Antioxidant and Anti-hyperlipidemic Effects of Bark Extract of Pinus eldarica in Dexamethasone-induced Dyslipidemic Rats

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ABSTRACT

Background & Objective: Although Pinus eldarica is considered as a pine with many valuable phytochemical constituents, little is known about the pharmacological effects of its bark extract. Therefore, the present study aimed to evaluate in vivo antioxidant activity and also the possible beneficial effects of the bark extract of P. eldarica on dexamethasone-induced dyslipidemia in rats.

Materials & Methods: Total phenolic content was determined using Folin-Ciocalteu method. The in vivo antioxidant assays included the measurement of hydroperoxides level and ferric reducing antioxidant power (FRAP) value in plasma samples of rats receiving intraperitoneal injections (IP) of plant extract (100, 200 and 400 mg/kg) for 28 days. For induction of dyslipidemia, dexamethasone (10 mg/kg) was subcutaneously administered during 8 days. Different doses of extract were given orally plus dexamethasone in three groups of animals. Serum lipids, blood glucose and malondialdehyde (MDA) levels and liver histopathology were assessed.

Results: High total phenolic content was determined as 375±1.2 mg gallic acid equivalent/ g of dried bark extract. The extract significantly decreased plasma hydroperoxides level at all doses and increased FRAP value at the dose of 400 mg/kg during in vivo antioxidant analysis. P. eldarica led to a significant reduction in serum levels of blood glucose, total cholesterol, triglyceride and MDA and improved liver histopathological changes at the doses of 200 and 400 mg/kg in dyslipidemic rats.

Conclusion: These findings suggest the potential antioxidant, antihyperlipidemic and antihyperglycemic activities for the bark extract of P. eldarica which may be due to the high amounts of phenolic compounds.

Keywords: Antioxidants, Dexamethasone, Hyperlipidemias, Lipid Peroxidation, Pinus eldarica

Introduction

Hyperlipidemia is considered as a risk factor in developing cardiovascular diseases (CVDs) such as atherosclerosis (1). Atherosclerosis or hardening of the arteries plays a prominent role in several deadly cardiovascular or cerebrovascular disorders (2).

Oxidative stress as a result of the overproduction of free radicals and low power of antioxidant defense system is also involved in the pathogenesis of CVD (3). In vasculature, reactive oxygen species (ROS) lead to nitric oxide (NO) inactivation, low-density lipoprotein (LDL) oxidation, and consequently endothelial dysfunction or apoptosis (4,5). Recent investigations have focused on herbal medicines and natural products for developing novel cholesterol lowering agents with antioxidant activities due to their desirable efficacy and relatively fewer complications (6).

Pine family (Pinaceae) includes a large number of widespread conifers with almost 250 species in 11 genera. Pinus as the most important genus in the Pinaceae family has about 115-120 species of coniferous trees and grows widely in different countries including Iran (7).
Material and Methods

Plant Material and Preparation of Extract

The barks of *P. eldarica* were obtained from pine trees in Isfahan (Isfahan Province of Iran) on July 2018. The specimen voucher (No. 3318) was authenticated by a botanist and located at the Herbarium of our School.

The hydroalcoholic extract was prepared by maceration method using ethanol (70%) at room temperature for 72 hr in three times. After filtration and removing the solvent with a vacuum rotary evaporator, the yield extract was freeze-dried and the extract powder was kept at -20°C.

Determination of Total Phenolic Content

Folin-Ciocalteu colorimetric assay was used for the measurement of total phenolic content in the bark extract of *P. eldarica* using a mixture solution of phosphomolybdate and phosphotungstate. In brief, 20 µL of the extract (2.5 mg/mL) was diluted with distilled water to 1.58 mL and mixed with Folin-Ciocalteu reagent (100 µL) and sodium carbonate (300 µL, 20%). After 2 hr incubation, the absorbance was determined using spectrophotometer at 765 nm. The results were calculated using a calibration curve of gallic acid and stated as milligram of gallic acid equivalent (GAE) per gram of the extract (16).

