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The Effect of Aqueous Extract of Pine Kindling on the Liver and Kidney Tissues of Diabetic Rats

Sulu Çam Çıra Ekstraktının Diyabetik Sıçanların Karaciğer ve Böbrek Dokuları Üzerindeki Etkisi

Ökkeş Yılmaz[1], Ersin Demir[2*], Halise Sarıgül[1]

[1]Firat University, Faculty of Science, Department of Biology, Elazig, Turkey.

[2]Duzce University, Faculty of Agriculture and Natural Sciences, Department of Agricultural Biotechnology, Duzce, Turkey.

ABSTRACT

In this study, the effect of aqueous extract of kindling wood obtained from pine trees on liver and kidney tissues of streptozotocin (STZ)-induced diabetic rats was investigated. Male Sprague Dawley rats were divided into five groups: 1) Control, 2) STZ rats 3) STZ rats treated with aqueous extract obtained from kindling wood of pine trees (respectively 100 g/L, 200 g/L and 400 g/L) for 10 weeks. According to our results, it was determined that this extract showed beneficial effects on oxidative stress (Malondialdehyde (MDA), Reduced glutathione (GSH), Oxidized glutathione (GSSG)) in liver and kidney tissues. Pine kindling extract showed generally positive effects on glycogen, cholesterol, alpha tocopherol, retinol, palmitic acid, stearic acid, oleic acid, linoleic acid, arachidonic acid and docosahexaenoic acid parameters in the liver and kidney tissues of diabetic rats. Pine kindling extract did not showed inhibitory effect on the amylase enzyme activity derived from bovine, porcine pancreas, fungi and bacteria during in vitro experiments. Pine kindling extract also did not exhibit inhibitor effect on α-glycosidase enzyme that hydrolyzes disaccharides.

Key Words

Amylase, glucosidas, oxidative stress, pine kindling wood, type-2 diabetes.

ÖZ

Bu çalışmada, streptozotosinin neden olduğu diyabette çam ağaçlarının çıra kısmından elde edilen sulu özütlerin, sıçan karaciğer ve böbrek dokuları üzerindeki etkisi araştırılmıştır. Erkek Sprague Dawley sıçanları beş gruba ayrıldı: 1) Kontrol, 2) STZ grubu 3) STZ grubu+Çam ağaçlarının çıra kısmından elde edilen sulu özütler (sırasıyla 100 g/L, 200 g/L ve 400 g/L) 10 hafta süre ile uygulandı. Çam çıra özütünün karaciğer ve böbrek dokusunda oksidatif stres (Malondialdehit (MDA), İndirgenmiş glutatyon (GSH), yükseltgenmiş glutatyon (GSSG)) üzerinde faydalı etkiler gösterdiği belirlendi. Diyabetik sıçanların karaciğer ve böbrek dokularında glikojen, kolesterol, alfa tokoferol, retinol, palmitik asit, stearik asit, oleik asit, linoleik asit, araşidonik asit ve dokosaheksaenoik asit parameterleri üzerinde çam çıra özütünün genel olarak olumlu etkiler gösterdiği tespit edildi. İn vitro ortamda yapılan çalışmalarda, sığır, domuz pankreası, mantar ve bakterilerden elde edilen amilaz enzimlerinin aktivitesi üzerine çam ekstraktının inhibitör etki yapmadığı gözlendi. Ayrıca disakkaritleri hidroliz eden α-glukozidaz enzimi üzerine de çam çırasının inhibitör etkiye sahip olmadığı saptandı.

Anahtar Kelimeler

Amilaz, glukozidaz, çam çıra, oksidatif stres, tip-2 diyabet.

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Correspondence to: E. Demir, Duzce University, Fac. of Agriculture and Natural Sciences, Dep. of Agricultural Biotechnology Duzce, Turkey.

