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Influence of *Olea oleaster* Leaves Extract on Some Physiological Parameters in Streptozotocin-Induced Diabetic Rats

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Abstract: The current study was intended to investigate the influence of wild olive (*Olea oleaster*) leaves extract on some physiological parameters in streptozotocin (STZ) induced diabetes in male Wistar rats after four weeks. The experimental rats were divided into four groups. Rats of the first group were served as normal controls. Rats of the second group were diabetic controls. Rats of the third group were diabetic rats, treated with olive leaves extract. Rats of the fourth group were non diabetic rats, treated with olive leaves extract. In diabetic rats of the second group, the levels of serum glucose, triglycerides, low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL), creatine kinase (CK) and lactate dehydrogenase (LDH) were significantly increased. However, the level of serum albumin was significantly decreased. Administration of olive leaves extract improved most observed physiological changes. Therefore, the results of this study proved the physiologically effective components of wild olive leaves extract which exert protective influence on diabetic rats.

Key words: *Olea Oleaster* Olive Leaves · Diabetes · Streptozotocin · Blood · Rats

INTRODUCTION

Diabetes mellitus (DM) is a metabolic syndrome distinguished by chronic hyperglycemia with disturbances of carbohydrate, protein and lipid metabolism resulting from deficiencies in insulin secretion, insulin action, or both. Its diagnosis is frequently indicated by the presence of symptoms such as polyuria, polydipsia and weight loss and is proved by measurement of abnormal hyperglycemia [1]. The World Health Organization (WHO) approximates that over 300 million people worldwide will have diabetes mellitus by the year 2025 [2]. Glycemic management in type 2 diabetes has become difficult with several pharmacological agents now available, raising concerns about their possible adverse effects and new uncertainties about the advantages of intensive glycemic control on macrovascular complications. As a consequence, many physicians are perplexed as to the best strategies for their patients [3-5].

There is no suitable efficient therapy to treat diabetes in contemporary medicine. Diabetes management by insulin therapy has several problems such as insulin resistance [6] and in chronic treatments causes anorexia nervosa, fatty liver and brain atrophy [7]. DM may be induced in experimental animals by destruction of β-cells of the pancreas with a single injection of streptozotocin (STZ). STZ has been used as diabetogenic factor in several studies [8-11].

Medicinal plants have often been a significant source for finding new remedies for human health problems. Many herbs have traditionally been recommended for diabetes treatment. Furthermore, antidiabetic effects of so various plants have been reported by several investigators [12, 13]. The olive tree *Olea oleaster* (family: Oleaceae) has been used for the treatment of different diseases. Olive leaves have been extensively used in traditional medicine in Mediterranean and European countries [14]. Animal studies on olive leaves extracts or their components have revealed several therapeutic effects such as hypoglycemic [15, 16], anti-tumor [17] and antimicrobial activity [18]. Thus, the current study was performed to examine the protective influence of wild olive (*Olea oleaster*) leaves extract on some physiological parameters in STZ-induced diabetic rats.

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MATERIALS AND METHODS

Animals: Eighty male Wistar rats, weighing 180.10 to 219.50 g were used in this study. The experimental animals were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Rats were acclimatized to the laboratory conditions for one week prior to the initiation of experimental treatments. The experimental animals were housed in standard plastic cages and maintained under controlled laboratory conditions of humidity (55±10), temperature (24±1°C) and 12:12 h light: dark cycle. Rats were fed *ad libitum* on normal commercial chow and had free access to water. The experimental treatments were conducted in accordance with ethical guidelines of the animal care and use committee of King Abdulaziz University.

Extraction of *Olea oleaster* Leaves: Fine qualities of *O. oleaster* leaves were directly collected from the outskirts of Taif city in Saudi Arabia during July 2015. This plant was scientifically defined by the herbarium of Biological Sciences Department, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. The collected samples were completely washed, air dried at room temperature and stored in dry plastic container until use for extraction processes. The aqueous extracts were prepared every two weeks. The dried samples of *O. oleaster* (150 g) were powdered, added to 6 liters of hot water. After 5 h, the mixture was slowly boiled for 1 h. 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 the selected plant were filtered using 250 mm filter papers (Whatman, England). 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 mean of *O. oleaster* leaves extract was 18.5%. Furthermore, this extract was stored in a refrigerator for subsequent experiments.

Induction of Diabetes: MD was induced in overnight fasted rats by intraperitoneal (IP) 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. Diabetes was allowed to develop and stabilize in these STZ-treated rats over a period of four days. Diabetes was defined in these rats using determination of fasting blood glucose levels. The blood glucose levels over than 300 mg/dL were considered as diabetic model rats.

Experimental Design: The experimental rats were randomly divided into four experimental groups, 20 of rats each. This study was continues for 4 weeks. The experimental groups were treated as follows:

- Rats of group 1 were served as normal controls.
- Diabetic rats of group 2 were served as diabetic controls.
- Diabetic rats of group 3 were orally supplemented with *O. oleaster* leaves extract at a dose of 300 mg/kg body weight/day.
- Rats of group 4 were orally supplemented with *O. oleaster* leaves extract at a dose of 300 mg/kg body weight/day.