Animals

Male Wistar albino rats weighing 250 ± 20 gr were maintained under the standard environmental conditions including room temperature range of 20-25°C and regular light/dark cycle of 12 hr/12 hr. They were allowed for free access to water and feed. The rats were acclimatized with the experimental environment for 1 week before the test. The experimental protocol was approved by the Biomedical Researches Ethics Committee of Isfahan University of Medical Sciences (ethical approval ID: IR.MUI.REC.1396.2.091).

In vivo Antioxidant Assay

For evaluation of the antioxidant effect of *P. eldarica* bark extract in rats, doses of 100, 200 and 400 mg/kg of the extract were administered daily intraperitoneally (i.p.) for 28 days (11). The control rats received i.p. injection of the vehicle daily. Each group consisted of 6 rats. At the end of the experiment, the blood was taken from orbital sinus plexus under anesthesia and the plasma was isolated for further testing.

Measurement of Hydroperoxides Concentration

The effect of *P. eldarica* bark extract on the plasma hydroperoxides of rats as the indicator for reactive molecules was measured using xylenol orange reagent-1 (FOX-1) method. In brief, the FOX1 reagent containing ammonium ferrous sulfate and xylenol orange in aqueous medium with sorbitol was added to 10 µL of the plasma samples. After incubation of mixture for 30 min at 37°C, the absorbance of solution was determined at 540-560 nm. The level of hydroperoxides in plasma samples was calculated in term of µmole/L of hydrogen peroxide (H₂O₂) equivalents using a standard curve of H₂O₂ (17).

Ferric Reducing Antioxidant Power Assay

Ferric reducing antioxidant power (FRAP) assay was used for evaluation of the total antioxidant capacity in rats’ plasma samples after treatment with different doses of *P. eldarica* bark extract. This colorimetric assay evaluates the reduction of ferric-tripryidyl triazine (TPTZ) complex in FRAP reagent to ferrous form. Briefly, plasma samples (10 µL) were mixed with FRAP reagent and incubated for 40 min at 37°C. Then, the absorbance was measured at 570 nm. The FRAP value in plasma samples was calculated in term of mmol/L of ferrous sulphate equivalents using a standard curve of FeSO₄ (18).

Evaluation of Antidyslipidemic Effects

Dexamethasone-induced dyslipidemia was used for evaluation of the possible antidyslipidemic effects of *P. eldarica* bark extract. Dexamethason (10 mg/kg) was injected subcutaneously (s.c.) for 7 days in dyslipidemic control rats (19). Different doses of the extract (100, 200 and 400 mg/kg) were given orally plus dexamethasone in the treatment groups. Control group received s.c. injection of normal saline and orally administration of the vehicle extract. The reference group received dexamethasone and standard treatment of atorvastatin (40 mg/kg, orally) for 7 days. At the end of the experimental period, the blood samples of overnight fasted rats were taken and the serum was isolated for biochemical analysis. Then, animals were sacrificed and the livers were separated and kept immersed in 10% formalin and processed for histopathological examinations.
Biochemical Analysis for Dyslipidemia

Serum lipid profile including triglycerides (TG), total cholesterol (TC) and high-density lipoprotein (HDL) was enzymatically estimated using particular biochemical kits (Pars Azmoon Co., Iran). The following formula was used to calculate LDL: LDL = Cholesterol - (Triglyceride/5) – HDL. The blood glucose level was measured using glucose oxidase method.

Lipid Peroxidation Assay

The serum lipid peroxidation was assessed through measuring malondialdehyde (MDA) concentration. First, the serum samples were mixed with trichloroacetic acid (20%). Then, H₂SO₄ was added for the dispersion of precipitate. After adding a solution of thiobarbituric acid (0.67%) in Na₂SO₄ to the samples and heating the solution in a boiling water bath for 1 hr, the mixture was rapidly cooled and mixed with n-butanol. The absorbance of the colored solution was detected at 532 nm using a spectrophotometer. Finally, the results were estimated in term of nmol/mL of MDA equivalents using a calibration curve of MDA tetrabutyl ammonium (20).

Histopathological Analysis

The formalin fixed liver samples were dehydrated and cleared in xylene. Then paraffin blocks were prepared and cut at 5 μm thickness, stained with hematoxylin & eosin and evaluated microscopically for histopathological changes.