E-Mail: ersncan.dmr@gmail.com

INTRODUCTION

Diabetes mellitus (DM) is defined as a metabolic disease characterized by chronic hyperglycemia due to defects in insulin metabolism, as well as various complications such as neuropathy, nephropathy and retinopathy occur in the future accompanied by disorders of carbohydrate, fat and protein metabolism [1]. In recent times, many antidiabetic agents occurred the plants have been discovered, but diabetes and its related complications continue to be a major medical problem both developed countries and in developing countries. Plants are an important source in the discovery of new antidiabetic compounds. Studies show that, plants play an important role in preventing complications of diabetes due to their antioxidant compounds. Detection of novel antidiabetic compounds with antioxidant properties may be great importance in the development of new therapeutic approaches for diabetes patients [2]. *Pine* is a coniferous forest tree belonging to the family Pinaceae. There are five species of *Yellow pine, Black pine, Aleppo pine, Red pine* and *Peanut pine* in Turkey. *Pine* grows in almost every region of Turkey. The majority of *pine* species have thick rough and cracked appearance. There is resin in the crust and wood part of the pine. *Pine* is one of the most common medicinal plants of the Mediterranean and Iran-Turan regions. The *Pine* is used for many years in Turkish folk medicine due to the antiseptic effects on respiratory system and urinary tract disorders [3]. Products obtained from *Pine* species in Turkey, are used particularly antiseptic, expectorant, respiratory and urinary tract diseases, rheumatism and skin diseases [4]. It has been reported that the extract of *Pinus densiflora* has the potential to prevent and/or heal obesity [5]. It has been observed that different *Pine* extracts have a positive effect on human health [6,7].

In this study, the parts obtained from *Pine* wood are called "çam çırası" among the people in Turkish and these parts are the richest place of *Pine* in terms of resin. In diverse regions of Turkey, especially diabetics, they express that they have consumed this water by putting this part of *Pine* resin into drinking water. People with diabetes who drink this water have expressed that they feel themselves good. In this study, the effect of aqueous *Pine* kindling extract on liver and kidney tissues of streptozotocin (STZ)-induced diabetic rats was investigated.

MATERIALS and METHODS

Sprague Dawley albino rats were obtained from Firat University Experimental Research Centers (FUDAM) and studies were conducted under appropriate experimental conditions in this center. All the experimental practices were conducted following the approval of the Ethical Committee of Animal Experiments at the Firat University, in accordance with the ethical rules of standard experimental animal studies (Decision number: 2012/121).

In experimental studies 49 pieces Sprague Dawley albino male rats were used weighing 364-473 g, 8-10 weeks old. These rats were randomly assigned to five groups.

1. Control group (n= 5): Any application was not performed.

2. Diabetic group (n= 11): Experimental Type-2 diabetes was generated by administering a single dose of 45 mg/kg STZ. The groups of rats were provided with food and water ad libitum.

3. Diabetes+Pine extract (Aqueous extracts from the obtained kindling wood of Pine trees ''Çam çıra'') group-100 g/L (n= 11): Experimental Type-2 diabetes was generated by administering a single dose of 45 mg/kg STZ. After one week the formation of diabetes, 100 g/L of *Pine* extract was added to the drinking water and this water was provided for drinking during the experiment.

4. Diabetes+Pine extract (Aqueous extracts from the obtained kindling wood of Pine trees ''Çam çıra'') group-200 g/L (n= 11): Experimental Type-2 diabetes was generated by administering a single dose of 45 mg/kg STZ. After one week the formation of diabetes, 200 g/L of Pine extract was added to the drinking water and this water was provided for drinking during the experiment.

5. Diabetes+Pine extract (Aqueous extracts from the obtained kindling wood of Pine trees ''Çam çıra'') group-400 g/L (n= 11): Experimental Type-2 diabetes was generated by administering a single dose of 45 mg/kg STZ. After one week the formation of diabetes, 400 g/L of *Pine* extract was added to the drinking water and this water was provided for drinking during the experiment. Diabetes was induced by single dose intraperitoneal injection of STZ (45 mg/kg), which dissolved in 0.1 M sodium citrate (pH= 4.5) [8]. After 72 hours following

STZ administration, blood samples were collected from tail vein, and samples were analyzed for blood glucose by using a glucometer (Smart Check). As a result, rats having a fasting blood glucose level between 140 and 200 mg/dL were considered to be Type 2 diabetic [9]. According to the above-mentioned criterion, rats diagnosed with diabetes, and then they were used in this study. This study was conducted 10 weeks. Throughout the study, small pieces obtained from the fatty parts of the *Pine* trees participated in drinking water of rats certain amount. The rats were allowed to drink from this water. At the end of the study, animals were decapitated according to ethical guidelines. The tissues were taken and washed with physiological saline solution (%0.9). The tissues were stored at -70°C until biochemical treatments.

Experimental Procedures

MDA measurement: The most important indicator of lipid peroxidation is MDA level. MDA level was measured spectrophotometrically by making some changes in the method described by Ohkawa et al. [10]. The MDA results were stated as nmol/g protein.

