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Effect of Olive Leaves Extract on Hepatorenal Function in Streptozotocin Diabetic Male Wistar Rats

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Abstract: The present study investigated the effect of olive leaves extract (*Olea oleaster*) on hepatorenal function in streptozotocin (STZ)-induced diabetes in male Wistar rats. 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, subjected to olive leaves extract. The lowest body weight gain was observed in diabetic rats of the second group. The levels of serum glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, creatinine, blood urea nitrogen and uric acid were significantly increased in diabetic rats of the second group. Treatments with olive leaves extract in diabetic rats demonstrated remarkable reducing and protecting effects of physiological changes. Therefore, the present study revealed to the importance of using olive leaves extract as promising complementary therapeutic agent against diabetes and its complications.

Key words: Olive Leaves · Diabetes · Streptozotocin · Liver · Kidney · Rats

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism linked with absolute or relative deficiency in insulin secretion and/or action [1].

Anxiety about this chronic disease is focused on serious diabetes-related complications which can influence multiple vital organ systems, thus leading to more severe and permanent pathological conditions such as nephropathy, hepatopathy, retinopathy, vasculopathy, neuropathy and cardiovascular diseases [2]. Continuing hyperglycemia enhances general oxidative stress and rises in the incidence of diabetic nephropathy and liver disease [3].

DM is now the most common cause of liver disease which is a chief cause of death in diabetic people [4]. Diabetic nephropathy is one of the upsetting worldwide health dilemmas at present leading to micro vascular (retinopathy, neuropathy and nephropathy) and macro vascular (heart attack, stroke and peripheral vascular disease) complications in many countries [5].

Many factors are involved in the onset of diabetic nephropathy; oxidative stress is believed to connect these factors [6]. In addition, free radicals prompted the development of liver diseases by stimulating hepatocyte apoptosis, hepatic inflammatory response and fibrogenesis [7].

Medicinal plants are extensively used and various studies have demonstrated that many species of medicinal plants with different compounds may be used as hypoglycemic agents. Medicinal plants offer a useful source of oral hypoglycemic compounds for the development of new pharmaceutical leads as well as dietary supplements to presented therapies [8]. The olive tree *Olea europaea* L. (family*:* Oleaceae) has been commonly recognized as one of the species with the highest antioxidant activity using its oil, fruits and leaves. The activity of the olive tree byproduct extracts in medicine and food industry is owing to the presence of some important antioxidant and phenolic constituents to prevent oxidative degradations. The olive tree has long been known as having antioxidant molecules, such as oleuropein, hydroxytyrosol, oleuropein aglycone and tyrosol [9, 10]. In addition, several studies have revealed

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the ability of olive leaves for treatment of diverse diseases [11-15]. Therefore, the current study was intended to investigate the effect of olive leaves extract on hepatorenal function in diabetic male Wistar rats.

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 Olive 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) [16]. 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: Diabetes mellitus was induced in overnight fasted rats by intraperitoneal (IP) injection of streptozotocin (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 estimated at the start of the experimental period, after 4 weeks using a digital balance. These weights were measured at the same time during the morning [17]. Moreover, the experimental animals were observed 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, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, creatinine, blood urea nitrogen (BUN) and uric acid. The level of glucose was measured using the method of Trinder [18]. The enzyme alanine aminotransferase (ALT/GPT) level was measured using the method of Reitman and Frankel [19]. Aspartate aminotransferase (AST/GOT) level was determined using the method of Reitman and Frankel [19]. The method of MacComb and Bowers [20] was carried out to determine the level of ALP. Total bilirubin concentration was determined according to the method of Doumas *et al*. [21]. Creatinine value was estimated according to the method of Larsen [22]. Blood

urea nitrogen (BUN) concentration was estimated according to the method of Patton and Crouch [23]. The method of Young [24] was used to determine the level of uric acid.

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.) and values were analyzed using one-way analysis of variance (ANOVA) and LSD tests to determine differences between the mean values of experimental groups. The results were considered statistically significant if the *P*-values were less than 0.05.

