BENEFICIAL AND POTENT EFFECT OF OLIVE LEAVES EXTRACT ON HYPERGLYCEMIC STATE, KIDNEY AND LIVER FUNCTION IN STZ-INDUCED TYPE 2 DIABETES MELLITUS

Abd El-Moneim M R Afify, Hossam S El-Beltagi*, Sayed A Fayad, Aber E El-Ansary

Biochemistry Dept, Faculty of Agriculture, Cairo University, P. Box 12613, Gamma st., Giza, Cairo, Egypt

ABSTRACT

To evaluate the effect of olive leaves extract (OLE) on type 2 diabetes mellitus (T2DM) induced by streptozotocin (STZ) in rats, male rats were allocated into four groups; normal control, diabetic control (45 mg/kg STZ), normal rats treated orally with OLE (17.8 mg/kg b.wt.) and diabetic rats treated with OLE. The changes in glucose level, kidney and liver function were investigated. OLE kept glucose level close to normal. There was a significant change between total protein and total albumin as the extract was found to keep them near normal level. Creatinine, urea, uric acid and liver function levels were very high in diabetic group. OLE significantly improved the levels of urea, creatinine, uric acid and kept liver function markers in normal range on treated diabetic rats. The present study submits that, OLE could be used to prevent T2DM complications such as nephropathy and liver damage through enhancing glycemic state.

KEYWORDS:
Glucose, kidney function, liver function, olive leaves extract, streptozotocin, T2DM

INTRODUCTION

Diabetes mellitus is a chronic, progressive and imperfect understood metabolic state that characterized by hyperglycemia (high blood sugar) which represents a major public health concern worldwide [1]. Mainly two types of diabetes mellitus are known; type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). Disturbances in insulin secretion, sensitivity to tissue actions of insulin, or both of them are supposed to be the commonest reasons leading to T2DM pathophysiology, diseases mainly arising from tissue insulin resistance which progress the complete loss of secretory activity of the pancreatic β-cells [1]. Hyperglycemia in T2DM can produce long-term complications such as cardiovascular and renal disorders, retinopathy and poor blood flow [2, 3].

Medicinal plants are a great source for economic value all over the world, where nature provided us with a very rich botanical wealth and large number of diverse types of plants [4, 5]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body and the most important of these compounds are alkaloids, flavonoids, tannins, terpenoids and phenolics [5, 6].

Olive leaves Olive (Olea europaea) have been used as a folk remedy for combating diseases due to they are rich in polyphenolic compounds as their contents may reach up to 40 g per kg of dry tissue [7]. Several studies reported that, polyphenols from olive represent natural anti-inflammatory agents and exhibit a wide range of interesting bioactivities such as antioxidant, antimicrobial, antiatherogenic, antitumoral, cytoprotective and cardioprotective properties [8-11].

The aim of the current investigation is to show the effect of olive leaves extract on hyperglycemia in STZ-induced rats as well as efficiency of liver and kidney function in vivo.

MATERIALS AND METHODS

Materials. STZ was acquired from Sigma Chemicals (St Louis, Mo, USA), in 97% purity. All other reagents used were of analytical grade.

Methods. Preparation of olive aqueous extract. Olive leaves (25g) were blended in 200 ml of water about 3-5 minutes. The whole solution was heated 2-3 minutes of boiling, then filtered using Double Rings Filter Paper 102. The leaf extract was separated and freeze-dried until used. It was redisolved in distilled water.

Induction of diabetes. For the evaluation of STZ diabetic effect, T2DM was induced by intraperitoneally injection of a single dose of STZ per body weight was (45 mg/kg b.wt.) dissolved in 0.01 M citrate buffer (pH 4.5) immediately before use. After STZ injection, rats had free access to food, water and were given 5% glucose solution to drink overnight to
encounter hypoglycemic shock. Rats were checked daily for the presence of glycosuria. Rats were considered to be diabetic if glycosuria was present for 3 consecutive days. Three days after STZ injection, fasting blood samples were obtained and blood sugar was determined (≥300 mg/dl).

Experimental animals. Twenty-four male Wistar rats weighing 120 ±10 g were purchased from animal house of Helwan station for experimental animals, Helwan, Egypt. The animals were retained in polyethylene cages in groups of 6 rats per cage in a controlled environment (25±2 ºC, 50-60% relative humidity, and 12-hour light-dark cycle) for two weeks for adaptation. Rats were divided into four equal groups of 6 rats each. All experimental treatments were approved by Cairo University Ethics Committee for the Care and Use of Experimental Animals in Education and Scientific Research (CU-IACUC) and the approval number was CUIIS1616.