GSH measurement: It was measured according to the method defined by Ellman [11]. The concentration of the reduced glutathione in the samples was calculated from a glutathione standard curve [12]. The amount of GSH was expressed as µg/g.

Protein measurement: The amount of protein was measured spectrophotometrically according to the method described by Lowry et al [13]. To create the calibration curve, bovine serum albumin was used as standard. Results were expressed as µg/g.

Lipid extraction: In the tissue, fatty acid, vitamins A, E and cholesterol extraction were performed according to the method defined by Hara and Radin [14]. In order to determine the fatty acid composition, 2% methanolic sulfuric acid was added to the samples and the samples were thoroughly mixed.This mixture was kept for about 15 hours at a temperature of 55°C for methylation. [15]. Analyzes were made by gas chromatography. For these analyzes, SP ™ -2380 capillary GC column (L×ID. 30 m×0.25 mm, df 0.20 µm) (Supelco, Sigma, USA) was used. Nitrogen gas was used as a carrier gas. In the analysis, first, the mixtures of the standard fatty acid methyl esters were injected and the retention periods of each fatty acid were determined. After the necessary programming, analysis of the fatty acid methyl esters belonging to the samples was made [16]. 5% methanol potassium hydroxide solution was added to the samples taken for cholesterol with fat soluble vitamins. After mixing, wait for 15 minutes at 85°C. The tubes were removed and cooled to room temperature and purified water was added and mixed. Non-soap lipophilic molecules were extracted with 2x5 mL hexane. The hexane phase was evaporated with a nitrogen gas.

Table 1. Hydrolysis of starch by amylase obtained from different sources according to time in Pine kindling extract environment.

Amylase	Pure water (mL)	Starch solution (5%) (mL)	Pine extract (100 g/L) (mL)	Pine extract (200 g/L) (mL)	Pine extract (400 g/L) (mL)	Enzyme (µL)
Control	1	0.5	1	1	1	-
Bovine pancreas amylase	1	0.5	1	1	1	100
Porcine pancreas amylase	1	0.5	1	1	1	100
Bacillus licheniformes amylase	1	0.5	1	1	1	100
Aspergilus oryzea amylase	1	0.5	1	1	1	100

Table 2. The enzyme test results for amylase isolated from different sources.

Enzymes	Lugol	Benedict
Control	+	-
Bovine pancreas amylase	-	+
Porcine pancreas amylase	-	+
Bacillus licheniformes amylase	-	+
Aspergilus oryzea amylase	-	+

One ml (50%+50%, v/v) was dissolved in acetone/methanol mixture and taken to autosampler vials. A mixture of acetonitril/methanol (60%+40%, v/v) was used as a mobile phase. Mobile phase flow rate was determined as 1.0 mL. UV detector was used for analysis. Supelcosil LC 18 (15×4.6 cm, 5 µm; Sigma, USA) was used as a column. Vitamin A 326 nm (nanometer), vitamin E 202 nm, Vitamin D and K 265 nm were measured in a detection wavelength [17, 18].

Glycogen measurement: Glycogen content was determined as described by Hassid and Abraham, [19]. Calculations were compared with standard glycogen.

Statistical Analysis: For statistical analysis, SPSS 15.0 (SPSS Inc., Chicago, IL, USA) package program was used. ANOVA test (one-way ANOVA) was used in comparison of control and experimental groups. The LSD test was used to compare the groups between themselves. Results were given as mean ± standard error.

RESULTS and DISCUSSION

In this study, we investigated the effects of *Pine* extract (Aqueous *Pine* kindling extracts -Çam çıra) on liver and kidney tissue, MDA, GSH, glycogen, total protein, tissue fatty acid composition, fat soluble vitamins (A and E) and cholesterol levels in diabetic rats. In preliminary studies, it was determined that the aqueous extract obtained from the wood part of the *Pine* was more effective than the extracts obtained from the leaves and shell parts of the *Pine* tree. In some studies, although some active ingredients obtained from leaf and shell parts of the tea have been used successfully in the treatment of diabetes. A preliminary study was conducted to determine whether this *Pine* aqueous extract was effective on digestion enzymes. In this study, the effect of *Pine* kindling extracts on bovine, pork and fungal amylase against starch digestion was tested. 100, 200, 400 g *Pine* aqueous extract was added to the one liter. It found that amylase activity was not inhibited. However, when starch was added to the *Pine* aqueous extraction medium, it was observed that starch was broken down effectively (Table 1). The control group gives a positive result with lugol, but gives a negative result to benedict. In the groups given amylase, the opposite of control was observed (Table 2).

