ABSTRACT

Objective: The oil of Juniperus communis (JC) which is among medicinal plants, has many pharmacological activities. In this study, the effects of JC oil on serum paraoxonase (PON1), pancreatic enzymes levels and lipid levels in experimental diabetic rats were investigated.

Materials and Methods: Thirty-two male Wistar-Albino rats (250-300g) were used. The rats were divided equally into four groups, control (C), diabetes (D), JC oil (J), and diabetes + JC oil (DJ). D and DJ groups were intraperitoneally (IP) injected with 45 mg/kg streptozotocin (STZ). JC oil was administered as 200 mg/kg/21 days by oral gavage in J and DJ groups.

Results: Total cholesterol (TC) and triglyceride (TG) levels were significantly decreased in the J and DJ groups when compared to C and D groups (p≤0.001). There was no difference in TG levels between D and control group (p≥0.05). Lipoprotein levels were not statistically significant between any group (p≥0.05). Comparing to the control group in the diabetes and DJ groups; significant decreased amylase levels and increased lipase levels (p<0.001) was observed. Paraoxonase activity in D group was statistically lower than in the other groups (p≤0.001). There is no significant difference between the C group and the J group (p>0.05). PON1 level has a significant elevation in the DJ in comparison with the D group (p≤0.05).

Discussion: As a result, JC oil caused an increase in antioxidant PON1 enzyme level and a decrease in lipid levels in diabetes. The data obtained are supportive that JC oil may be a potential protective effect against diabetes-associated complications.

Keywords: Diabetes, Juniperus communis oil, Paraoxonase, Lipoproteins, Lipids, Pancreatic enzymes

INTRODUCTION

Diabetes mellitus (DM) is a diverse group of metabolic diseases that is characterized by chronic hyperglycaemia and abnormalities in carbohydrate, lipid and protein metabolism. Chronic and postprandial hyperglycaemia is mainly responsible for the acute, short term, and late complications that affect all organs and systems in the body (Benjamin et. al. 2016). Diabetes and its complications is a leading cause of death and paralysis, reducing total and healthy life expectancy (Alberti and Zimmet, 1998). DM increases the risk of microvascular damage (eg, retinopathy, nephropathy and neuropathy) (Alexandru et al., 2016). Hyperglycaemia in the course of diabetes contributes to the oxidative stress, causing the redox balance of the body to change into excessive production of reactive oxygen species (Prasath et al. 2013).
There are a number of plants that have been proved worldwide to be useful for treatment of DM disease and known to have hypoglycaemic activities (Akdogan et al., 2012; Yegin et al. 2013; Çiftçi et al. 2017). The mechanisms, by which the herbs generally act, are not fully established, but most of medicinal plants possess antioxidant activities. These plants have been shown to be effective by this property is various conditions including diabetes, cancer, memory deficit, Alzheimer’s disease, atherosclerosis, diabetes and cardiovascular diseases. Antioxidant activities of herbal medicines are also effective in reducing the toxicities of toxic agents or other drugs (Karimi et al., 2015).

JC oil is used as a herbal medicine because it has therapeutic advantages and used for treatment of many diseases such as arthritis, diabetes, cancer and kidney disorders. JC oil has many pharmacological activities including hepatoprotective, antibacterial, anti-inflammatory and antidiabetic activities (Seca and Silva, 2005). Oil obtained from JC fruit by means of hydroxylation method was found to be composed of monoterpene hydrocarbon (22.97%), oxygenated monoterpenes (14.86%), sesquiterpene hydrocarbon (32.43%), oxygenated sesquiterpenes (6.75%), diterpene (1.35%), and others (21.62%) (Tuzmen and Hafizoğlu 2004). Paraoxonase (PON_1) is a serum esterase that synthesized in the liver and associated with the “good” cholesterol high density lipoprotein (HDL). Population diabetes mellitus was shown to have marked reductions in serum paraoxonase activity without having a significantly lower HDL cholesterol concentration and this shows that serum paraoxonase activity is low in insulin-dependent diabetics (Mackness et al., 2002; Çiftçi et al., 2017). PON_1 is a potent anti-diabetic enzyme that exerts this protection against diabetes through its antioxidative characteristics and by its insulin stimulation properties on β-cells. The PON_1 activity and concentration are highly variable in humans. Both quality and quantity of the enzyme in serum are important in an individual for risk of developing cardiovascular disease (Koren-Gluzer et al., 2011).

