabetic rats treated with LD and HD of olive leaves extract. The level of serum insulin was statistically decreased in rats supplemented with LD (32.5%) and HD (25.2%) of olive leaves extract. The levels of serum glucose, insulin, total protein and albumin were

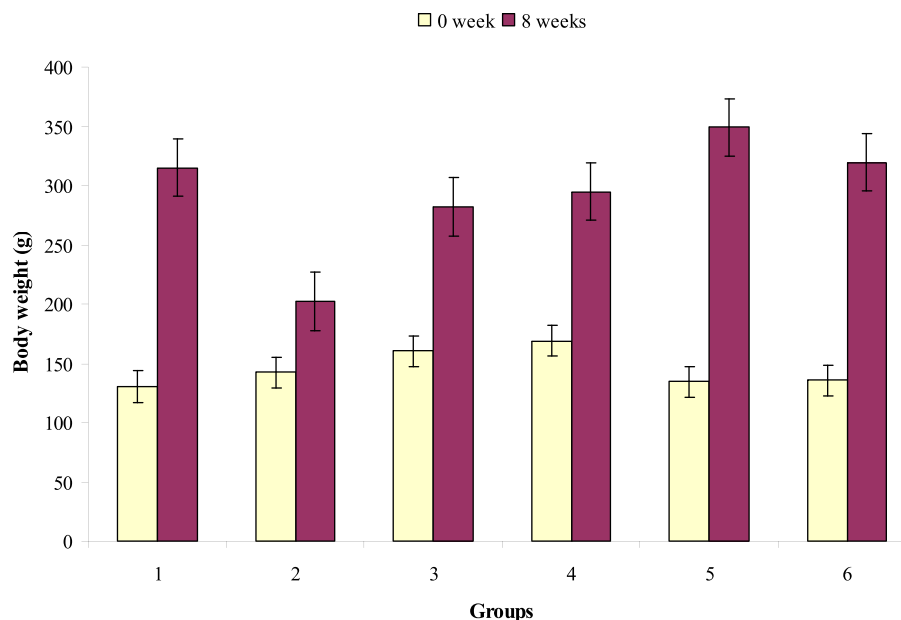


Fig. 1. Changes of body weight after eight weeks in control (group 1), STZ (group 2), STZ plus LD of olive leaves extract (group 3), STZ plus HD of olive leaves extract (group 4), LD of olive leaves extract (group 5) and HD of olive leaves extract (group 6) treated rats.