Statistical Analysis

The results were stated as the mean ± standard error of mean (SEM). The experimental groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey post-hoc test using the SPSS 18 (SPSS Inc., Chicago, IL., USA). The P-value < 0.05 was considered as the level of significance.

Results

Total Phenolic Content

Total phenolic content of P. eldarica bark extract was determined as 375 ± 1.2 mg GAE/ gr of the dried plant extract.

Effect of P. eldarica Bark Extract on Hydroperoxides Concentration in Normal Rats

FOX1 method was used for evaluation of the effect of hydroalcoholic extract of P. eldarica bark on plasma hydroperoxides level in rats. The administration of P. eldarica bark extract for 28 days reduced the plasma hydroperoxides levels significantly at all doses of 100 (P<0.01), 200 (P<0.01) and 400 mg/kg (P<0.001) compared to the control group (Figure 1).

Effect of P. eldarica Bark Extract on FRAP Value in Normal Rats

The FRAP value was used for the expression of plasma total antioxidant capacity in rats. P. eldarica bark extract increased the FRAP value significantly at the dose of 400 mg/kg compared to the control group (P<0.05) after 28 days treatment (Figure 2).

Effect of P. eldarica Bark Extract on Biochemical Parameters in Dyslipidemic Rats

Table 1 illustrates the effect of treatment with P. eldarica bark extract on biochemical factors during dyslipidemia induced by dexamethasone. Significant elevations were observed in serum blood glucose (P<0.01), TG (P<0.05), TC (P<0.05), and LDL level (P<0.05) along with a major decrease in HDL level (P<0.05) after administration of dexamethasone. Atorvastatin as a reference antihyperlipidemic drug reduced all the serum parameters of dyslipidemia and also increased HDL level significantly. Treatment of animals with P. eldarica bark extract at the doses of 200 and 400 mg/kg significantly reduced serum blood glucose (P<0.05), TG (P<0.01) and TC (P<0.001 and P<0.01, with respect to the doses of extract). However, no significant changes were observed in LDL and HDL levels after the treatment with P. eldarica bark extract.

Effect of P. eldarica Bark Extract on MDA Level in Dyslipidemic Rats

As indicated in Figure 3, there is a significant elevation in the serum MDA level as an indicator of lipid peroxidation in dyslipidemic rats compared to that in normal animals (P<0.05). Administration of atorvastatin and doses of 100 and 200 mg/kg from P. eldarica bark extract decreased the MDA level significantly (P<0.01).

Effect of P. eldarica Bark Extract on Liver Histopathology

Morphological examination of liver sections after administration of high dose of dexamethasone revealed histological changes including cellular swelling, fatty degeneration and diffused steatosis (Figure 4B). Normal architecture was observed in the liver of the control animals (Figure 4A). Treatment with atorvastatin (Figure 4F) and P. eldarica bark extract at the dose of 100 mg/kg (Figure 4C), 200 mg/kg (Figure 4D) and 400 mg/kg (Figure 4E) showed partially reduction in the histopathological changes of liver induced by dexamethasone.
## Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>BG (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>TC (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
<th>LDL-C (mg/dL)</th>
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<tr>
<td>Normal control</td>
<td>153.6 ± 5.1</td>
<td>129.1 ± 9.6</td>
<td>104.8 ± 5.5</td>
<td>44.2 ± 8.0</td>
<td>38.9 ± 2.2</td>
</tr>
<tr>
<td>Dexamethasone dyslipidemic control</td>
<td>184.3 ± 8.2†</td>
<td>155.2±13.4€</td>
<td>139.3±10.1€</td>
<td>30.3 ± 4.9€</td>
<td>59.3 ± 8.2‡</td>
</tr>
<tr>
<td>Dexamethasone + atorvastatin (40mg/Kg)</td>
<td>170.5 ± 5.1†</td>
<td>115.2±14.5**</td>
<td>100.8±10.5**</td>
<td>47.9 ± 12.6**</td>
<td>42.4 ± 5.3**</td>
</tr>
<tr>
<td>Dexamethasone + <em>P. eldarica</em> (100mg/Kg)</td>
<td>180.1 ± 10.5†</td>
<td>145.4±19.1</td>
<td>115.1±16.1</td>
<td>30.1 ± 4.5€</td>
<td>56.1 ± 3.1‡</td>
</tr>
<tr>
<td>Dexamethasone + <em>P. eldarica</em> (200mg/Kg)</td>
<td>158.3 ± 13.3†</td>
<td>124.6±6.7**</td>
<td>93.3±11.3***</td>
<td>31.7 ± 9.3€</td>
<td>53.7 ± 3.2‡</td>
</tr>
<tr>
<td>Dexamethasone + <em>P. eldarica</em> (400mg/Kg)</td>
<td>159.2 ± 18.1†</td>
<td>126.3±3.2**</td>
<td>100.2±18.7**</td>
<td>40.1 ± 5.4</td>
<td>54.3 ± 4.5‡</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD (n=6). Tukey post hoc analysis, †P<0.05 versus normal control, ‡P<0.01, ††P<0.001 versus normal control; *P<0.05 and **P<0.01 versus dexamethasone-induced dyslipidemic control. BG, blood glucose; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