The aim of this study is to investigate the effect of JC oil with antioxidant/oxidant status, and antidiabetic properties (hiperglisemia, hyperlipidaemia) on serum paraoxonase and pancreatic enzymes activity, lipid and lipoprotein levels in rats with experimentally diabetes induced rats.

### MATERIALS and METHODS

#### JC oil

The cold infiltration form which was produced by JC oil, was purchased from Nature Derman Food, Drugs Firm, Bursa, Turkey. JC oil was dissolved in distilled water containing 0.5% sodium carboxyl methyl cellulose (CMC).

#### Animals

In this study, 32 male Wistar-Albino rats weighing 250-300 g were acquired from the Experimental Animal Unit of Van Yuzuncu Yil University, Faculty of Medicine. Subjects were divided equally into four experimental groups, control (C), diabetes generated (D), JC oil given (J), diabetes induced, and JC oil given (DJ). The rats were accommodated in cages, in which feed and fresh water were present all the time, in the rooms adjusted for a temperature of 22±2 °C, applied for 12 hours in darkness / light during the three weeks trial period. Experiments conducted according to ethical rules and carried out under the supervision of Van Yuzuncu Yil University Animal Experiments Local Ethics Committee (2016-04).

#### Experimental animal design

Diabetes was induced in D and DJ groups by injecting 45 mg/kg single dose of streptozotocin (Sigma, Germany) dissolved in citrate buffer PH: 4.5 intraperitoneally (IP) (Vardi et al. 2005). 200 mg/kg of JC oil extract was administered to the rats of J group, DJ group with intragastric gavage (Petlevski et al., 2001). Experimental animal desing were given in Table 1.

#### Control group (C): Eight rats were randomly selected as the control group. 45mg/kg single dose of physiological serum was injected intraperitoneally (IP). The feed was collected at 8± AM daily, and removed from rats of this group and rats were given distilled water with 0.5% of carboxymethyl cellulose (CMC) orally by gavage at 10± AM.

#### Diabetes group (D): It contains eight male rats which exposed to single dose of 45 mg/kg of streptozotocin in citrate buffer intraperitoneally. After 72 hour of injection, blood glucose was measured using glucometer (eBsensor, Taiwan), and rats with more than 300 mg/dl blood glucose were considered diabetic. Also, this group was feed only standard diet. The feed was collected at 8± AM daily, and removed from rats of this group and every rat were given distilled water (contents 0.5% of CMC) orally by gavage at 10± AM.
**Table 1. Preparation of the experimental animal**

<table>
<thead>
<tr>
<th>Control group (C)</th>
<th>Diabetes group (D)</th>
<th>Juniperus communis oil (J)</th>
<th>Diabetes + J. communis oil (DJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water (contents 0.5% of CMC)</td>
<td>STZ (45mg/kg)</td>
<td>(200 mg/kg of J oil and dissolved in distilled water (contents 0.5% of CMC)</td>
<td>STZ (45mg/kg) + (J oil and dissolved in distilled water (contents 0.5% of CMC)</td>
</tr>
</tbody>
</table>

**JC oil group (J):** Every day, the feed of rats was collected at 8am AM. *Juniperus communis* oil was given dissolved distilled water (contents 0.5% of CMC) and eight rats were applied orally by gavage for 21 days as 200 mg/kg/day at 10am AM.