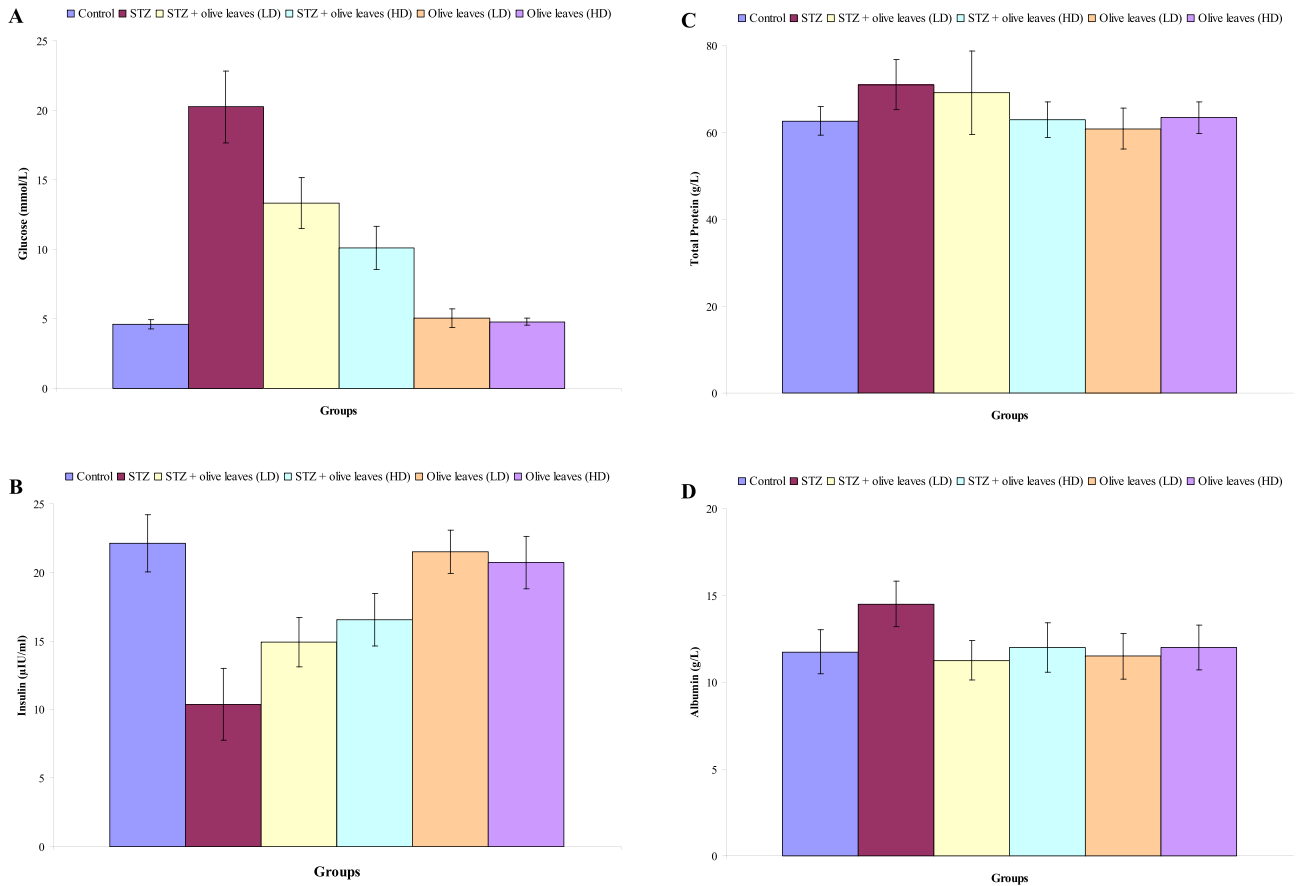


Fig. 2. (A–D) The levels of glucose (A), insulin (B), total protein (C) and albumin (D) in serum from control, STZ, STZ plus LD of olive leaves extract, STZ plus HD of olive leaves extract, LD of olive leaves extract and HD of olive leaves extract treated rats.

statistically unchanged in normal rats treated with LD (group 5) and HD (group 6) of olive leaves extract.

The levels of serum triglycerides, cholesterol, HDL-C, LDL-C and VLDL-C in all groups were presented in Fig. 3A–E. Serum triglycerides (45.6%), cholesterol (35.1%), LDL-C (19.4%) and VLDL-C (33.3%) were enhanced in diabetic rats (group 2) compared with normal control rats. Likewise, the levels of serum triglycerides (31.6%), cholesterol (12.7%), LDL (12.9%) and VLDL (25.9%) were increased in diabetic rats supplemented with LD of olive leaves extract (group 3). Serum HDL-C level was decreased in diabetic rats of group 2, while this parameter was unchanged in rats of group 3. Furthermore, insignificant alterations were observed in the levels of serum triglycerides, cholesterol, HDL-C, LDL-C and VLDL-C in diabetic rats of groups 4 and non diabetic rats of groups 5 and 6.

As it is shown in Fig. 4A–F, the levels of serum CK were significantly enhanced in diabetic rats (79.8%), and diabetic rats exposed to LD (62.8%) and HD (24.8) of olive leaves extract. The levels of serum LDH were significantly increased in diabetic rats (51.7%) and diabetic rats supplemented with LD of olive leaves