Body Weight Determinations: The body weights of rats were determined at the start of the experimental period and after 4 weeks using a digital balance. Therefore, the experimental animals were noted for signs of abnormalities throughout the period of study.

Blood Serum Analyses: After four weeks, rats were fasted for 8 hours; water was not restricted and anaesthetized with diethyl ether. Blood specimens were collected from orbital venous plexus in non-heparinized tubes. Blood specimens were centrifuged at 2500 rpm for 15 minutes and the clear samples of blood serum were separated and stored at –80 °C. These serum samples were used to determine the levels of glucose, total protein, albumin, triglycerides, cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), creatine kinase (CK) and lactate dehydrogenase (LDH).

The level of glucose was measured using the method of Trinder [19]. The method of Peters [20] was used to determine the level of t otal protein. The method of Doumas *et al*. [21] was used to estimate the level of albumin. To estimate the triglycerides value, Fossati and Prinicip method [22] was used. The method of Richmond [23] was used to measure the level of cholesterol. The method of Seguchi *et al*. [24] was used to determine the level of HDL-C. The level of serum LDL-C was measured according to the equation of Friedewald *et al*. [25].

LDL-C = Total cholesterol- HDL- triglycerides/ 5

Serum VLDL-C was evaluated using the following equation:

VLDL-C = Triglycerides/2.175

The value of CK was estimated according to the method of Hørder *et al*. [26]. The method of Weishaar [27] was used to measure the value of LDH.

Statistical Analysis: The data were analyzed using the Statistical Package for Social Sciences (SPSS for windows, version 22.0). Each value is expressed as mean ± standard deviation (S.D.). Data were analyzed using one-way analysis of variance (ANOVA), followed by LSD tests to determine differences between the mean values of experimental groups. Statistical significance was considered at $P < 0.05$.

RESULTS

Significant increase in the level of serum glucose was observed in diabetic rats of group 2 (+333.6%, $P < 0.0001$) compared with normal control rats of group 1. Insignificant changes were noted in the levels of serum glucose in diabetic (group 3) and non diabetic (group 4) rats treated with *O. oleaster* extract compared with normal control rats of group 1.

In comparison with control rats of group 1, there were no significant changes in the levels of serum total protein in STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats (Fig. 1).

There was a significant decrease in the level of serum albumin in the diabetic rats of group 2 ($P = 0.019$). However, there were no significant differences in the levels of serum albumin in diabetic rats of group 3 and non diabetic rats of group 4 compared with rats of group 1 (Fig. 2).

In comparison with control rats, there were no significant changes in the levels of serum cholesterol noted in all treated groups (Fig. 3).

In comparison with control rats of group 1, the level of serum triglycerides was significantly elevated in diabetic rats of group 2 (+ 54.8%, $P = 0.001$). Additionally, insignificant alterations of serum triglycerides levels were observed in rats of groups 3 and 4 compared with rats of group 1 (Fig. 4).

The level of serum HDL-C was insignificantly altered in rats of groups 2, 3 and 4 compared with normal control rats of group 1 (Fig. 5).

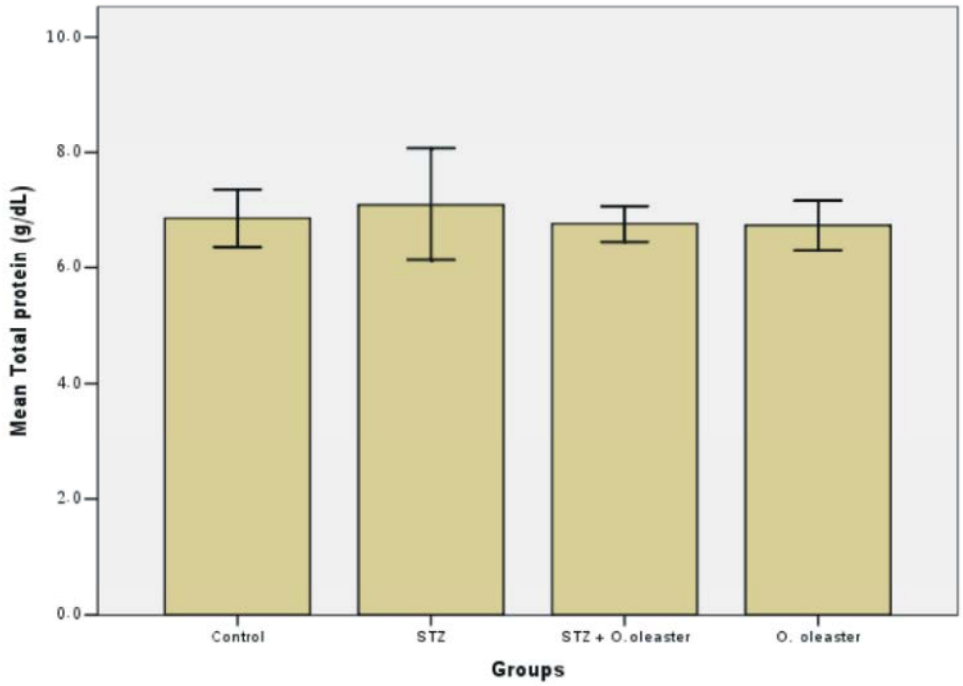

Fig. 1: Level of serum total protein in control, STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats after four weeks. Error bars: ±1 standard deviation