When liver tissue findings were evaluated (Table 3); total protein levels were significantly partially higher in diabetes and *Pine* extract 100 groups ($p<0.05$) compared to control group, although *Pine* extract 200 and *Pine* extract 400 groups were significantly higher ($p<0.01$, $p<0.001$). When the liver tissue findings were evaluated, it was found that changes in GSH levels in *Pine* all extract (100, 200 and 400 g/L) groups were not statistically significant compared with control group ($p>0.05$), but GSH levels were significantly decreased in the diabetes group ($p<0.01$). Findings were evaluated, it was found that changes in GSSG levels in all *Pine* extract concentrations were not statistically significant ($p>0.05$), but the change in diabetes group was significantly increased ($p<0.001$). Changes in MDA levels in *Pine* extract 100 and *Pine* extract 400 groups were found to be statistically significant ($p<0.05$) when compared with control group. But, it was found that the level of the *Pine* extract 200 group was not statistically ($p>0.05$) significant compared with the control group, and the change in the diabetic group was significantly increased ($p<0.001$).

Liver glycogen synthesis and breakdown, blood glucose homeostasis and changes in glycogen metabolism are important mechanisms. The excess glucose from the liver is converted to glycogen, but the body's glycogen storage capacity is limited, so the remaining glucose is directed to fatty acid synthesis. Glycogen is a short-term energy storage molecule in animal organisms and its level in tissues is dependent on insulin activity. Streptozotocin which causes damage to β-cells producing insulin, causes a marked decrease in insulin levels in the metabolism. Glucose metabolism is dependent on insulin. When the level of insulin decreases in circulation, glycogen level also decreases in liver tissue. In our study, it was observed that the glycogen level decreased in diabetic group but the glycogen level did not decrease importantly in the *Pine* extract group (Table 3). We can say that this effect has emerged due to the beneficial effects of active compounds of *Pine* extract on insulin metabolism in the pancreas. When the studies are examined, it was determined that plant active compounds have such effects on insulin metabolism [20]. In addition, according to the results of fatty acid analysis, the amount of palmitic acid was increased in groups given *Pine* extract compared to the diabetes group (Tables 3 and 4). From here, can be reach the following conclusion. Depending on nutrition or other factors, increased glucose in the blood is metabolized by liver cells. These metabolic processes are glycolysis, glycogen synthesis, pentose phosphate pathway and uronic acid pathways respectively. Glucose taken into the cell is primarily used in the production of ATP energy. Then, metabolic activity of glucose is carried out according to the sig-

Table 3. The effect of aqueous extract of wood part of *Pinus sp.* on biochemical parameters in rat liver tissue (mg/g)

Groups / Parameters	Control	Diabetes	D+ Pine Extract-100 g/L	D+ Pine Extract-200 g/L	D+ Pine Extract-400 g/L
Total protein	191.23±14.45	208.14±7.85^{b}	204.85±7.07^{b}	220.89±13.70c	270.05±9.39d
GSH (µmol/g)	3.33±0.11	2.54±0.11^{c}	3.35±0.08^{a}	3.28±0.1^{a}	3.24±0.1a
GSSG (µmol/g)	0.45±0.02	0.73±0.02^{d}	0.51±0.02^{a}	0.54±0.02^{a}	0.53±0.01a
MDA (µmol/g)	227.40±4.09	313.45±4.42^{d}	209±7.37^{b}	219.18±8.05^{a}	239.67±8.64b
Retinol	274.61±4.57	228.96±20.96^{c}	323.99±23.32^{c}	330.22±14.93^{c}	252.53±24.25b
Glycogen	89.40±3.34	66.63±1.90^{c}	85.33±2.65^{a}	92.09±3.38^{a}	83.11±2.16^{a}
α-Tocopherol	16.45±3.03	8.01±1.25^{d}	11.80±1.89^{b}	14.76±1.16^{a}	8.24±0.98^{d}
Cholesterol	1.27±0.07	1.01±0.12^{b}	1.51±0.15^{c}	1.83±0.15^{d}	1.06±0.09^{d}
Palmitic acid (16:0)	1.15 ± 0.40	1.01± 0.28^{b}	1.76±0.28^{c}	1.96±0.28^{d}	1.54±0.18^{b}
Stearic acid (18:0)	1.06±0.38	0.96±0.18^{b}	1.56± 0.30^{c}	1.87±0.24^{c}	1.05±0.18^{a}
Oleic acid (18:1)	0.35±0.09	0.33±0.08^{a}	0.56±0.14^{b}	0.46±0.07^{b}	0.44±0.04^{b}
Linoleic acid (18.2)	0.11±0.06	0.96±0.18^{b}	1.64±0.26^{d}	1.63±0.21^{d}	1.14±0.17^{d}
Arachidonic acid (20:4)	1.62±0.64	1.38±0.25^{b}	2.49±0.39^{d}	2.85±0.40^{d}	2.08±0.27^{d}
Docosahexaenoic acid (22:6)	0.45±0.19	0.309±0.07^{b}	0.646±0.10^{b}	0.79±0.11^{c}	0.60±0.08^{c}