extract (25.5%), while the level of serum LDH was unchanged in diabetic rats treated with HD of olive leaves extract. In comparison with control values, the levels of serum SOD (52.5%), GSH (62.1%) and CAT (55.9%) were statistically declined in diabetic rats (group 2). Similarly, 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 decreased in diabetic rats subjected to LD and HD of olive leaves extract respectively. The levels of serum MDA were markedly evoked in diabetic rats of group 2 (183.6%), diabetic rats treated with LD of

olive leaves extract (109.4%) and diabetic rats exposed to HD of olive leaves extract (58.4%). Moreover, insignificant changes in the levels of serum SOD, GSH, MDA and CAT were noted in normal rats treated with LD (group 5) and HD (group 6) of olive leaves extract.

Fig. 5A and B shows the levels average of liver IRS1 and IRA in all experimental groups. The levels of IRS1 were significantly declined in diabetic rats subjected to normal diet (73.6%), and diabetic rats supplemented with LD (52.3%) and HD (35.9%) of olive leaves extract compared with normal control rats. In comparison with normal control rats, notable decreases in the level of IRA were observed in diabetic rats exposed to normal diet (84.2%), LD of olive leaves extract (52.5%) and HD of olive leaves extract (38.14%). Additionally, non diabetic rats supplemented with LD and HD of olive leaves extract showed insignificant changes in the levels of IRS1 and IRA compared with normal control rats (Fig. 5).