RESULTS

The percentage change of body weight gain in normal control rats was +24.6% after four weeks. The percentage change of body weight gain in rats treated with *O. oleaster* was +7.2%. Significant decrease (-6.4%) in the values of body weight gain was observed in diabetic rats fed with normal diet. The change of body weight gain was +11.4% in diabetic rats supplemented with *O. oleaster* extract.

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 normal control rats of group1, statistically increases in the level of serum ALT were observed in diabetic rats of group 2 (+77.6%, $P < 0.0001$) compared with normal control rats of group1. Furthermore, the level of ALT was statistically unchanged in rats of groups 3 and 4 compared with normal control rats of group1 (Fig. 1).

The level of serum AST was significantly increased in diabetic rats of group 2 (+17.8%, $P = 0.002$). This parameter was statistically unchanged in rats of groups 3 and 4 compared with normal control rats of group1 (Fig. 2).

In comparison with control data of group 1, the levels of serum ALP was significantly raised in diabetic rats of group 2 (+111.7%, $P < 0.0001$). Moreover, there were no significant changes observed in the levels of serum ALP in diabetic rats of group 3 and non diabetic rats of group 4 (Fig. 3).

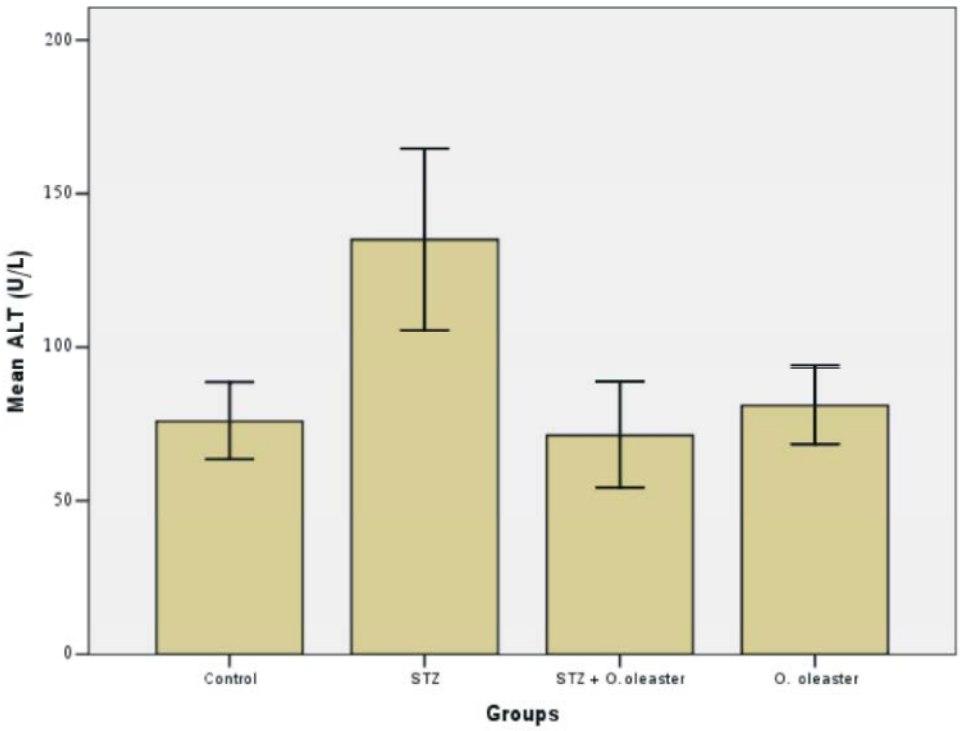

Fig. 1: Level of serum ALT in control, STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats after four weeks. Error bars: ±1 standard deviation