**Diabetes and JC oil group (DJ):** Diabetes was induced in this group by injecting single dose of 45 mg/kg Streptozotocin (Sigma, Germany) dissolved in citrate buffer intraperitoneally given. After 72 hours, blood glucose has been examined using glucometer using strips and glucometer (eBsensor, Taiwan) and rats with more than 300 mg/kg blood glucose were considered diabetic. Every day, the feed of rats was collected at 8am AM. *Juniperus communis* oil was given dissolved distilled water (contents 0.5% of CMC) was applied orally by gavage to those rats with blood glucose levels of 300 mg/dL and above, at 21 days as 200 mg/kg/day at 10am AM.

**Sample collection**

After 21 days of the experimental regime, blood was withdrawn from heart puncture into both jell tubes and EDTA tubes under ketamine anesthesia. Blood collected in jell tubes were centrifuged (3000 rpm at 4°C) for 10 minutes. Then the separated serum was transferred to eppendorf tubes. Blood glucose in serum and Hb1Ac levels in total blood were measured on the same day. The serum was stored at -20°C until analysis of other parameters.

**Biochemical analysis**

At the end of the study, biochemical parameters of serum glucose, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), total cholesterol (TC), total triglycerides (TG), amylase and lipase were determined using commercial kits (Roche, Germany) and biochemical autoanalyzer model HITACHI/PA800 supplied by Roche, Germany. HbA1c was also determined from EDTA tubes blood at the same day using commercial kits and COBAS Integra 800 autoanalyzer supplied by Roche, Germany. Spectrophotometric method was used in the determination of paraoxonase activity in serum. This enzyme was examined using commercial kit from Rel assay, Gaziantep, Turkey (Catalog Number 1027010).

**Statistical analysis**

At the end of the study, all data from groups for every biochemical parameter were analyzed using SPSS program pack 13.0. Comparisons among groups were done using nonparametric Kruskal-Wallis test first and then the Mann-Whitney U-test as the post hoc test correction was used. Data were given as mean ± SD (standard deviation) and p-values<0.001 and ≤0.05 were considered statistically significant, respectively.

**RESULTS**

The levels of serum glucose, HbA1c, total cholesterol, total triglycerides, lipoproteins (HDL-C, LDL-C and VLDL-C), exocrine pancreatic functions (amylase and lipase), and paraoxonase in all experimental groups were shown in Table 2.

There is a significant increase in serum glucose level in the D group when compared to control group (p≤0.001). No significant difference can be seen in glucose level between control group and JC group (p>0.05). Glucose level was decreased significantly in the DJ group when compared to D group (p≤0.001) (Table 2).

Level of HbA1c in Table 2 were significantly increased only in the D group and decreased significantly in DJ group when compared to the C group (p≤0.001). There is no significant difference between the C group and JC oil group (p≥0.05). In comparison between both the D and the DJ groups, a significant decrease can be seen in the DJ group (p≤0.001).

Serum total cholesterol level was considerably increased in the D group comparison with C group (p≤0.001). Level of total cholesterol in the JC group is significantly elevated when compared to the C group (p≤0.001). A significant decrease in total cholesterol level can be seen in the DJ group compared to the D group (p≤0.001) (Table 2).

Serum triglyceride levels between the C group and the D group showed no significant difference (p≥0.05). Serum triglyceride level was decreased significantly in the JC group (J), when compared with the C group (p≤0.001). It is also significantly
decreased in the DJ group and the J group in comparison with the D group (p≤0.001) (Table 2).

Serum levels of lipoprotein (HDL-C, LDL-C, VLDL-C) showed no significant difference between the C group and both the D group and J groups. Also, no significant difference can be seen between D group and DJ group (p≥0.05) (Table 2).

Serum levels of amylase enzyme showed statistically significant lowering in the D group when it is compared to the C group (p≤0.001). No significant difference has been detected between the C group and J group (p≥0.05). Also, there is no significant difference between the D group and the DJ in amylase activity (p≥0.05) (Table 2).

Serum lipase enzyme level showed statistically significant elevation in diabetes group (D), when compared to control group (C) (p≤0.05). But there is no significant difference between the C group (C) and the J group and between the D group and the DJ in lipase activity (p≥0.05) (Table 2).