Histopathological examination of the pancreas of normal control rats showed the normal structure, the histological appearance of the pancreatic (Langerhans) islet cells showed no histopathological alterations (Fig. 6A). Similar observations were noted in pancreatic tissues of non diabetic rats treated with LD (Fig. 6G) and HD (Fig. 6H) of olive leaves extract. The diabetic pancreatic sections from group 2 showed several changes including a mild and severe degeneration and damage of Langerhans islet with an obvious of β -cells necrosis and destruction, and a decrease of its number (Fig. 6B–D). Histopathological examination of pancreatic sections from groups 3 and 4 showed a mild degeneration and damage of Langerhans islet.

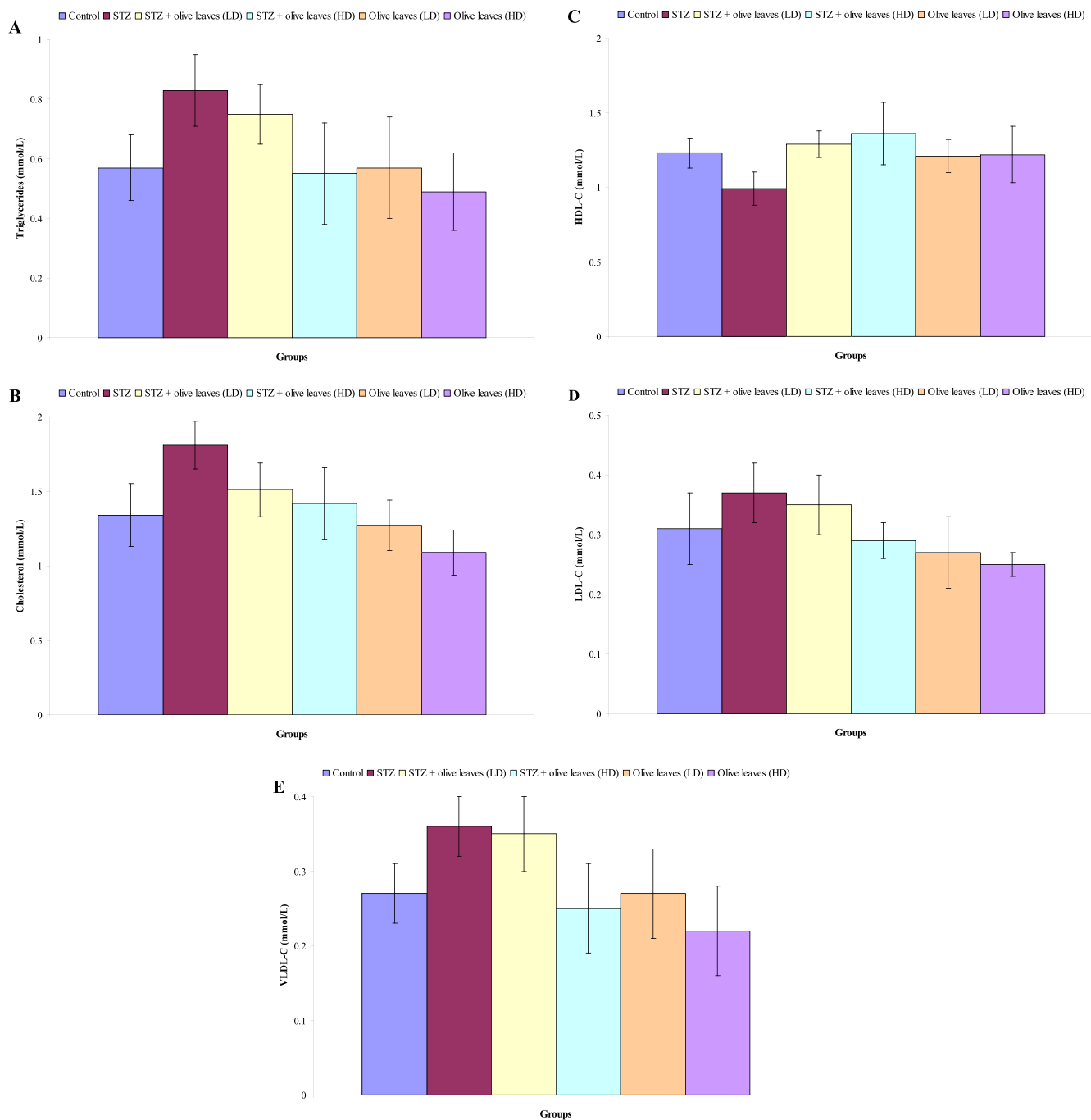


Fig. 3. (A–E) The levels of triglycerides (A), cholesterol (B), HDL-C (C), LDL-C (D) and VLDL-C (E) in serum from control, STZ, STZ plus LD of olive leaves extract, STZ plus HD of olive leaves extract, LD of olive leaves extract and HD of olive leaves extract treated rats.