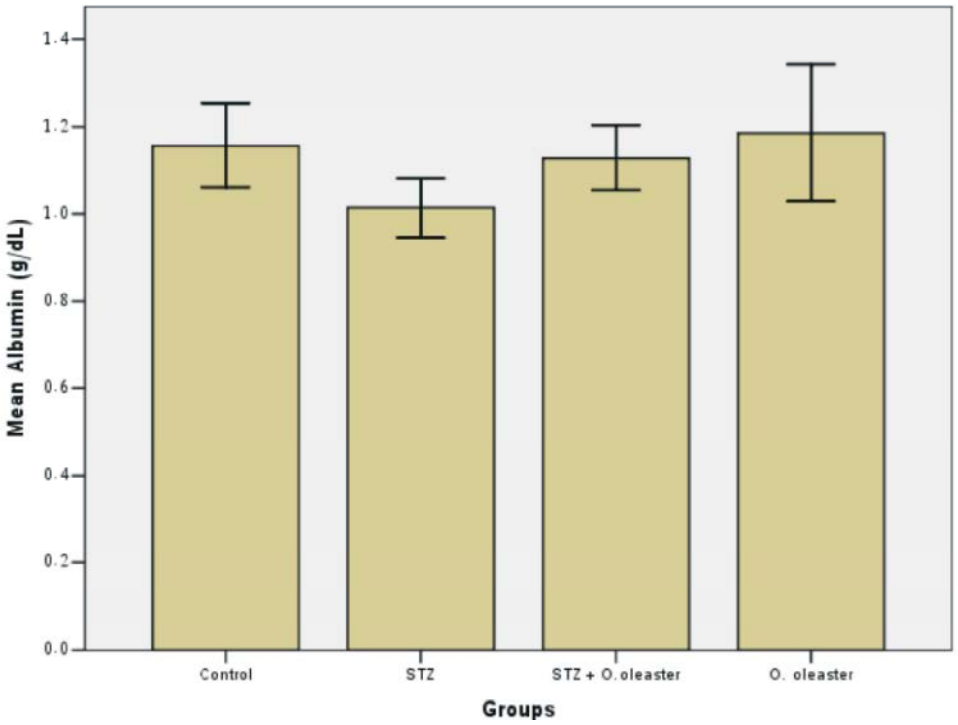

Fig. 2: Level of serum albumin in control, STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats after four weeks. Error bars: ±1 standard deviation

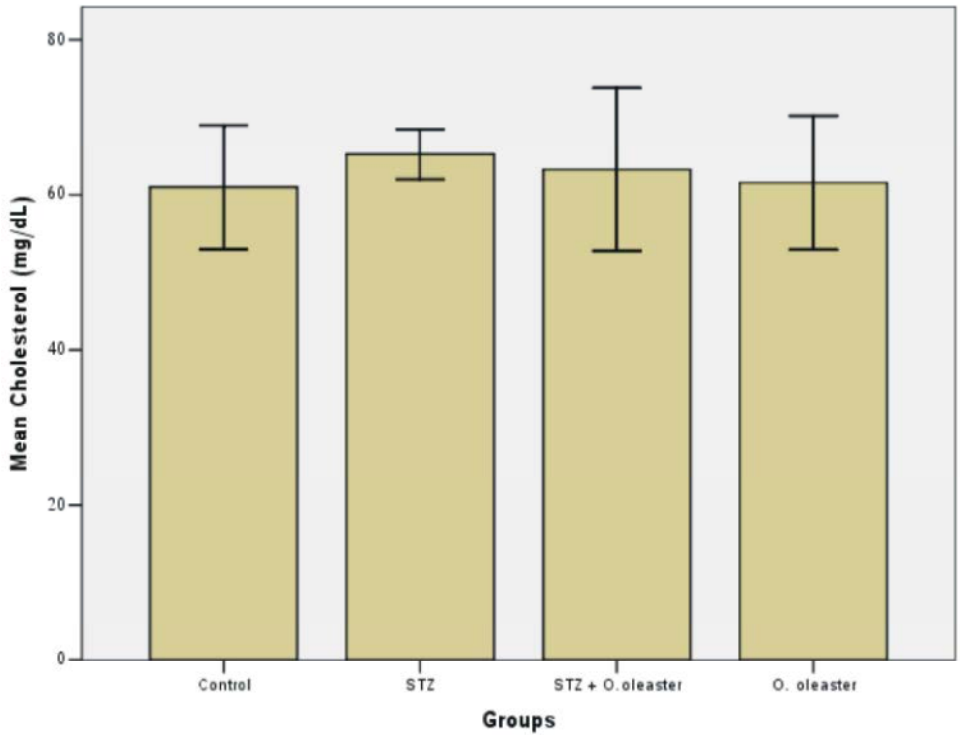

Fig. 3: Level of serum cholesterol in control, STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats after four weeks. Error bars: ±1 standard deviation