(One-way analysis of variance (ANOVA) LSD post hoc test, P<0.05), a: The difference between the groups is not statistically significant (p>0,05), b: The difference between the groups is statistically significant (p<0,05), c: The difference between the groups is statistically more significant (p<0,01), d: The difference between the groups is statistically most significant (p<0,001).

Table 4. The effect of aqueous extract of wood part of Pinus sp. on biochemical parameters in rat kidney tissue (mg/g).

Groups / Parameters	Control	Diabetes	D+ Pine Extract-100 g/L	D+ Pine Extract-200 g/L	D+ Pine Extract-400 g/L
Total protein	121.86±11.17	57.41±1.62^{d}	66.8±7.74^{d}	59.91±2.92^{d}	72.14±4.97^{d}
GSH (µmol/g)	3.56±0.37	2.70±0.25^{c}	3.60±.28^{a}	4.56±0.58^{b}	2.92±0.40^{c}
MDA (µmol/g)	29.37±9.04	34.40±.14^{d}	25.66±3.41^{a}	27.56±2.50^{a}	45.46±8.55^{b}
Retinol	0.96±0.53	12.5±10.42^{d}	0.62±0.24^{b}	0.97±0.65^{a}	1.34±0.84^{b}
α-Tocopherol	11.17±0.70	27.06±63.62^{c}	36.86±9.51^{d}	46.48±11.28^{d}	76.13±12.86^{d}
Cholesterol	0.91±0.08	0.86±0.05^{a}	0.81±0.05^{a}	0.81± 0.04^{a}	1.05±0.04^{b}
Palmitic acid (16:0)	0.14±0.02	0.30±0.07^{d}	0.26±0.04^{b}	0.21±0.06^{b}	0.23±0.01^{b}
Stearic acid 18:0	0.07±0.005	0.18±0.01^{b}	0.20±0.015^{b}	0.16±0.02^{b}	0.15±0.004^{b}
Oleic acid (18:1)	0.17±0.017	0.06±0.012^{a}	0.11±0.013^{b}	0.08±0.012^{a}	0.08±0.002^{a}
Linoleic acid (18.2)	0.24±0.05	0.37±0.03^{b}	0.30± 0.03^{b}	0.27±0.04^{a}	0.34±0.09^{b}
Arachidonic acid (20:4)	0.13±0.03	0.73±0.12^{d}	0.81±0.11^{d}	0.68±0.06^{c}	0.62±0.02^{c}
Docosahexaenoic acid (22:6)	0.07±0.02	0.14±0.04^{d}	0.10±0.01^{c}	0.11±0.03^{c}	0.09±0.02^{b}

(One-way analysis of variance (ANOVA) LSD post hoc test, P<0.05), a: The difference between the groups is not statistically significant (p>0,05), b: The difference between the groups is statistically significant (p<0,05), c: The difference between the groups is statistically more significant (p<0,01), d: The difference between the groups is statistically most significant (p<0,001).

nal transmitted process [21]. Our findings support both events. According to the diabetes group, the high levels of glycogen and palmitic acid in the control and *Pine* extract groups are consistent with the above mentioned information (Tables 3 and 4).