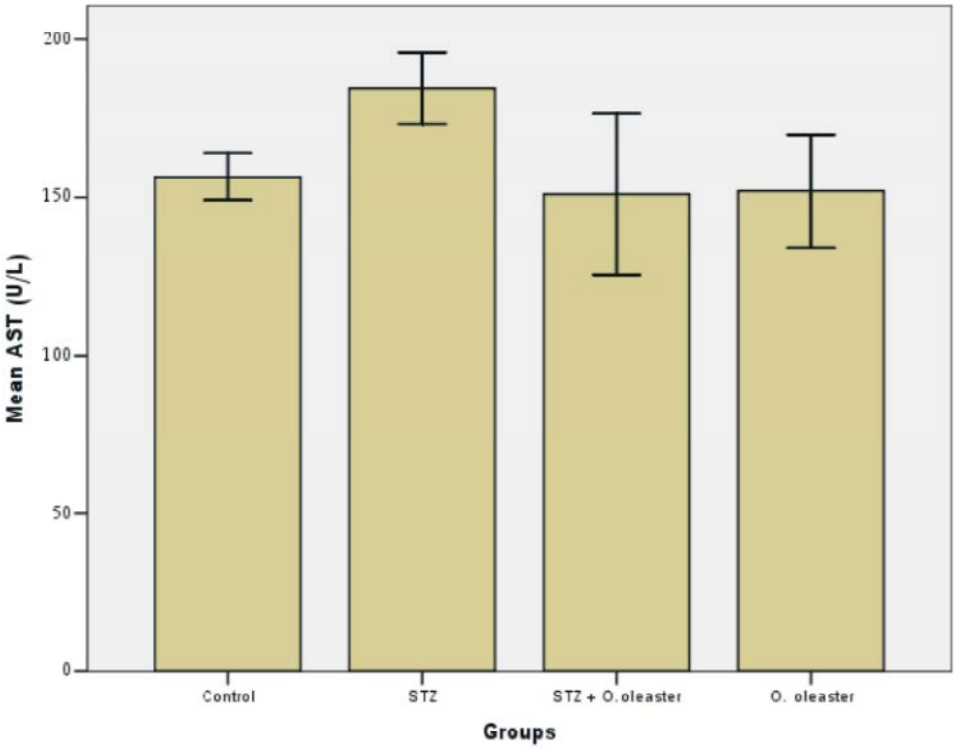

Fig. 2: Level of serum AST in control, STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats after four weeks. Error bars: ±1 standard deviation