Experimental design. The animals were randomly allocated into four groups: normal control, diabetic control (45 mg/kg STZ), normal rats treated with olive leaves extract (17.8 mg/kg b.wt.) and diabetic rats treated with OLE (17.8 mg/kg b.wt.). OLE was dissolved in distilled water and then given by oral gavage administration five days a week during the study period. The dose was calculated as 20 mg oleuropein/kg b.wt. Throughout this period, the rats were fed on a diet (TD.94045 AIN Diet) and water. The vitamin mix (AIN-93-M) (1%) and mineral mix (AIN-93G-MX) (3.5%).

Biochemical analysis. Blood samples were collected from orbital sinus under anesthesia as follows: about 3 ml in EDTA-coated tubes and about 2 ml in plain tubes, centrifuged at 3000 rpm for 10 min at 4 ºC to get plasma and serum respectively. Rats were sacrificed by cervical dislocation at the end of the study period.

Determination of glucose level. The estimation of glucose level was carried out as illustrated by Trinder [12].

Total albumin and total protein (TP) contents. Total albumin was assayed in serum according to the method of Doumas et al. [13], while total protein was determined by the method of Gornall et al. [14].

Creatinine, urea and uric acid contents. Determination of creatinine [15] and urea [16] were assayed in serum by diagnostic kit method. Where, uric acid was assayed by the method of Fossati et al. [17].

Alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzyme activities. ALP enzyme activity was determined by the method of Belfield and Goldberg [18]. The activities of AST and ALT were assayed according to the method of Reitman and Frankel [19].

Statistical analysis. Values are presented as means ± SEM. Statistical analysis was carried out by using the “costat” statistic computer program. Statistical analysis was based on One-way analysis of variance ANOVA followed by student-Newman Keuls test, and least significant difference (LSD) at P < 0.05. A Pearson product-moment correlation coefficient was used to describe the relationships between enzymatic and non-enzymatic antioxidants.

RESULTS AND DISCUSSION

Impact of olive leaves extract on the levels of glucose, total albumin and total protein in different groups. Table (1) showed the effect of olive leaves extract on STZ-induced rats. Diabetic group revealed a significant increase in the level of glucose (283.28 mg/dl) however, the levels of total albumin and total protein significantly decreased (30.6 and 42.05 g/l, respectively) as compared to normal group. Diabetic rats supplemented with OLE showed improvements in these levels where, it decreased the high level of glucose (55.30 mg/dl) and increased the levels of total albumin and total protein (41.70 and

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of olive leaves extract on the levels of glucose, total albumin and total protein in different groups</td>
</tr>
<tr>
<td>Groups</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Normal control</td>
</tr>
<tr>
<td>Diabetic control</td>
</tr>
<tr>
<td>Normal OLE</td>
</tr>
<tr>
<td>Diabetic OLE</td>
</tr>
<tr>
<td>OLE</td>
</tr>
<tr>
<td>LSD 0.05</td>
</tr>
</tbody>
</table>

Values are mean ±SEM. Each group contains 6 rats. The mean values with different small letter within a column indicate significant differences (p<0.05). The mean values with different capital letter within a row indicates significant differences (p<0.05).
Currently, diabetes mellitus is characterized as a chronic metabolic disease conjugated with great prevalence and global public health concern where, it is caused by absolute or relative deficiency in insulin and/or reduction in insulin activity [20].

The deficiency in insulin secretion or insulin tolerance increase the level of blood glucose and cause vital damage to body systems such as, nerves and blood vessels [21].

The present study showed a high increase in glucose level (283.28 mg/dl). However, the levels of total albumin and total protein decreased. These results are similar to the results of Wang et al. [20] who observed an increase in blood glucose level. In the same context, Laaboudi et al. [22] found an increase in glucose level and a decrease in total protein level in diabetic group, they demonstrated that the oral administration of olive and leaves extract decreased the levels of glucose, and increased total protein in treated rats. Al-Janabi et al. [23] revealed that the use of OLE improved the levels of blood glucose, total protein, and albumin of diabetic rats. In diabetes stats, a chronic metabolic disorders like increased blood glucose level rising from the lack in insulin secretion, action, or both of them. In addition, increased protein metabolism, albuminuria and microproteinuria may attribute to the reduction in serum levels of total protein and albumin [24]. According to the current results, treatment of diabetic rats with OLE led to decreasing the level of glucose (116.42 mg/dl). This result is in parallel with the observation of Komaki et al. [25] who found that, humans treated with olive leaf extract revealed a significant decrease in blood glucose compared with untreated controls.