Serum paraoxonase-1 (PON1) enzyme activity was significantly lower in the D group than in the C group (p≤0.05). There is no significant difference between the C group and the J group (p≥0.05). PON1 level has a significant elevation in the DJ in comparison with the D group (p≤0.05) (Table 2).

Table 2. Serum glucose, hemoglobin A1c, total cholesterol, total triglyceride, lipoprotein, lipase, amylase and paraoxonase levels of rats in groups.

<table>
<thead>
<tr>
<th></th>
<th>Control (C)</th>
<th>Diabetes (D)</th>
<th>J/C oil (J)</th>
<th>Diabetes+J/C oil (DJ)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>156±37 a</td>
<td>636±83 c</td>
<td>206±33 a</td>
<td>497±118 b</td>
<td>0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>3.78±0.13 a</td>
<td>8.04±0.76 c</td>
<td>3.80±0.12 a</td>
<td>6.89±1.59 b</td>
<td>0.001</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>56±9 a</td>
<td>76±10 c</td>
<td>65±6 b</td>
<td>60±3 ab</td>
<td>0.001</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>88±1 b</td>
<td>100±3 b</td>
<td>55±2 a</td>
<td>46±5 a</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>52±13 a</td>
<td>58±4 a</td>
<td>57±7 a</td>
<td>57±6 a</td>
<td>0.455</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>20±7 a</td>
<td>25±4 ac</td>
<td>30±6 c</td>
<td>25±4 ac</td>
<td>0.019</td>
</tr>
<tr>
<td>VLDL-C (mg/dL)</td>
<td>18±2 b</td>
<td>20±6 b</td>
<td>12±5 a</td>
<td>10±1 a</td>
<td>0.001</td>
</tr>
<tr>
<td>Amylase U/L</td>
<td>1073±75 b</td>
<td>692±253 a</td>
<td>1074±71 b</td>
<td>676±91 a</td>
<td>0.001</td>
</tr>
<tr>
<td>Lipase (U/L)</td>
<td>7.63±1.30 a</td>
<td>20.88±7.77 b</td>
<td>8.87±2.80 a</td>
<td>23.00±7.67 b</td>
<td>0.001</td>
</tr>
<tr>
<td>PON1 (U/L)</td>
<td>33.07±31.94 b</td>
<td>3.17±4.94 a</td>
<td>54.13±26.43 b</td>
<td>36.45±18.73 b</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Differences between mean values with different letters are significant (P≤0.001-0.05).

HbA1c, Hemoglobin A1c; TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; VLDL-C, very low density lipoprotein cholesterol; PON1, paraoxonase. Data were expressed as mean±SD.

DISCUSSION

Diabetes mellitus and its complications are among today’s most common diseases and the incidence is increasing. Among the complications high blood cholesterol and triglyceride levels are two of the fundamental reasons underlying serious health problems, primarily atherosclerosis and heart diseases, of people today. For this reason, retaining the blood cholesterol levels at normal levels is important to stop the progression of numerous diseases. Many traditional plant treatments for diabetes have been used all over the world; in this study we used JC plant oil as anti-diabetic plant that used to prevent diabetes mellitus disease. Moreover, Swanston-Flatt et al. (1990) studied the effect of several plants including JC on glucose homeostasis. These plants have been used as traditional treatments for diabetes mellitus.

Several studies showed that plants may cause an increase in beta cells in the pancreas by activating regeneration of insulin secreting cells (Abdel-Moneim et al., 1997). Additionally, fibre content of plants is known to interfere with the carbohydrate content in the process of intestinal absorption lowering blood glucose levels (Nelson et al., 1991). JC has a strong antioxidant activity among traditional plants. This activity might have preventive effects against protein glycation. This activity has also roles in alleviating diabetic macro vascular and micro vascular complications (Asgary et al., 2014).
In addition to JC oil, another critical reason, behind the inhibitory effect of JC plant on glucose and HbA1c, is that this plant contains flavonoids which are a group of polyphenolic compounds ubiquitously found in plants including fruits and vegetables. Natural flavonoids can be used as alternative blockers of cellular glucose uptake in vitro (Jae, 1999).