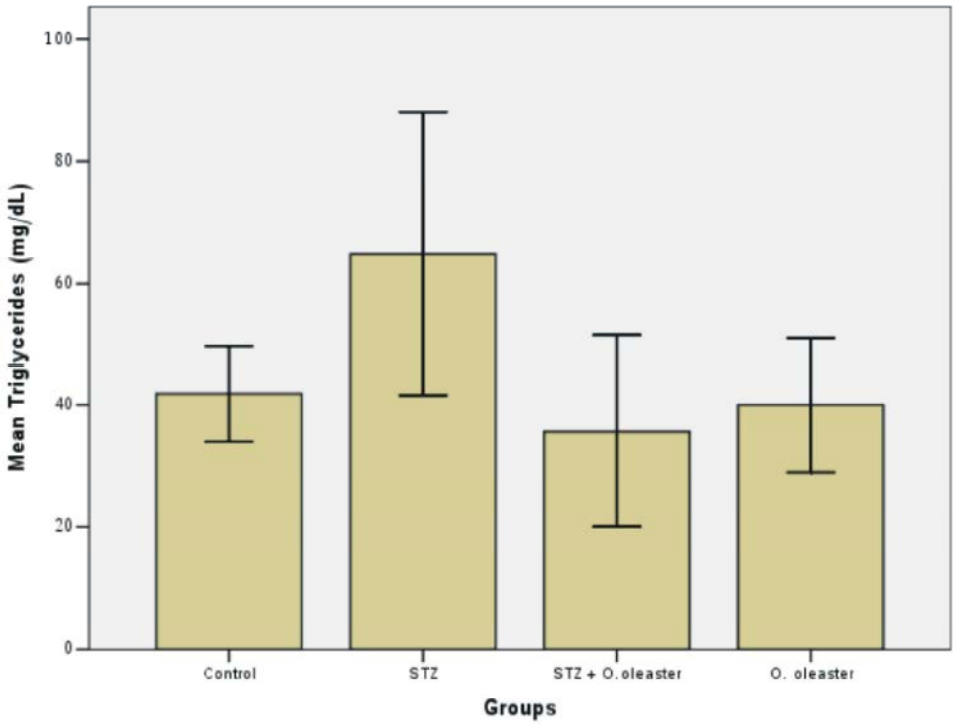

Fig. 4: Level of serum triglycerides in control, STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats after four weeks. Error bars: ±1 standard deviation

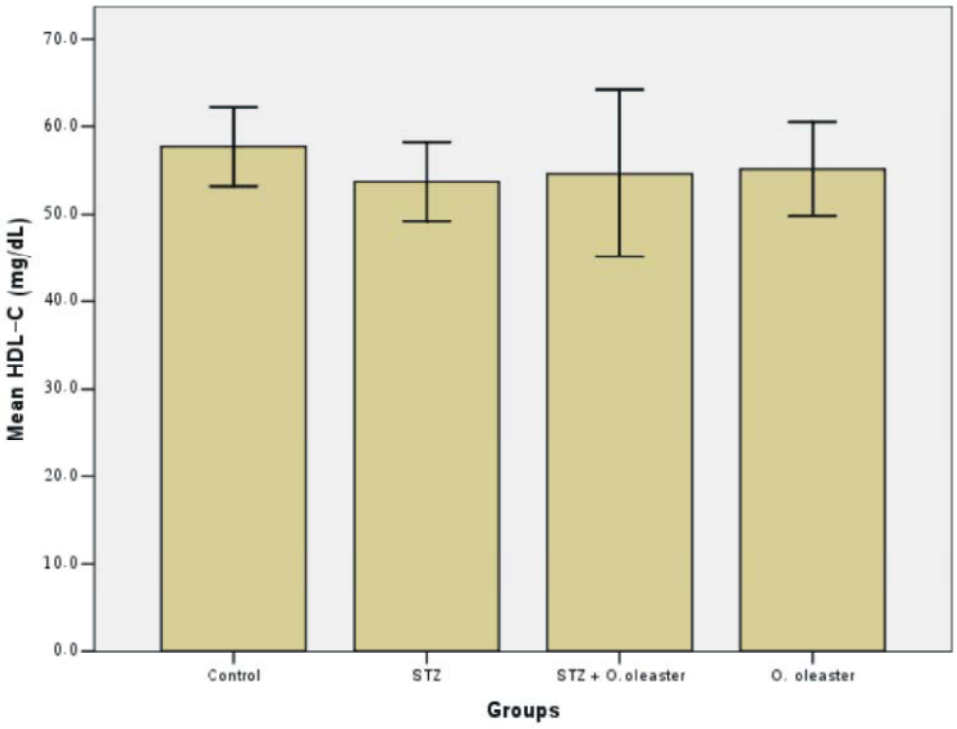

Fig. 5: Level of serum HDL-C in control, STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats after four weeks. Error bars: ±1 standard deviation