4. Discussion

DM is a chronic condition that grows the most, especially in developing countries. The disease is highlighted for the severity of its complications, in addition to being considered a public health problem in terms of population growth and aging, greater urbanization, the increasing prevalence of obesity and sedentarism, as well as the increased survival rate from people with DM (Whiting et al., 2011). In the present study, the extract of olive leaves (200 and 400 mg/kg body weight) was investigated for antidiabetic activity on STZ diabetic rats. significant declines of body weight gain in STZ-diabetic rats were noted after eight weeks.

Similar observations were noted in many experimental diabetes researches (Zari and Al-Attar, 2007; Al-Attar, 2010b; Al-Attar and Zari, 2010; Salahuddin et al., 2010; Iweala et al., 2013; Jayaprasad et al., 2015, 2016; Zhang et al., 2016). An increase in body weight implies that anabolic effects have overridden the catabolic ones. No variation means protection against weight loss. Decrease in body weight would mean that catabolism has persisted. The destruction of β -cells and disorder of insulin secretion in the diabetic state causes physio-metabolic abnormalities such as a decrease in body weight gain and increase in food and water intake and urine volume. The diabetic rats induced by STZ also showed these changes (Kang et al., 2006). STZ induced diabetes

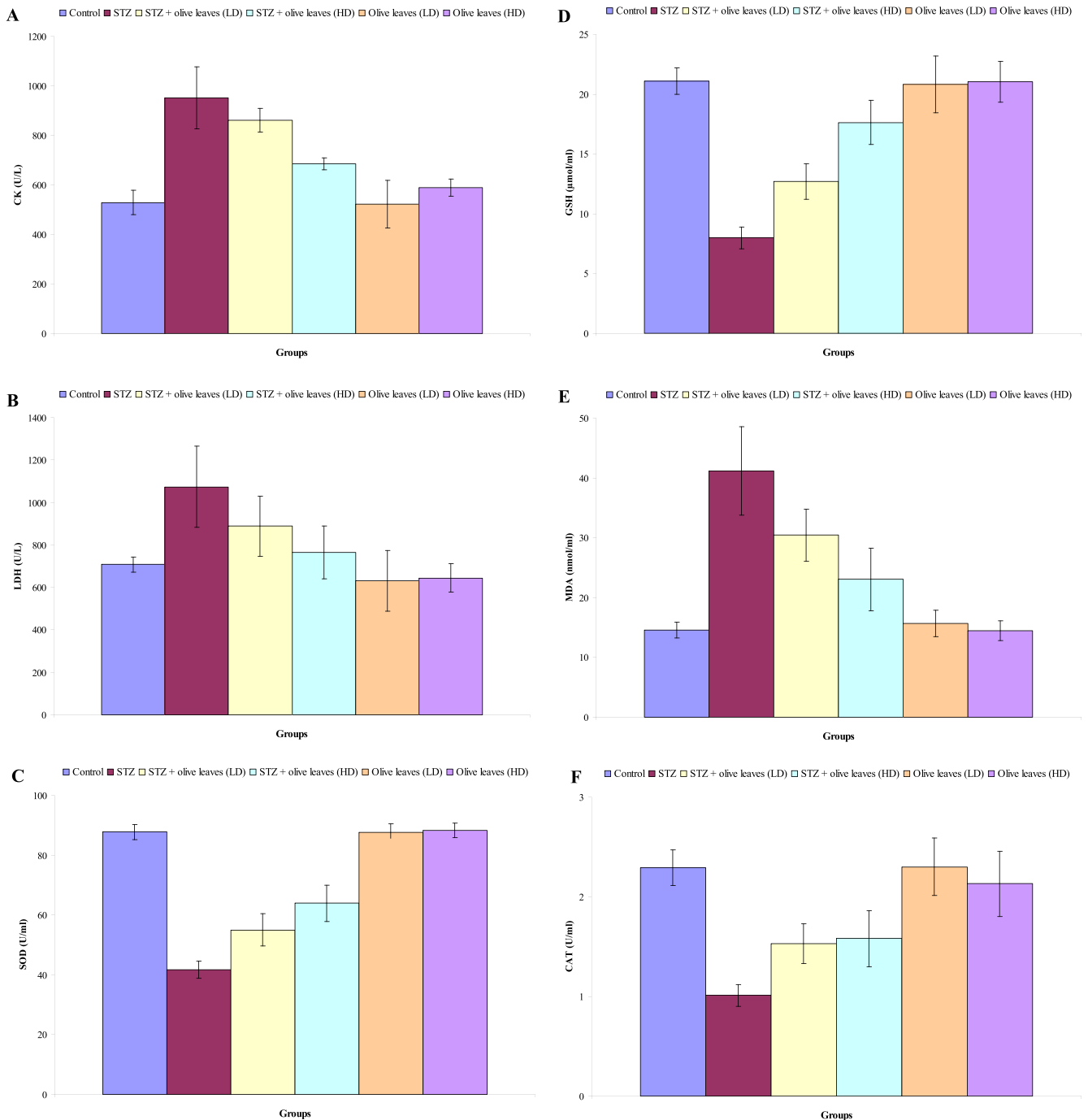


Fig. 4. (A–F) The levels of CK (A), LDH (B), SOD (C), GSH (D), MDA (E) and CAT (F) in serum from control, STZ, STZ plus LD of olive leaves extract, STZ plus HD of olive leaves extract, LD of olive leaves extract and HD of olive leaves extract treated rats.

is characterized by severe weight loss (Al-Shamaony et al., 1994). The decrease in body weight in diabetic rats might be the result of protein wasting due to unavailability of carbohydrate as an energy source (Chen and Ianuzzo, 1982). Increased food consumption and decreased body weight observed in diabetic rats in comparison to normal rats indicates a polyphagic condition and weight loss due to excessive breakdown of tissue proteins (Chatterjea and Shinde, 2002).

In the present study, the levels of serum glucose, insulin, total protein, albumin, triglycerides, cholesterol, LDL-C and VLDL-C were significantly higher, while the level of HDL-C was statistically

decreased in diabetic rats of group 2. These observations are generally in agreement with other investigations on STZ and alloxan induce relative influences (Zari and Al-Attar, 2007; Al-Attar, 2010b; Al-Attar and Zari, 2010; Kasetti et al., 2010; Salahuddin et al., 2010; Amraie et al., 2015; Achi et al., 2016; Hu et al., 2016; Zhang et al., 2016).

The present hyperglycemia is confirmed by statistically decreases of serum insulin and the levels of liver IRS1 and IRA, and histopathological alteration of pancreatic islets. However, several studies showed that the hyperglycemia associated with many changes of pancreatic islets structure (Yazdanparast et al., 2005;