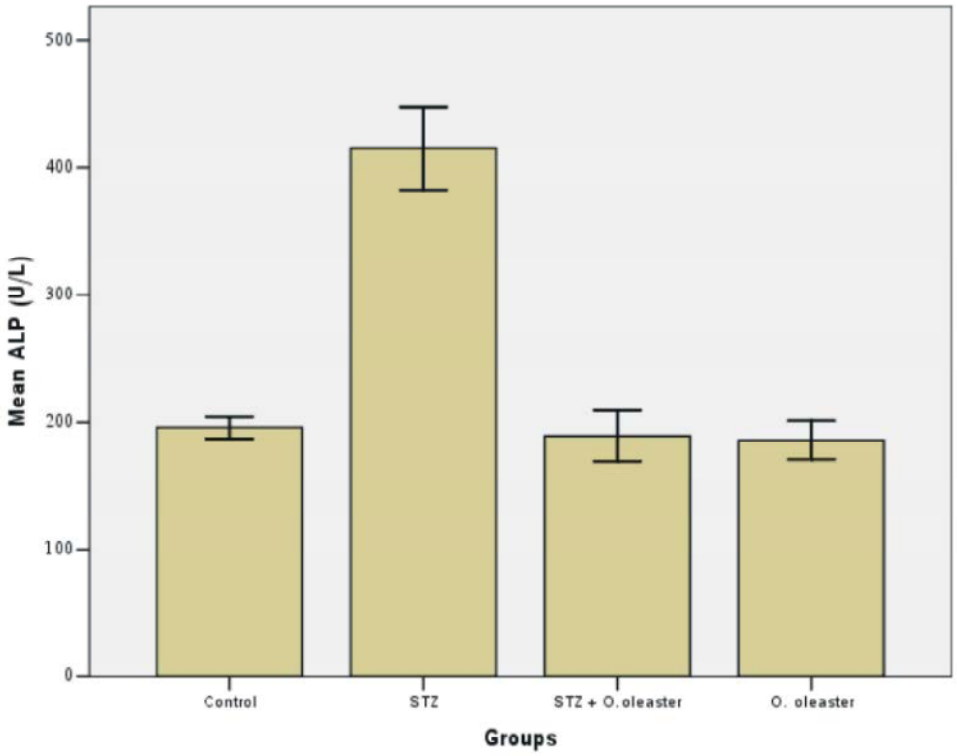

Fig. 3: Level of serum ALP in control, STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats after four weeks. Error bars: ±1 standard deviation

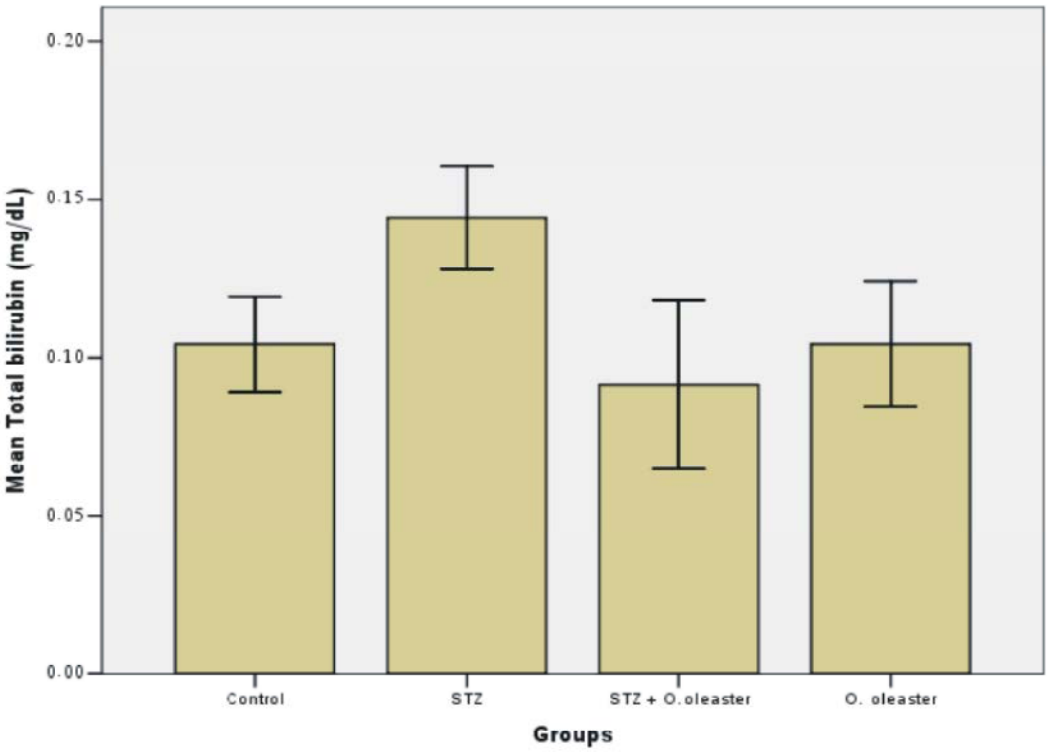

Fig. 4: Level of serum total bilirubin in control, STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats after four weeks. Error bars: ±1 standard deviation