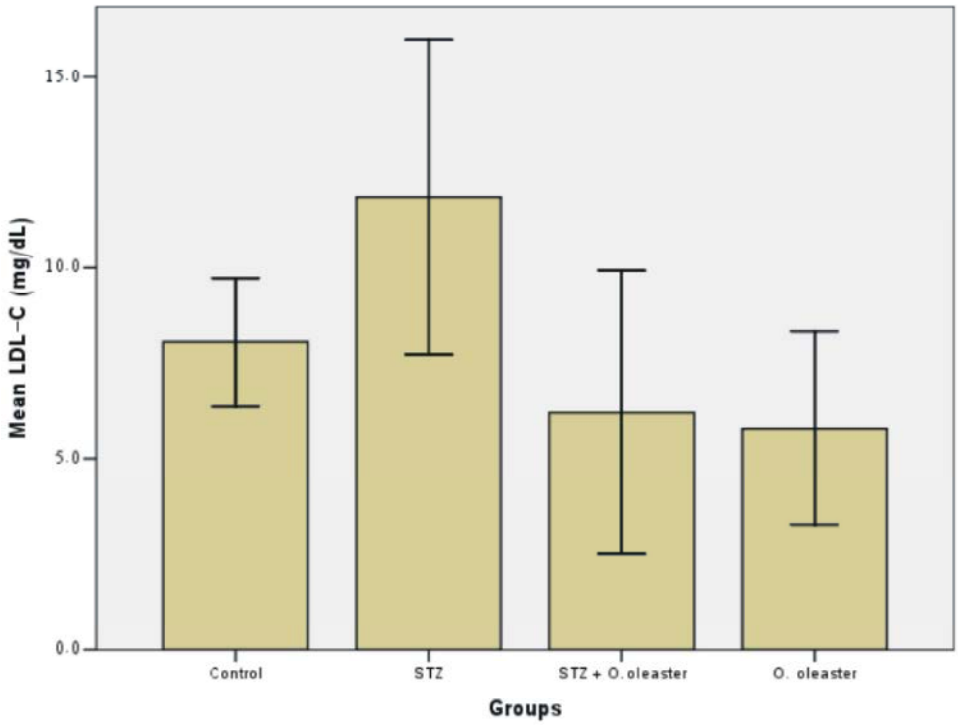

Fig. 6: Level of serum LDL-C in control, STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats after four weeks. Error bars: ±1 standard deviation

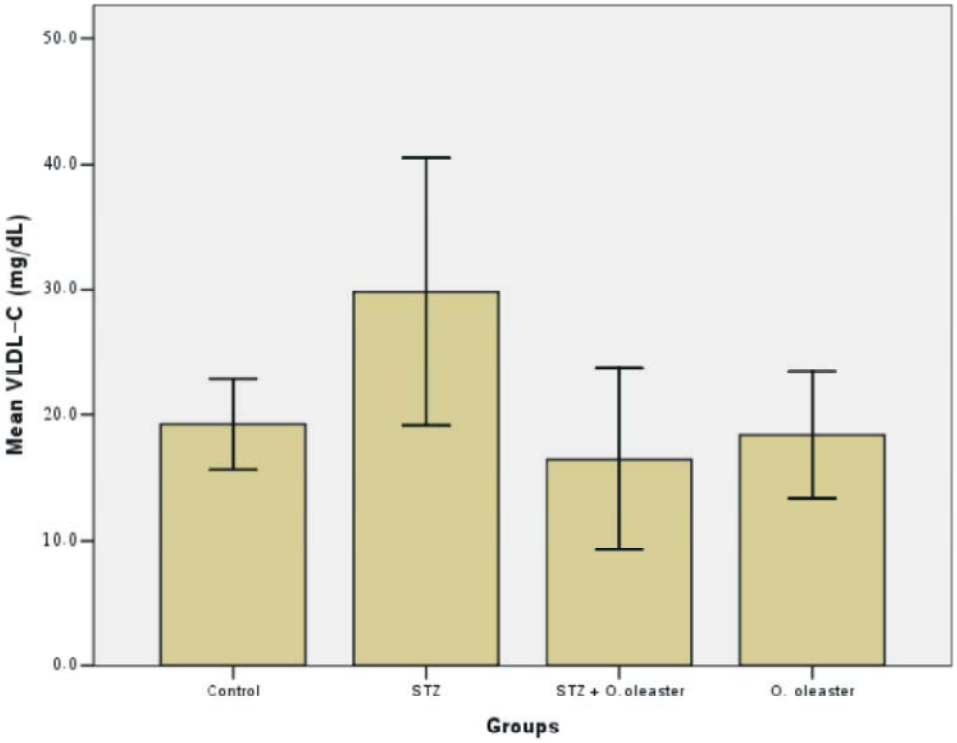

Fig. 7: Level of serum VLDL-C in control, STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats after four weeks. Error bars: ±1 standard deviation