Antioxidant phytoconstituents are the secondary constituents or metabolites naturally found in plants such as fruits and vegetables. Plants produce a various types of antioxidant compounds such as carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols and tocotrienols. Several synthetic antidiabetic drugs like glipalamide, metformin, and repaglinide also possess antioxidant activity, so this shows a similarity-between natural-antioxidant substances and synthetic drugs (Venkatesh and Sood, 2011).

In the current study, cholesterol, triglyceride, were significantly decreased after the treatment of diabetic rats with the JC plant oil. The inhibitory effects of this plant are consistent with many other studies.

Another study showed that JC oil also decreased the cholesterol levels in diabetic rats but in that study, cholesterol containing diet was used to induce hypercholesterolemia, then they used different doses of the plant oil detecting different hypocholesterolemic activities (Akdogan et al., 2012).

Constituents in JC are important in cholesterol reducing activity. Several mechanisms were suggested to clarify the inhibitory effect of plant sterols and stanols against cholesterol levels. First, plant sterol competes with cholesterol to unite with micelle thus leading to lower cholesterol absorption. Second, plant sterol competes with cholesterol to enter through the cell membrane in the intestines, thus leading to low cholesterol absorption. Finally, by the action of these substances, the soluble cholesterol transforms into non-soluble cholesterol, which prevents absorption. Thus, cholesterol is excreted through feces (Williams and Gokool, 2005).

In this study, the level of serum triglycerides was decreased significantly in diabetic rats after using the plant. Diabetic dyslipidaemia is regarded as a major risk factor for diabetic complications and has been widely investigated. Macro and micro angiopathies in diabetic dyslipidaemia are characterized by nephropathy, neuropathy, retinopathy, and complications belonging to cardiovascular, cerebrovascular and peripheral vascular systems (Habib and Aslam, 2003).

In the present study, the level amylase was decreased in the diabetes in comparison to control group. The same conclusion was reported by other previous researchers and it was suggested that low serum amylase in diabetes was associated with increased risk of metabolic abnormalities (Nakajima et al., 2011). The reduction in the serum pancreatic amylase was recorded in both types of diabetes, which amounted to 71% for type 1 diabetics and 49% for type 2 diabetics. On the other hand, the reduction in serum amylase in type 2 diabetes was higher in patients with longer duration of illness (59%) and in patients with low serum insulin value (79%), while reduction in serum lipase was only detected in patients with very low serum insulin (34%) (Augsteen et al., 2005).

In the current study, a slight decrease in the levels of amylase can be seen in diabetic rats after the treatment JC oil; however, this decrease was not statistically significant. Other previous studies showed that JC oil exhibited a strong inhibitory effect on amylase enzyme (Najafian et al., 2011; Jumepaeng et al., 2013).

Substances that are inhibitors for α-amylase enzyme are available in plants such as polyphenolic compounds and glycoproteins (Babu et al., 2013). The inhibition of amylase enzyme by JC may be due to the presence of some secondary metabolites such as lignans, coumarins, sterols, aliphatic compounds, and other terpenes in the hydro alcoholic extract. Leaves and fruits of JC also contain relatively high amounts of monoterpen hydrocarbons such as α-pinene, limonene and β-myrcene which they result in lowering amylase enzyme (Lohani et al., 2013).

The natural bioactive compound available in JC including luteolin, β-pinene, and myrcene may also be inhibiting amylase enzyme activity. It was known that luteolin can cause a reduction in amylase enzyme activity (Kim et al., 2000). Steroids that are found in JC may also cause inhibition of amylase activity because of their effect on pancreatic secretion (Barzilai et al., 1986; Jelenkovic et al., 2014).