There are two possible mechanisms proposed to explain the hypoglycemic effect of olive leaf extract oleuropein: improving glucose-induced insulin release and increasing peripheral uptake of glucose [26]. Another way that might exert the hypoglycemic effect by olive leaf extract is through the inhibition of pancreatic amylase activity [25]. Olive leaf extract may also inhibit starch digestion and glucose uptake or stimulate hepatic glycogen synthesis, culminating in reduced hyperglycemia [27]. The same authors suggested that, starch digestion by intestinal enzymes and the inhibition of disaccharases at the level of the intestinal mucosa may underlie the hypoglycemic effect of the olive leaf extract.

Impact of olive leaves extract on the levels of creatinine, urea and uric acid in different groups.

The levels of creatinine, uric acid and urea in diabetic group showed significant increases (9.75, 14.13 mg/dl and 48.11 g/l, respectively) as compared to normal group. While, the group treated with OLE possessed enhancement in these levels (4.75, 8.63 mg/dl and 36.43 g/l, for creatinine, uric acid and urea respectively) after 10 weeks of treatment. Regarding to the present study, diabetic rats possessed increased levels of urea, creatinine and uric acid. These results are in agreement with the results of Salahuddin and Katary [28] who mentioned that, the diabetic rats revealed an elevation in the level of creatinine and urea. Also Al-Attar and Alsalmi [29] detected a statistically increases in the levels of creatinine and uric acid in diabetic rats. In the treated diabetic group, the levels of creatinine were significantly decreased compared to diabetic group, while the level of uric acid was unchanged compared with normal control rats. Laaboudi et al. [22] confirmed that the administration of OLE decreased the levels of creatinine, urea and uric acid in STZ diabetic male rats. The increases of creatinine, urea and uric acid in diabetic group related to the progressive renal damage and diabetic nephropathy [30]. In addition, the high production of urea may result from the increase of protein catabolism in liver and plasma [31]. Moreover, the increase in uric acid may be related to the decrease in total protein level in diabetic rats, which may have led to muscle wasting and an elevated release of purine, the main source of uric acid [22].

The remediation of diabetic rats with OLE led to reduction in the levels of urea, creatinine and uric acid. The study of Al-Attar et al. [32] showed that, the administration of OLE can prevent severe alterations of renal haematobiochemical markers and disruptions of its histological structure. Tavafi et al. [33] studied the effect of olive leaf extract on gentamicin-induced nephrotoxicity in rats where, they found that the olive leaf extract ameliorated gentamicin nephrotoxicity throughout the antioxidant activity, increase of renal glutathione (GSH) content and increase of renal antioxidant enzymes activity.

**TABLE 2**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Creatinine (mg/dl)</th>
<th>Urea (g/l)</th>
<th>Uric (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero time (10 weeks)</td>
<td>Zero time (10 weeks)</td>
<td>Zero time (10 weeks)</td>
</tr>
<tr>
<td>Normal control</td>
<td>1.42 ± 0.30</td>
<td>35.28 ± 1.10</td>
<td>4.87 ± 0.28</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>1.33 ± 0.44</td>
<td>36.02 ± 3.58</td>
<td>4.71 ± 0.17</td>
</tr>
<tr>
<td>Normal + OLE</td>
<td>1.25 ± 0.25</td>
<td>34.01 ± 1.14</td>
<td>5.26 ± 0.49</td>
</tr>
<tr>
<td>Diabetic + OLE</td>
<td>1.13 ± 0.13</td>
<td>36.43 ± 2.40</td>
<td>4.92 ± 0.19</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>1.48</td>
<td>10.40</td>
<td>6.84</td>
</tr>
</tbody>
</table>

Values are mean ±SEM. Each group contains 6 rats. The mean values with different small letter within a column indicate significant differences (p<0.05). The mean values with different capital letter within a row indicates significant differences (p<0.05).
The present findings showed highly increased activity of AST and a moderate significant increase in ALP and ALT activities in the diabetic control group (Table 3). AST activity has shown a 2 fold increase in diabetic control rats at the end of the experiment. However, a significant decrease of AST activity in diabetic rats treated with OLE (10.67 U/l) compared to diabetic control. The present results indicated that, treatment with OLE didn’t permit the ALP activity from rising in the plasma of diabetic treated group (39.50 U/l) compared to diabetic control group (47.58 U/l). ALP activity in treated normal group (36.50 U/l) was very close to normal group (37.33U/l). At the same time, diabetic rats exhibited a significant increase in ALT activity (47.58 U/l) after 10 weeks of treatment, while it was 37.00 U/l at zero time. Besides, the OLE treated diabetic group recorded a significantly lower value (39.50 U/l) at the end of the experiment compared with a diabetic control group (Table 3).