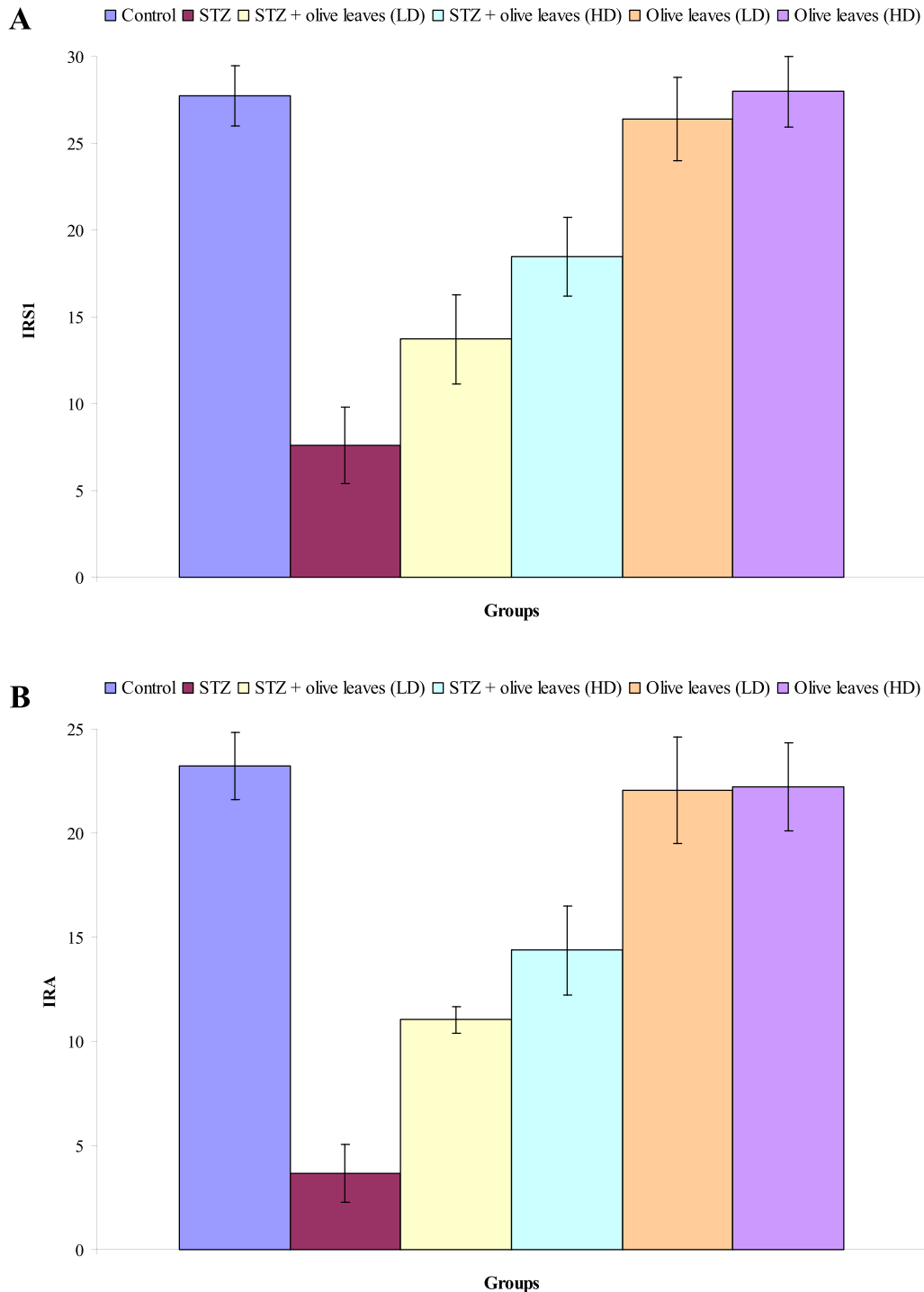


Fig. 5. (A and B) The levels of IRS1 (A) and IRA (B) in liver from control, STZ, STZ plus LD of olive leaves extract, STZ plus HD of olive leaves extract, LD of olive leaves extract and HD of olive leaves extract treated rats.

Eliakim-Ikechukwu and Obri, 2009; Qadori, 2011; Mohammadi and Naik, 2012; Rifaai et al., 2012; Fasola et al., 2016; Wahba et al., 2016).

The adequate binding of insulin to its receptor is influenced by the concentration of circulating insulin, the concentration of receptors and the affinity of the receptor for insulin (Kahn, 1994). In DM and other insulin resistant states such as obesity, higher concentrations of circulating insulin will decrease insulin receptor (IR) concentration in a dose-dependent manner in a process known as down regulation (Gavin et al., 1974). IRS molecules are key medi-

ators in insulin signaling and play a central role in maintaining basic cellular functions such as growth, survival and metabolism. They act as docking proteins between the IR and a complex network of intracellular signaling molecules (Sesti, 2000). At the molecular level, insulin resistance is correlated with impaired insulin signaling. This can involve dysfunction of cell surface components, such as IR as well as intracellular components like IRS 1 family of docking proteins and other elements of insulin signaling and glucose transport pathway (Lamothe et al., 1998). Hepatic insulin resistance is manifested by the blunted ability of insulin