STZ is widely used in the creation of experimental diabetes. STZ selectively destroys insulin-producing β cells in the pancreas, resulting in hyperglycemia due to lack of insulin. As a result of depletion of antioxidant system in diabetes, lipid peroxidation level is increased in tissues due to oxidative stress. In our study, we found a significant increase in the level of lipid peroxidation in the liver and kidney tissues of diabetic rats. This is consistent with previous study findings [22]. The effect of *Pine* extract on both hyperglycemia and oxidative stress has not been evaluated so far. Therefore, this study is the first on the subject. However, extracts obtained from different parts of the *Pine* showed beneficial effects on both hyperglycemia and oxidative stress parameters. In our study, we found that *Pine* extract reduced the level of MDA, which is an important indicator of lipid peroxidation, in both liver and kidney tissue. Glutathione (GSH), the most important antioxidant molecule in the cells, has a very physiological function other than its antioxidant properties. In order to maintain detoxification of free radicals within the cell, oxidized glutathione must be recycled to the reduction form. With the reaction of NADPH, oxidized glutathione (GSSG) is converted to reduced glutathione (GSH) again [23]. 5-carbon ribose and NADPH production occur on pentose phosphate pathway. NADPH is a molecule used in all reductive reactions [24].

It was found that GSH level was decreased and GSSG level was increased in diabetic group. We found that

the *Pine* extract applied to diabetic rats (especially low dose) supports the antioxidant capacity of the cell by showing beneficial effects on glutathione metabolism (Table 3 and 4). Since our study was the first, we did not find any literature on the subject. However, it has been determined that active compounds derived from different pine species have beneficial effects on glutathione metabolism [25, 26]. If glucose enters the cell regularly, pentose phosphate and other metabolic pathways work better. In our findings, it was found that the amount of GSH was higher in control group and *Pine* extract groups compared to diabetes group. In addition, although the amount of GSSG was high in the diabetes group, control and *Pine* extract decreased in the groups (Table 3, 4). The active compounds found in *Pine* extract may have activated the pentose phosphate pathway in liver tissue in a regular and effective way.

The levels of retinol in liver tissue was found to be significantly increased ($p<0.01$) in *Pine* extract 100 and 200 compared to the control group. It was found that retinol levels were significantly decreased ($p<0.01$) in the diabetic group compared to the control group. In the liver tissue changes in glycogen levels were not statistically significant ($p>0.05$) for the *Pine* all extracts when compared with the control group. In the liver tissue, glycogen levels were significantly decreased ($p<0.01$) in the diabetic group compared with the control group. In the liver tissue, the change in the α-tocopherol level in the Pine extract 200 group was not statistically significant ($p>0.05$) compared with the control group. In the liver tissue, the change in the α-tocopherol level in the Pine extract 100 group was statistically significant ($p<0.05$) compared with the control group. In liver tissue, the level of tocopherol was significantly ($p<0.001$) decreased in diabetes and Pine extract 400 groups compared to the control group. In the liver tissue, it was determined that cholesterol levels decreased significantly ($p<0.001$) in the Pine extract 400 group compared to the control group. In the liver tissue, cholesterol level was increased significantly ($p<0.01$, $p<0.001$) in the Pine extract 100 and Pine extract 200 groups compared to the control group. In the liver tissue, cholesterol level was decreased partially ($p<0.05$) in the diabetes group compared to the control group (Table 3).

When the 16:0 (mg/g) level was compared to the control group, it was determined that the level difference was statistically significant in diabetes and Pine extract 400 groups ($p<0.05$), significantly higher in Pine extract 100 and Pine extract 200 groups ($p<0.01$, $p<0.001$). When the 18:0 (mg/g) level was compared to the control group, It was determined that the level difference was statistically insignificant in Pine extract 400 groups ($p>0.05$), partially reduced in the diabetes group ($p<0.05$), significantly higher in Pine extract 100 and Pine extract 200 groups ($p<0.01$). When the 18:1 (mg/g) level was compared to the control group, it was determined that the level difference was statistically insignificant in diabetes groups ($p>0.05$), partially high in Pine extract 100, Pine extract 200 and Pine extract 400 groups ($p<0.05$). When the 18:2 (mg/g) level was compared to the control group, It was determined that the level difference was partially reduced in the diabetes group ($p<0.05$), significantly increased in Pine extract 100, Pine extract 200 and Pine extract 400 groups ($p<0.001$). When the 20:4 (mg/g) level was compared to the control group, it was determined that the level difference was partially reduced in the diabetes group ($p<0.05$), significantly increased in Pine extract 100, Pine extract 200 and Pine extract 400 groups ($p<0.001$). When the 22:6 (mg/g) level was compared to the control group, it was determined that the level difference statistically significant in diabetes and pine extract 100 groups ($p<0.05$), significantly increased in Pine extract 200 and Pine extract 400 groups ($p<0.01$) (Table 3).