In the current study, the level of lipase was increased significantly in diabetes. The same results were found in some previous studies. It was known that nearly 25% of diabetic subjects showed an
increase in the pancreatic level to an extent that, the authors suggest that pancreas levels should be regarded as a parameter for diabetic diagnosis (Steinberg et al., 2014). Despite these findings occasional diabetic lipase reduction can be seen. In type 2 diabetes, the reduction in lipase activity was only detected in patients with very low serum insulin level (Augsteen et al, 2005).

In this study, the treatment of diabetic rats showed no considerable effect on lipase activity; however, this result disagrees with some studies that deal with the activity of anti-diabetic plant on lipase activity. A large study concluded that most of anti-diabetic plants have also anti-lipase activities. The study suggested using those plants for the treatment of obesity and diabetic dyslipidemia due to their inhibitory effects on lipase activity. Further studies about the effect of JC on lipase activity may clarify the exact effect of this plant (Young and Hui, 2005).

The present study showed that diabetes was associated with a significant reduction in serum paraoxonase activity, this result has been shown also by other researchers (Gupta et al., 2011; Kota et al., 2013; Yegin et al., 2013; Çiftçi et al. 2017). Serum Paraoxonase-1 (PON1) is an antioxidant enzyme that associated with high-density lipoproteins. Low PON1 activity has been found in oxidative stress-associated processes as dyslipidaemia, advancing age, diabetes mellitus, and smoking. In diabetes, oxidative stress is related to the degree of insulin resistance, a key component of the metabolic syndrome (Senti et al., 2003). The decreased activities of serum Paraoxonase-1 (PON1), an antioxidant enzyme has been shown to be related with dyslipidemia, diabetes mellitus, advanced age, hypertension, low HDL and increased oxidative stress (Turkoglu et al. 2008; Yegin et al., 2013).

In the current study, there is a significant decrease of PON1 activity in the diabetes group compared to control group. After giving JC oil to the diabetic rats, PON1 activity was increased significantly. This stimulatory effect on paraoxonase enzyme activity is shown for the first time and regarded as the main discovery for this study. Decreased serum PON1 activity in diabetes may play an important role the in development of premature atherosclerosis and thereby cardiovascular diseases (Ferretti et al., 2001).

Strong antioxidant property of JC is regarded as a common cause of increasing activity of PON levels (Hoferl et al., 2014). Phytochemicals with antioxidant activity such as coumarins, phenylpropanoids, cinnamon acids, diterpenes, flavonoids, monoterpenes, lignans, tannins and triterpenes also are beneficial in protecting diabetes or diabetic complications (Montonen et al., 2004). Among the antioxidant substances, activities of radical scavenging of flavonoids are well understood (Lohani et al., 2013).

PON1 levels have clinical significance, it was known that high-density lipoproteins (HDL) reduce the accumulation of lipid peroxides in LDL mainly due to paraoxonase enzyme activity proven via in vitro studies. PON1 is responsible for the most of antioxidant properties of HDL, and because PON1 is antioxidant and anti-inflammatory enzyme so deficiency of this enzyme results in inflammation and oxidative stress (Litvinov et al., 2012).

**CONCLUSION**

In conclusion, this study showed that JC oil enhances anti-diabetic activity by reducing both glucose and glycosylated hemoglobin levels. High blood cholesterol and triglyceride level are one of the fundamental reasons underlying serious health problems, primarily atherosclerosis and heart diseases, of people now a days. The oil of this plant showed that it could play a role in alleviating diabetic dyslipidemia as it reduced plasma lipid levels. Finally, the plant caused an increase in the paraoxonase activity in diabetic rats leading to increase in antioxidant activities in diabetic subjects.

The increase of antioxidant PON1 enzyme level and a decrease in lipid levels in diabetes make of JC oil a potentially protective against the complications of diabetes.

**ACKNOWLEDGMENTS**

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**Ethical approval:** The study was approved by judgement with 05.05.2016-2016-04 reference number of Van Yuzuncu Yil University Animal Experiments Local Ethics Committee.

**Conflict of Interests:** The authors declared that there is no conflict of interests.
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