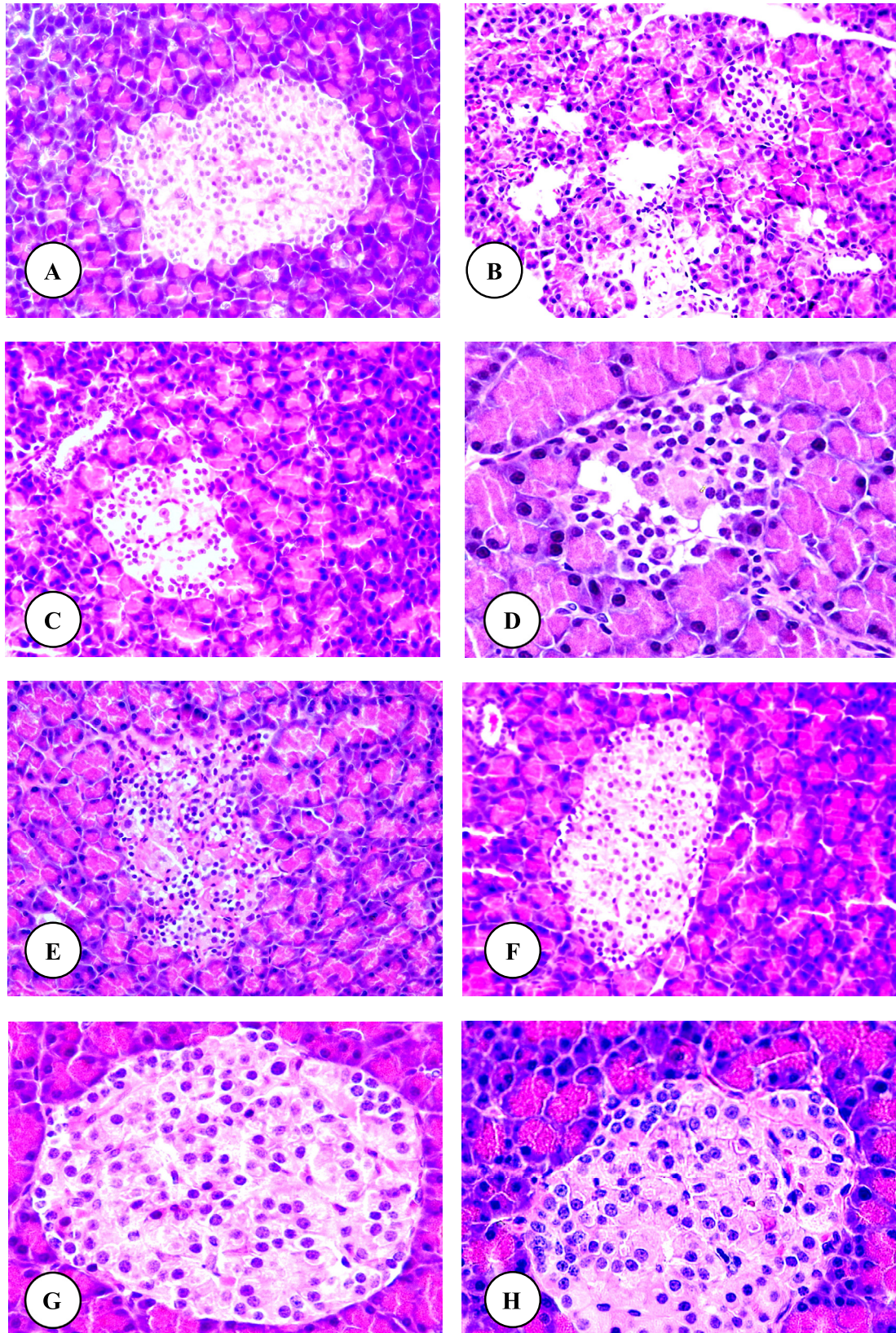


Fig. 6. (A–H) Photomicrographs of pancreas sections in each group. (A) control (X200), (B, C and D) STZ (X200, X200 and X400), (E) STZ plus LD of olive leaves extract (X200), (F) STZ plus HD of olive leaves extract (X200), (G) LD of olive leaves extract (X200) and (H) HD of olive leaves extract (X200) treated rats.

to activate its receptor kinase and its downstream targets, resulting in incomplete suppression of hepatic glucose production and therefore a manifest hyperglycemia (Diaz-Castroverde et al.,

2016). From the present study, the resistance of insulin could be to insulin binding defect caused by the decline of IR level. Another explanation for this case is to the affinity or defects at the level

of effector molecules such as glucose transports and enzymes involved in glucose metabolism (Sechi and Bartoli, 1997; Kim et al., 2000).

The present increase in the levels of serum total protein, albumin, triglycerides, cholesterol, LDL-C and VLDL-C with the decrease in the level of serum HDL-C indicate disturbances in protein and lipid metabolism in diabetic rats. Hyperproteinemia and hyperalbuminemia associated with dysfunctions of liver and kidney, and the increased rate of body water loss. An increase in serum albumin indicates poor liver function or impaired synthesis and it may be either in liver cells damage or diminished protein intake (Kalaiselvi et al., 2015). Dyslipidemia, characterized by abnormally elevated plasma triacylglycerol and cholesterol concentrations, is an established risk factor in the development of coronary heart disease (Al-Attar, 2010c). A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency, are responsible for the observed accumulation of lipids (Rajalingam et al., 1993). The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the blood. Further, it has been reported that diabetic rats treated with insulin shows normalized lipid levels (Pathak et al., 1981). Additionally, it is well known that in uncontrolled DM, there will be increase in triglycerides, cholesterol, LDL-C and VLDL-C with decrease in HDL-C, all of which contribute to the coronary artery disease seen in some diabetic patients (Palumbo, 1998; Arvind et al., 2002). Moreover, The diabetics have abnormal lipid metabolism due to insulin deficiency in the body as a result of STZ induced damage to pancreatic β cells. Insulin can activate lipoprotein lipase, the enzyme lipoprotein solver. On the diabetic condition of the enzyme lipoprotein lipase activity decreased so that the levels of lipoproteins in the blood increases (Johnston and Gass, 2006).