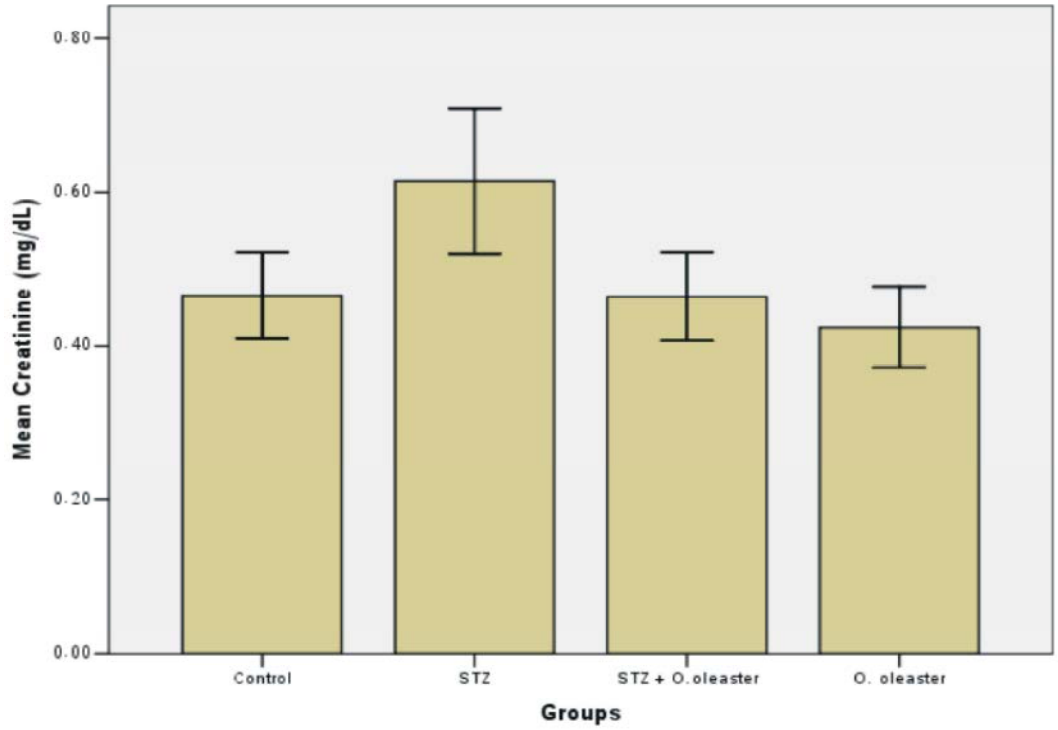

Fig. 5: Level of serum creatinine in control, STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats after four weeks. Error bars: ±1 standard deviation