Diabetes mellitus is a group of metabolic diseases characterized by hyperlipidemia, hyperglycemia caused by defects in insulin secretion, insulin effect, or both. Insulin regulates blood sugar homeostasis in the normoglycemic state, plus additionally Δ6 and Δ9 desaturase activities but cannot perform the regulatory task in hyperglycemic conditions. The liver is one of the organs that keep glucose levels within normal limits [27]. Changes in the composition of fatty acids in the tissue of diabetes, Delta 5 desaturase, Delta 6 desaturase and delta 9 desaturase activities were noted to be responsible for the defect [28]. In the living system, fatty acid biosynthesis is carried out by a complex of seven enzymes called the “fatty acid synthetase” system. Fatty acid synthesis begins with Malonyl CoA. In fatty acid synthesis, chain elongation occurs with the addition of two carbons to the malonyl CoA molecule. In order to participate in the synthesis of Acetyl CoA fatty acid, a carbon dioxide molecule must first join the molecule

via Acetyl CoA Carboxylase and form malonyl CoA. Palmitic acid is the precursor molecule of long chain fatty acids, which is the normal product of the "fatty acid synthetase" system. In addition to these, 18C stearic acid occurs as the result of adding two more carbon atoms or longer chain fatty acids occurs as the result of adding more carbon. Palmitic and stearic acid can also turn into monoenoic acid. In the liver and adipose tissues of vertebrates and other Aerobic organisms, there are enzyme systems that synthesize a pair of bonds. As a result of the activity of this system is composed of palmitoleic and oleic acids. This synthesis stage is influenced by insulin hormone [29].

In this study, it was found that palmitic and stearic acid levels, which are saturated fatty acids in liver tissue of diabetes group, decreased compared to control group, increased both fatty acid values in Pine extract groups (Tables 3). In kidney tissue, the levels of palmitic and stearic acid increased in the diabetes group compared to the control group, at the same time the levels of both fatty acids increased in the Pine extract groups (Table 4). When similar studies are evaluated, it is observed that there are different results in both tissues [30-32]. These results may be due to defects in insulin metabolism. However, the effects of treatment antidiabetic agents are tested in such studies; these agents have shown beneficial effects on tissue fatty acid metabolism due to the positive effects on insulin metabolism [30, 33].

Diabetes inhibits delta-6-desaturase, which converts linoleic acid into gamma linolenic acid, the precursor of arachidonic acid. Gamma linolenic acid level is decreased in diabetes. Eventually, the levels of dihomogamma linolenic acid and arachidonic acid also are decrease. The synthesis of unsaturated fatty acids takes place in the liver's microcosomes. It has been shown that Δ6 desaturase in the liver, the key enzyme of fatty acid desaturation, is suppressed in diabetes mellitus and is rapidly restored by insulin therapy [27, 28].

In this study, it was found that the levels of arachidonic acid in the liver tissue decreased in the diabetes group compared to the control group, but the levels of arachidonic acid increased as a result of Pine extract. However, level of arachidonic acid was lower than those of diabetes and Pine extracts in the kidney tissue (Table 3 and 4). When the literature is assessed, there are different results for both tissues. These results can be expressed as a result of deterioration in insulin metabolism. [27, 30, 32].

It was found that total protein levels were significantly higher ($p<0.001$) in the diabetes and Pine extract group compared to the control group. In kidney tissue, it was found that changes in the GSH levels in the Pine extract 100 compared to the control were not statistically significant ($p>0.05$) and that the change in the Pine extract 200 were statistically significant ($p<0.05$) and that the GSH levels in the Diabetes+ Pine extract 400 decreased significantly ($p<0.01$). It was found that changes in MDA level in kidney tissue, of control, was not statistically significant ($p>0.05$) compared to Pine extract 100 and Pine extract 200 and this value increased significantly with in Pine extract 400 ($p<0.05$) and that MDA levels in diabetes group were significantly higher ($p<0.001$). In the kidney tissue, it was determined that retinol levels increased significantly ($p<0.001$) in the diabetes group compared to the control group. In the kidney tissue, retinol levels were decreased significantly ($p<0.001$) in the Pine extract 100, Pine extract 200 and Pine extract 400 groups compared to the diabetes group. In the kidney tissue, retinol level was decreased significantly ($p<0.05$) in the Pine extract 100 group compared to the control group. In the kidney tissue, it was determined that retinol level change insignificantly ($p>0.05$) in the Pine extract 200 group compared to the control group. In the kidney tissue, retinol level was increased significantly ($p<0.05$) in the Pine extract 400 group compared to the control group. In kidney tissue, a significant increase of α-tocopherol levels was observed in Pine extract 100, Pine extract 200, Pine extract 400 ($p<0.001$) and diabetes groups ($p<0.01$) compared to control group (Table 4).