The results of the current work showed significant increase in ALP, AST and ALT in diabetic group. These findings are generally in accordance with previous experimental diabetes studies [29]. In diabetes mellitus, a significant decrease in levels of total protein and albumin and increases of levels of ALT, AST and ALP indicated the damage happen to liver cells in diabetic group [34]. Sakr et al. [35] mentioned that, treating rats with STZ caused structural alterations in the liver. These alterations

### TABLE 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALP (U/l)</th>
<th>AST (U/l)</th>
<th>ALT (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero time</td>
<td>10 weeks</td>
<td>Zero time</td>
</tr>
<tr>
<td>Normal control</td>
<td>30.59 ± 2.84</td>
<td>34.59 ± 0.94</td>
<td>10.50 ± 1.32</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>31.28 ± 2.54</td>
<td>59.76 ± 2.07</td>
<td>9.25 ± 0.43</td>
</tr>
<tr>
<td>Normal + OLE</td>
<td>32.12 ± 1.93</td>
<td>30.61 ± 2.14</td>
<td>11.5 ± 1.5</td>
</tr>
<tr>
<td>Diabetic + OLE</td>
<td>33.75 ± 0.99</td>
<td>36.89 ± 3.09</td>
<td>9.25 ± 0.75</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>10.59</td>
<td>7.16</td>
<td>3.86</td>
</tr>
</tbody>
</table>

Values are mean ±SEM. Each group contains 6 rats. The mean values with different small letter within a column indicate significant differences (p<0.05). The mean values with different capital letter within a row indicates significant differences (p<0.05).

**FIGURE 1**

Correlation between: A. creatinine (mg/dl) and ALP (U/l), B. creatinine (mg/dl) and ALT (U/l), C creatinine (mg/dl) and AST(U/l), D. ALP (U/l) and AST(U/l).
include leucocytic infiltrations, congestion of blood vessels, and cytoplasmic vacuolization of hepatocytes. Of these liver enzymes, ALT is most closely related to liver fat accumulation and consequently ALT has been used as a marker of non-alcoholic fatty liver disease (NAFLD) [36, 37].

Regarding to the liver enzymes activities, the OLE-treated group improved the activities of ALP, AST and ALT (Table 3). The obtained results of Hamad [38] showed that, olive leaf extract have very high phenol content and possess strong antioxidant activity and significant effect on liver damages induced by CCl₄ administration, which result in improved serum ALT and AST and increased serum total antioxidant capacity in comparison with CCl₄ treated group. So, the improving effect of olive may related to its antioxidant activity.

**Pearson correlation between lipid profile and heart function parameters.** A Pearson product-moment correlation coefficient was computed to estimate the relationship between different kidney and liver function parameters. The results suggest that that 18 out of 36 correlations were statistically significant.

Statistical analysis confirmed these results, indicating a significant positive correlation between both creatinine and ALP \((r^2 = 0.70, p \leq 0.05)\), and between creatinine and ALT \((r^2 = 0.54, p \leq 0.05)\) (Fig1 A and B). Statistical analysis indicated a significant positive correlation between creatinine and ALT \((r^2 = 0.77, p \leq 0.05)\), and between ALP and AST \((r^2 = 0.77, p \leq 0.05)\) (Fig1 C and D). Results of Al-Attar and Alsalmi [29] exhibited a positive correlation between creatinine and ALP, creatinine and ALT, creatinine and ALT, ALP and AST.

At the same time, the present study showed a strong significant positive correlation between uric acid and ALP \((r^2 = 0.795, p \leq 0.05)\), and a significant negative correlation between uric acid and total protein \((r^2 = 0.51, p \leq 0.05)\) (Fig2 A and B). Laaboudi et al. [22] also found a correlation between uric acid and total protein.

**CONCLUSIONS**

Olive leaves extract showed an ameliorative effect on the diabetic state, through improving kidney function, liver function, and exerting a hypoglycemic effect. So, using OLE as a medicinal product can be beneficial to diabetic individuals. At the same time, it will be a less cost and more safe than using organic solvents.

**ACKNOWLEDGEMENTS**

Authors would like to show appreciation to Faculty of Agriculture, Cairo University, Department of Biochemistry for continuing cooperation to support research that provided facilities necessary to accomplish the most wanted objectives of the research.

**REFERENCES**