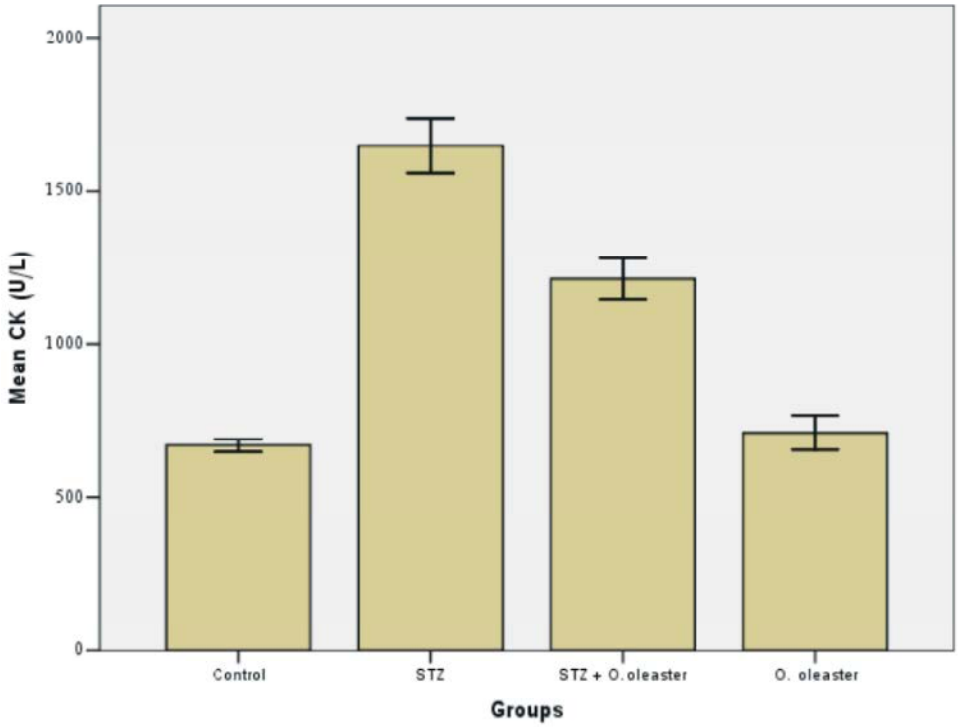

Fig. 8: Level of serum CK in control, STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats after four weeks. Error bars: ±1 standard deviation

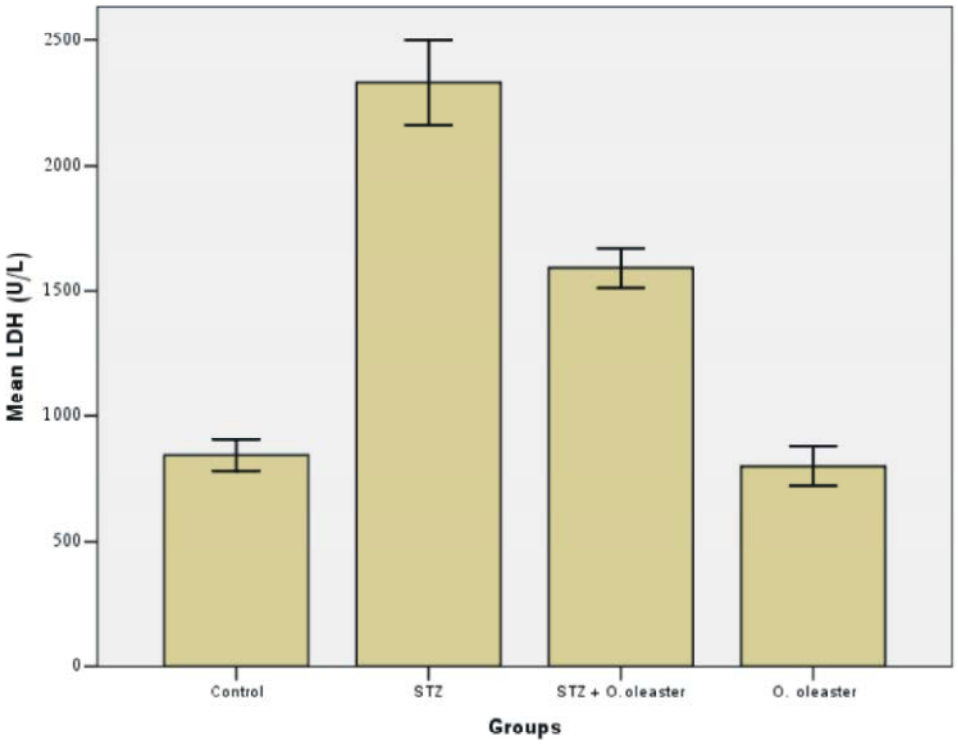

Fig. 9: Level of serum LDH in control, STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats after four weeks. Error bars: ±1 standard deviation