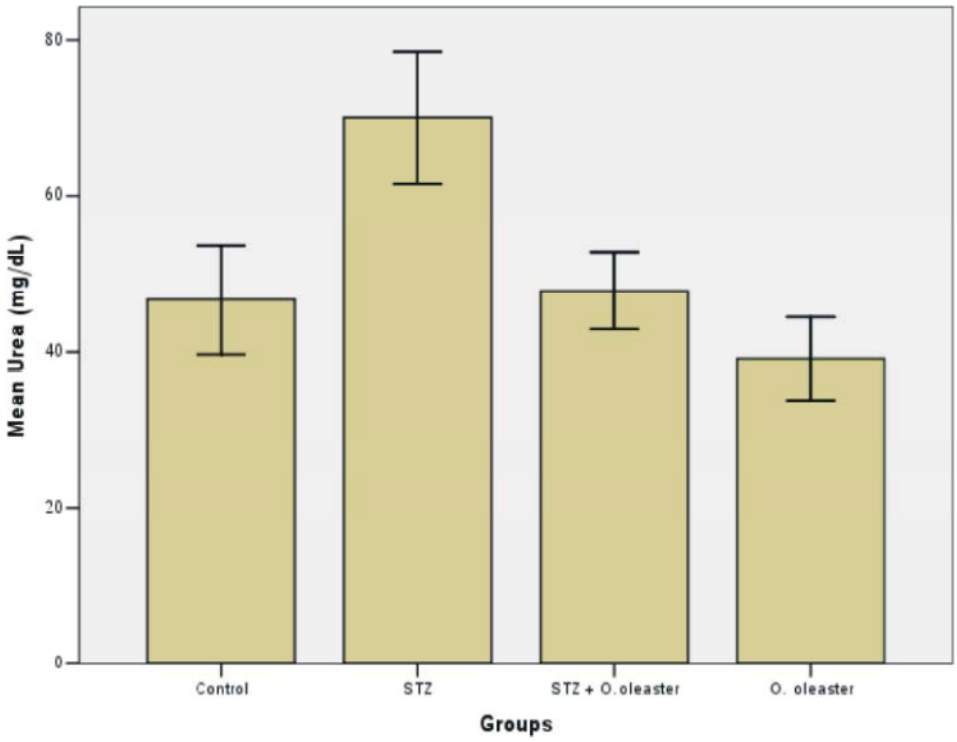

Fig. 6: Level of serum BUN in control, STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats after four weeks. Error bars: ±1 standard deviation

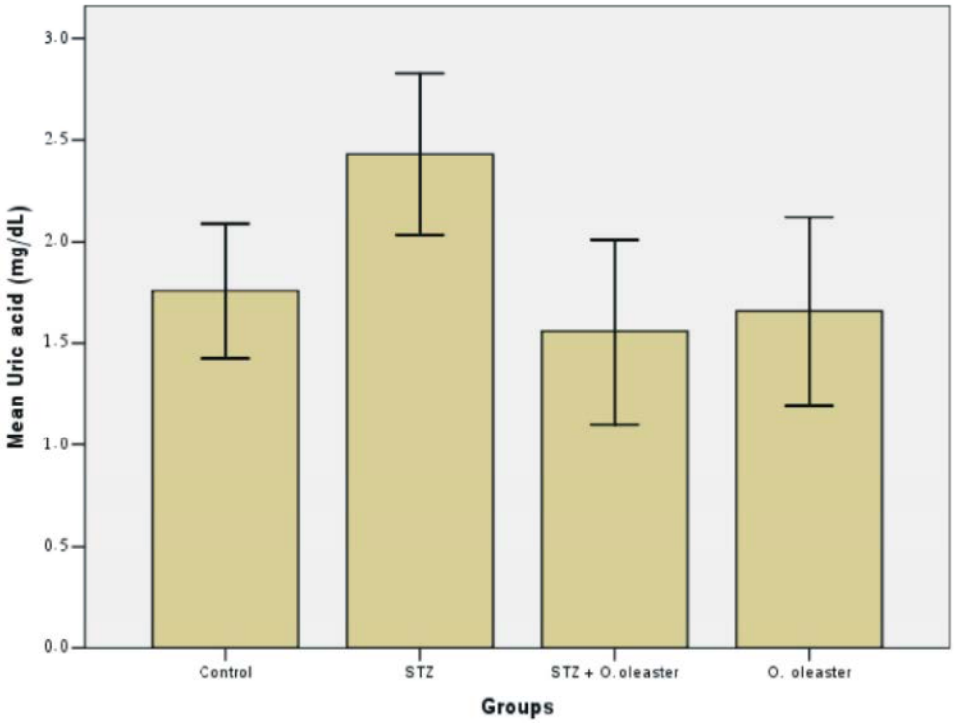

Fig. 7: Level of serum uric acid in control, STZ, STZ plus *O. oleaster* extract and *O. oleaster* extract treated rats after four weeks. Error bars: ±1 standard deviation

Fig. 4 showed the level of serum total bilirubin in all experimental groups. The level of serum total bilirubin was statistically evoked in diabetic rats of group 2 (+40.0%, $P < 0.0001$) compared with normal control rats of group1, while there were no significant alterations in the levels of serum total bilirubin in rats of groups 3 and 4.

The measured levels of serum creatinine in all experimental groups are given in Fig. 5. In comparison with control rats, the level of serum creatinine was markedly increased in diabetic rats of group 2 (+29.8%, $P < 0.0001$). Insignificant change was found in the level of serum creatinine in rats of groups 3 and 4.