The present study showed that the levels of serum CK and LDH were significantly enhanced in control diabetic rats. The serum level of muscle enzymes is a marker of the functional status of muscle tissue and varies widely in both pathological and physiological conditions. CK has since become an important clinical marker for myocardial damage. Increased serum level of LDH is usually found in cellular death and/or leakage from cells or in some cases it is a useful marker of myocardial or pulmonary infarction (Onyeneke et al., 2007). However, the present high activity of serum CK and LDH demonstrated that the cellular membranes integrity of myocardial tissues may be disturbed. Moreover, several studies showed that the levels of CK and LDH were significantly increased in diabetic rats (Alnahdi, 2012; Nasry et al., 2013; Giribabu et al., 2016; Mohammed et al., 2016; Suanarunsawat et al., 2016).

The present study showed that STZ induced oxidative stress which confirmed by the increases of serum GSH, SOD and CAT levels, and a decrease of MDA level. Moreover, several investigations showed that these parameters were differed and changed in diabetic rats compared with normal control rats (Sen et al., 2011; Kharazi-Nejad et al., 2014; Adewoye and Adele, 2015; Gao et al., 2015; Miao et al., 2015; Nwaehujor et al., 2015; Roy et al., 2015). In the past two decades, it has become increasingly clear that oxidative stress plays a major role in the pathogenesis of a number of human diseases such as atherosclerosis, chronic renal failure, ischemia/reperfusion injury, neurodegenerative diseases, hypertension, cancer and DM (Mates et al., 1999). Although the pathophysiology of diabetic complications is multifactorial, animal and human studies suggest a role for oxidative stress via an increased formation of reactive oxygen species (ROS) (Jennings et al., 1987; Low and Nickander, 1991). Oxidative stress is a constant feature of uncontrolled diabetes in humans and animals (Horie et al., 1997). Additionally, the decrease in antioxidant enzymes activity under diabetic conditions could be due to glycation of these enzymes, which occurred at persistently elevated

blood glucose levels (Taniguchi, 1992). However, oxidative stress is the result of a redox imbalance between the generation of ROS and the compensatory response from the endogenous antioxidant network. There is no consensus concerning the changes in the activities of antioxidant enzymes in diabetic rats (Almeida et al., 2012).

In spite of the previous studies focused on the effect of olive leaves in diabetic animals, the present study revealed for the first time the mechanism action of two doses (LD and HD) of olive leaves extract in diabetes induced by STZ in male rats. Oleuropein, a phenolic secoiridoide, is used as a well-known compound of extracts and its concentration is significantly high in olive leaves and fruit. Additionally, olive leaves contain hydroxytyrosol, tyrosol, and caffeic acid were identified as the major active components. Moreover, olive leaves contain p-coumaric acid, vanillic acid, vanillin, luteolin, diosmetin, rutin, luteolin-7-glucoside, apigenin-7-glucoside and diosmetin-7-glucoside have been identified as therapeutic agents delaying the progression of advanced glycation end products-mediated inflammatory diseases such as diabetes (Chandler et al., 2010).

The present study showed that the treatment of diabetic rats with LD and HD of olive leaves extract improved the alteration levels of glucose, insulin, IRS1 and IRA, protein and lipid profiles, cardiac markers, oxidation processes, and protected the pancreatic histopathological changes. Furthermore, the most improvements were noted in diabetic rats subjected to HD followed by LD of olive leaves extract. From the present study, it is obviously that the role of olive leaves extract on hyperglycemia may be attributed to one or more of the following factors: (1) The inhibition of oligosaccharides and polysaccharides digestion with lowering secretion of their specific hydrolytic enzymes. (2) The diminution of intestinal glucose and other monosaccharides absorption rate. (3) The enhancement of glycogenesis, and decline of glycogenolysis and gluconeogenesis processes. (4) The increase of cellular glucose uptake. (5) The improvement of β -cells and its regeneration with the increase of insulin secretion. (6) The protection and improvement of insulin receptors, and reduction and suppression of insulin resistance. These explanations of hypoglycemic role of olive leaves extract are partially consistent with several previous investigations (Pari and Saravanan, 2002; Eidi et al., 2009; Wainstein et al., 2012; Moghaddam et al., 2013; Laaboudi et al., 2016). Moreover, the antioxidant activities of LD and HD of olive leaves extract were confirmed by the present evaluations of oxidative stress markers in diabetic rats. However, several studies showed that the hypoglycemic effect of olive leaves extract was attributed to the antioxidant properties of its constituents (Al-Azzawie and Alhamdani, 2006; Jemai et al., 2009; El-Amin et al., 2013; Farah, 2015; Laaboudi et al., 2016). Furthermore, the antioxidant activities of olive leaves extract led to inhibition of physiological, molecular and histopathological alterations in diabetic rats. Accordingly, it can be concluded that olive leaves extract has a beneficial influence on DM and its complications. Finally, additional experimentation is required to elucidate the influences of different concentrations and doses of olive leaves extract and its constituents as promising therapeutic agents for DM and its complications.

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