Serum LDL-C level was statistically evoked in diabetic rats of group 2 (+46.9%, $P = 0.007$) compared with normal control rats of group 1. Insignificant changes were noted in the level of serum LDL-C in rats of groups 3 and 4 (Fig. 6).

Noticeably increase of serum VLDL-C was observed in diabetic rats of group 2 (+54.4%, $P = 0.001$). This parameter was insignificantly altered in rats of groups 3 and 4 compared with normal control rats of group1 (Fig. 7).

Remarkable increases in the levels of serum CK were observed in diabetic rats of groups 2 (+146.3%, $P < 0.0001$) and 3 (+81.3%, $P < 0.0001$) when compared to normal control rats of group 1. The level of serum CK was unchanged in rats of group 4 (Fig. 8).

Fig. 9 showed the level of serum LDH in all experimental groups. The levels of serum LDH were notably evoked in diabetic rats of groups 2 (+176.9%, $P < 0.0001$) and 3 (+88.5%, $P < 0.0001$) when compared to normal control rats of group 1, while this parameter was statistically unchanged in rats of group 4 compared with normal control rats of group 1.

DISCUSSION

Chronic diseases are the main cause of death and disability worldwide. They include diabetes mellitus, heart disease, stroke, cancer, arthritis, obesity and respiratory diseases. The prevalence of chronic diseases have elevated steadily among people of all ages in recent years. The WHO [28] projects increase in deaths and illness because of chronic diseases in low- and middle-income countries up to 2030. Diabetes mellitus is a chronic progressive metabolic disorder, common and costly. Probable complications include heart attack, stroke, kidney failure, leg amputation, vision loss and nerve injury [29]. Treatment with medicinal plants is considered very safe as there is no or minimal side effects. The golden fact is that, use of herbal treatments is independent of any age groups and the sexes [30]. Leaves, flowers, stems, roots, seeds, fruit and bark can all be components of herbal medicines [31-35]. The present increase levels of serum glucose, triglycerides, LDL-C and VLDL-C with the decrease level of albumin in diabetic rats of group 2 indicate disturbances in carbohydrates, lipid and protein metabolism due to diabetes. Similar observations were detected by many authors in different diabetic studies [36-39]. Hypoproteinemia and hypoalbuminemia are associated with dysfunctions of liver and kidney. Albumin is synthesized by the liver and as such, it represents a major synthetic protein and is a marker of the ability of the liver to synthesize proteins [40]. It is synthesized in the liver that is dependent on protein intake subject to feedback regulation by the plasma albumin level. The changing levels of serum albumin, thus, provide valuable indices of severity, progress and prognosis in hepatic disease [41]. In diabetes the circulating albumin level is depressed. Albumin degradation and relative extra vascular distribution volume are likewise decreased about 35% in diabetes [42].

Dyslipidemia, characterized by abnormally elevated plasma triacylglycerol and cholesterol concentrations, is an established risk factor in the development of coronary heart disease [43]. Diabetes mellitus and hyperlipidemia are two major factors involved in the development of cardiovascular disease. The medical treatment and reduction of the effects of these conditions are key modalities in the prevention of heart disease [44- 46]. Defects in insulin action and increases in glucose can lead to higher amounts of lipoproteins in the blood. The subsequent increase in lipids, secondary to a state of glucose intolerance, adds to the progression of atherosclerosis and cardiovascular disease. As well, even slight increases in lipid levels in such diabetic patients are associated with a substantial increase in cardiovascular disease, more so than the general population [47].

Any disturbance in lipoprotein metabolism is reflected in the lipid profiles of blood plasma and liver. Since the liver has a major role in the metabolism of lipoprotein, any derangement in its activity leads to alterations in the lipid profile of blood plasma. Several factors may play a role in the accumulation of lipids in the liver. An increase in the level of free fatty acids in blood plasma, as a result of mobilization of fat from adipose tissue or from the hydrolysis of lipoprotein, chylomicrons or triglycerides by lipoprotein lipase in extrahepatic tissues [48], leads to their increased uptake and esterification in the liver. Since the production of lipoprotein does not keep pace with the increased free fatty acids, triglycerides and cholesterol accumulate in the liver. Fat accumulation in the liver may also occur if there is a derangement in the production of lipoprotein, especially its apoprotein part [48]. Elevated concentrations of plasma HDL-C protect the arterial wall from the development of atherosclerotic plaque facilitated by reverse cholesterol transport [49]. In plasma, HDL-C concentrations are modulated in a number of ways, including the uptake of the entire HDL particle [50, 51], the selective uptake of cholesteryl ester by the liver and steroidogenic organs via scavenger receptor B class 1, SR-B1, [50, 52] and the transfer of individual components