The level of serum BUN was significantly increased in diabetic rats of group 2 compared with control level in normal rats of group 1 (+48.9%, $P < 0.0001$). However, the level of serum BUN was significantly decreased in rats of group 4 ($P < 0.022$). The level of serum BUN was unchanged in rats of group 3 (Fig. 6).

Fig. 7 represented the levels of serum uric acid in all experimental groups. The level of serum uric acid was statistically enhanced in diabetic rats of group 2 (+33.3%, $P = 0.002$) compared with normal control rats of group 1. Insignificant alterations of serum uric acid levels were observed in diabetic rats of group 3 and non diabetic rats of group 4.

DISCUSSION

Hyperglycemia has been a classical risk factor in the development of diabetes mellitus and its complications. Thus, control of blood glucose levels is critical in the early treatment of diabetes mellitus [25-27]. Nature has been a source of medicinal agents since the beginning of time. Herbal medicine is still the most common source for primary health care of about 65-80% of the world's population [28], mostly in developing countries, owing to better cultural acceptability, better compatibility with the human body and fewer side effects. In spite of the use of a lot of oral hypoglycemic drugs, all of them are expensive and demonstrate limited efficacy and certain adverse effects. Comparatively very less side effects and low cost of phytochemicals from natural resources open new avenues for the treatment of diverse diseases including diabetes mellitus [27].

In the present study, significant decreases of body weight gain in STZ-diabetic rats were observed after 4 weeks. These observations are supported by the findings of many experimental investigations [29-33]. 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 [34]. STZ-induced diabetes is characterized by severe weight loss [35]. The decrease in body weight in diabetic rats might be the result of protein wasting due to unavailability of carbohydrate as an energy source [36]. 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 [37].

The present study showed that STZ induced an elevation in the levels of serum ALT, AST, ALP and total bilirubin in rats, since necrosis or membrane damage releases these enzymes into circulation, which agrees with the previously reported results [38]. Serum levels of these parameters are very sensitive markers employees in diagnosis of liver diseases [39]. Individuals with diabetes mellitus have a higher incidence of liver function test abnormalities than non diabetic ones [40, 41]. Shakoori *et al.* [42] reported that the increase of blood enzymatic activity is either due to (1) leakage of theses enzymes from hepatic cells and thus raising levels in blood, (2) increased synthesis and (3) enzyme induction of these enzymes. Moreover, ALT, AST, ALP and total bilirubin will leak into the serum resulting in elevating their serum concentrations. Rajesh and Latha [43] also reported that the elevated levels of these parameters were indicative of cellular leakage and loss of the functional integrity of the cell membranes.

The present study evaluated kidney function by measuring the levels of serum creatinine, BUN and uric acid. The present enhancement of these biochemical parameters confirmed renal dysfunction in untreated diabetic rats. Creatinine, BUN and uric acid are waste products of protein metabolism that need to be excreted by the kidney, therefore a marked increase of these parameters, as observed in this study, confirms an indication of functional damage to the kidney [44]. Hyperglycemia induces oxidative insult in renal tubular epithelial cells and that injury initiates tubulointerstitial fibrosis, a characteristic feature of diabetic nephropathy, which then progressively results in renal failure [45, 46]. Nephropathy is reported to develop in 30-40% of patients with diabetes mellitus and has become a leading cause of end stage renal failure worldwide [47, 48]. Diabetic nephropathy is characterized by structural as well as functional abnormalities [49]. Poor glycemic control and

accumulation of advanced glycation end products play a significant role in the development of diabetic nephropathy [50]. Additionally, Mestry *et al.* [50] demonstrated that untreated STZ-diabetic rats display a pronounced impairment in renal function which is confirmed by the enhancement of serum levels of creatinine, BUN and uric acid.