It was found that the changes in the levels of cholesterol in the kidney tissue were not statistically significant for Pine extract 100, Pine extract 200 and diabetes groups ($p>0.05$), but the change in the Pine extract 400 was found to be statistically significant ($p<0.05$) when compared to the control group. In the kidney tissue, the changes in the palmitic acid (16:0) levels in the Pine extract 100, 200 and 400 ($p<0.05$), and diabetes groups ($p<0.001$) were statistically significant when compared with the control group. In the kidney tissue, the changes in the stearic acid (18:0) level in the Pine extract 100, 200, 400 and diabetes groups ($p<0.05$) were statistically significant when compared with the control group. In the kidney tissue, the changes in oleic acid (18:1) levels in Pine extract 200, Pine extract 400 and diabetes groups were not statistically significant ($p>0.05$), but the change in the Pine 100 was found to be statistically significant ($p<0.05$) when compared to the control group.

The changes in linoleic acid (18:2) levels in the Pine extract 200 were not statistically significant ($p>0.05$) in the kidney tissue but the changes in the Pine extract 100, 400 and diabetes groups were found to be statistically significant ($p<0.05$) when compared to the control group. In the kidney tissue, the levels of arachidonic acid (20:4) in Pine extract 100, 200, 400 and diabetes groups were found to increase statistically ($p<0.01$) compared with control group. In the kidney tissue, the levels of docosahexaenoic acid (22:6) in Pine extract 100, 200, 400 and diabetes groups were found to increase statistically ($p<0.01$) compared with control group ($p<0.05$) (Table 4).

Vitamin A is necessary for vision, growth, reproduction, embryo development, blood production, immune system and tissue cell differentiation. In liver tissue, retinol levels decreased in diabetic group compared to control, and retinol levels increased in Pine extract groups. In the kidney tissue, retinol levels were elevated in the diabetes group compared to the control group, but different results were obtained in the groups of Pine extract (Tables 3 and 4). It is reported that levels of retinol in diabetic rats have increased in the liver due to decreased levels of retinol binding protein in the liver and kidney tissue [34]. Retinol levels are affected by diabetes conditions [35]. The protein activity of the retinol binding protein depends on insulin metabolism. According to the findings, we can say that changes in retinol level are caused by disorders in retinol-binding protein and insulin metabolism.

High-fat resolution antioxidant vitamin E acts as a free radical scavenger that protects polyunsaturated fatty acids against lipid peroxidation. In our study, it was found that the levels of tocopherol decreased in the diabetes group compared to the control group in liver tissue but increased in the groups with Pine extract. In kidney tissue, the level of tocopherol is increased in all groups compared to the control group (Table 3 and 4). Takitani et al. [36], and Miyazaki et al. [37], reported that Alpha-tocopherol levels were elevated in liver tissue. Cholesterol is a biomolecule that is the precursor of steroid hormones and bile acids but also involved in the structure of plasma membranes and myelin. It has been found that total cholesterol levels decrease in the liver and kidney tissue in diabetes [38]. In this study, we found that total cholesterol levels decreased in the diabetes group compared to the control group in both tissues. It can be said that aqueous extract of wood part of Pine affects the total amount of cholesterol in the liver and kidney tissues depending on the dose (Table 3 and 4). This result may be the result of the effect of diabetes on cholesterol metabolism. An important way to maintain cellular cholesterol homeostasis is reverse cholesterol transport through cholesterol carriers. ATP-binding cassette protein A1 (ABCA1) is an important rate-controlling protein in reverse cholesterol transport. Several reports have shown that expression of the ABCA1 protein varies under diabetic conditions. Decreased levels of free cholesterol in peripheral tissues may be due in part to increased expression of the ABCA1 protein [38]. As a result of the experimental data obtained, pine extract may be effective in preventing some complications arising from hyperglycemia. In particular, for patients with metabolic origin and ongoing insulin production in the pancreas may be use of this type of herbal medicine. Scientific examination of herbal active extracts and determination of their useful aspects will contribute positively to the national economies of the drug industry.

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