of HDL by cholesterol ester transfer protein (CETP) and phospholipid transfer protein (PLTP) [53]. A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency, are responsible for the observed accumulation of lipids [54]. 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 [55]. Additionally, it is well known that in uncontrolled diabetes, 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 [56, 57]. Moreover, diabetes has 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 [58]. The present study demonstrated that the supplementation of *O. oleaster* extract significantly decreased the levels of serum glucose, triglycerides, LDL-C and VLDL- in diabetic rats. The above mentioned results revealed that this extract can improve the metabolic processes of carbohydrates and lipids due to their chemical constituents. Plant sterols (phytosterols) and plant stanols (phytostanols) are a large group of compounds that are found exclusively in plants. There are numerous mechanisms in which plant sterols and stanols prevent the absorption of and thus reduce cholesterol [59].

The current study showed that the levels of serum CK and LDH were significantly enhanced in diabetic rats. Serum CK was first used as a diagnostic aid in progressive muscular dystrophy [60]. It has since then become important clinical marker for muscle damage. The serum CK levels in healthy individuals depend on age, race, lean body mass and physical activity [60-62]. LDH is a cytoplasmic enzyme found in the cells of all major organs, including the heart, the liver, the kidneys, the skeletal muscle, the brain, red blood cells and the lung [63, 64]. It is responsible for converting lactic acid into pyruvic acid, an essential step in producing cellular energy [63]. Because LDH is present in almost all body tissues, LDH test is usually used to detect tissue damage or inflammation [65]. As a result of necrosis, the levels of diagnostic indicators of myocardial infarction, such as CK and LDH, increase in the serum [66, 67]. The pathogenesis of diabetes-induced cardiomyopathy involves metabolic derangements, such as hyperglycemia and hyperlipidemia that produce glycation of interstitial proteins, such as collagen and, in turn, lead to myocardial stiffness and impaired contractility [68 - 70]. An increase in oxidative stress contributes to the characteristic morphological and functional abnormalities that are also associated with diabetic cardiomyopathy [71]. However, the present increase of serum CK and LDH levels in diabetic rats may be due to the injury of cardiac muscle tissues. Moreover, several investigations showed that the levels of CK and LDH were significantly increased in diabetic rats [72-74].

Olive leaves contain several active components which have been identified as therapeutic agents delaying the progression of advanced glycation end products-mediated inflammatory diseases such as diabetes [75]. The oleuropein and tannins in olive leaves are act as α-glucosidase inhibitors, reducing the absorption of carbohydrates in the gut [15]. Moreover, the extract of olive leaves was shown to have an inhibitory effect on the postprandial blood increase in glucose in diabetic rats [76]. El and Karakaya [77] reported that there have been two possible mechanisms suggested to explain the hypoglycemic effect of the olive leaves extract: (1) oleuropein improved glucose-induced insulin release and (2) increased peripheral uptake of glucose. The oleuropein in olive leaves has been shown to accelerate the cellular uptake of glucose, leading to reduced blood glucose [78]. Since oleuropein is a glycoside, it could potentially access a glucose transporter such as a sodium-dependent glucose transporter found in the epithelial cells of the small intestine, thereby permitting its entry into the cells [79]. The hypoglycemic effect of olive leaves extract was attributed to the antioxidant properties of its constituents [15, 80 & 81]. Mousa *et al.* [82] concluded that the olive leaves extract is having hypoglycemic effect and improves alterations associated with diabetes mellitus probably due to its many potentially bioactive compounds. In another study, Sakr *et al.* [83] concluded that the ameliorative effect of olive leaves extracts against diabetes mellitus in rats may be attributed to the presence of its phenolic compounds.

In this study, it is clearly that the treatment of diabetic rats with *O. oleaster* leaves extract attenuated the greatly physiological alterations. It seems that olive leaves extract has a favorable influence on diabetes mellitus and its complications. Additional studies are required to establish the efficacy of various concentrations of olive leaves extract and its constituents as potential therapeutic agents for diabetes mellitus and to elucidate their mechanisms of action on diabetic models induced by STZ and other diabetogenic factors.

CONCLUSIONS

The current study was intended to investigate the influence of wild olive (*Olea oleaster*) leaves extract on some physiological parameters in STZ-induced diabetes in male Wistar rats. In diabetic rats, the levels of serum glucose, triglycerides, LDL-C, VLDL, CK and LDH were significantly increased. However, the level of serum albumin was significantly decreased. Administration of olive leaves extract improved most observed physiological changes. Therefore, it may be concluded that olive leaves extract has a favorable influence on diabetes mellitus and its complications. The results of this study proved the physiologically effective components of olive leaves extract which exert protective influence on diabetic rats.

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