The present study demonstrated the effects of *O. oleaster* leaves extract on diabetes mellitus induced by STZ in male rats. Oleuropein is used as a well-known compound of extracts and its concentration is significantly high in olive leaves and fruits. Furthermore, olive leaves have hydroxytyrosol, tyrosol and caffeic acid were identified as the main active components. In addition, olive leaves contain p-coumaric acid, vanillic acid, vanillin, luteolin, diosmetin, rutin, luteolin-7-glucoside, apigenin-7-glucoside and diosmetin-7-glucoside which have been recognized as therapeutic agents delaying the progression of advanced glycation end products-mediated inflammatory diseases such as diabetes mellitus [51].

Oleuropein and tannins in olive leaves are working as á-glucosidase inhibitors, reducing the absorption of carbohydrates in the gut [52]. In addition, olive leaves extract was demonstrated to have an inhibitory effect on the postprandial blood increase in glucose in diabetic rats [53]. El and Karakaya [54] indicated that there have been two probable mechanisms suggested to explain the hypoglycemic effect of the extract: (1) oleuropein improved glucose-induced insulin release and (2) increased peripheral uptake of glucose. The oleuropein in olive leaves has been revealed to accelerate the cellular uptake of glucose, leading to reduced blood glucose [55]. Since oleuropein is a glycoside, it might potentially access a glucose transporter such as a sodium-dependent glucose transporter found in the epithelial cells of the small intestine, thus permitting its entry into the cells [56]. Research points to an interaction between dietary flavonol monoglucosides with the intestinal sodium-dependent glucose transporter and inhibited Na-independent glucose uptake [57, 58]. Several studies demonstrated that the hypoglycemic effect of olive leaves extract was attributed to the antioxidant properties of its components [59, 60].

Similarly, the favorable effect of olive leaves extract on STZ-diabetic male rats was examined by Mousa *et al.* [61]. They found that the level of blood glucose was improved by olive leaves extract in diabetic rats. The activities of liver enzymes, ALT and AST, were raised in diabetic rats. Treatment with olive leaves extract decreased the activities of these enzymes. They concluded that the olive leaves extract is having hypoglycemic effect and improves changes linked with diabetes mellitus possibly because of its several potentially bioactive constituents. In addition, the oral administration of olive tree (fruit and leaves) extract with high polyphenols content contributes to blood glucose level decreasing in diabetic rats compared to the diabetic control rats. Moreover, treatment with olive tree extract reduced significantly the levels of creatinine, urea, uric acid, ALT and AST [62]. Sakr *et al.* [63] demonstrated that the levels of serum glucose, ALT and AST were also significantly increased in STZ-diabetic male rats. After diabetic rats treated with olive leaves extract, an improvement was noted in the biochemical parameters. They concluded that the beneficial effect of olive leaves extracts against diabetes mellitus in rats might be attributed to the presence of its phenolic compounds.

The antidiabetic effects and stress oxidant improvement of olive leaves ethanolic extract (100 mg/kg body weight) in alloxan-induced diabetic rats were investigated by Ben Salah *et al*. [64]. The olive leaves ethanolic extract displayed a significant decrease in blood glucose in diabetic rats. Furthermore, the extract prevented body weight loss in diabetic rats. The plant extract tended to decrease ALT and AST activities toward the normal levels. An amelioration effect was noted in antioxidant state in liver and kidneys of diabetic rats treated with olive leaves extract. Thus, they demonstrated that the ethanolic extract of olive leaves possesses an antihyperglycemic activity on alloxan-diabetic rats and reduced the adverse effect of oxidative response.

CONCLUSIONS

The current study was intended to investigate the effect of olive leaves extract on hepatorenal function in diabetic male Wistar rats. The results showed that the lowest body weight gain was observed in diabetic rats of the second group. Furthermore, the levels of serum glucose, ALT, AST, ALP, total bilirubin, creatinine, blood urea nitrogen and uric acid were significantly increased in diabetic rats of the second group. Treatments with olive leaves extract in diabetic rats demonstrated remarkable reducing and protecting effects of physiological changes. Therefore, the present study revealed to the importance of using olive leaves extract as promising complementary therapeutic agent against diabetes and its complications.

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