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**Figure 1.** Plasma hydroperoxides concentrations (means ± SEM, N=6) after 28 days in rats treated with *P. eldarica* bark extract (100–400 mg/kg). †††P<0.001 and **P<0.01 versus control group treated with normal saline.

**Figure 2.** Plasma FRAP value (means ± SEM, N=6) after 28 days in rats treated with *P. eldarica* bark extract (100–400 mg/kg). *P<0.05 versus control group treated with normal saline.
Figure 3. Effect of administration of *P. eldarica* bark extract (100-400 mg/kg) on serum MDA concentration (means ± SEM, N=6) in dexamethasone-induced dyslipidemic rats. *P*<0.05 versus normal group, **P*<0.01 versus dexamethasone-induced dyslipidemic group. MDA: Malondialdehyde.

Figure 4. Representative photomicrograph of liver sections. Normal control group presenting normal histological structure (A), Dexamethasone-induced dyslipidemic group displaying diffused vesicular steatosis (B), *P. eldarica* bark extract treated groups with doses of 100 mg/kg (C) and 200 mg/kg (D) showing moderate vesicular steatosis and 400 mg/kg showing mild vesicular steatosis (E), atorvastatin treated group showing low histopathological changes in hepatic architecture (F); H & E, ×40 magnification

**Discussion**

Findings of the present investigation demonstrated that the hydroalcoholic extract of *P. eldarica* bark has antioxidant, hypoglycemic and antihyperlipidemic effects in dexamethasone-induced dyslipidemia. It is well known that oxidative stress, hyperglycemia, dyslipidemia and metabolic syndrome are considered as common problems during long-term treatment with high doses of glucocorticoids which are mainly due to the high production of free radicals, insulin resistance and abnormalities in lipid metabolism (21,22).

In this study, high amounts of phenolic compounds were detected in *P. eldarica* bark extract suggesting its potential therapeutic efficacy in various oxidative-induced disorders especially cardiovascular diseases. Our results indicated in vivo antioxidant activities for *P. eldarica* bark extract through reducing the plasma...
hydroperoxides concentration and improving the total antioxidant capacity in normal rats and also declining the lipid peroxidation in dyslipidemic rats. Similarly, Bargi et al., discussed the protective effects of Pinus eldarica extract against the oxidative damage of hippocampal tissues in pentylenetetrazole-induced seizure model among rats (23).

These antioxidant properties may be related to the great quantities of phenolic compounds containing hydroxyl groups with high reactivity to metal ions and free radicals (15).

In the study of Sadeghi and co-workers which evaluated the bark, seed and needle of Pinus eldarica for the antioxidant components, the highest amount of total polyphenols was identified in the bark extract suggesting the valuable potential of this part of the plant for clinical use (24).

In the present study, the data related to assessing the antidyshlipidemic effect revealed the ability of Pinus eldarica bark extract in decreasing serum TG, TC and blood glucose level and also improving the liver histopathological changes in dexamethasone-induced dyslipidemic rats.

Some investigations have described antidiabetic, anti-atherosclerotic and anti-hyperlipidemic activities for different parts of Pinus species including leaves, nuts, branches and barks. In this regard, antidiabetic effect has been reported for the essential oil of Pinus koraiensis leaves and the bark extract of Pinus pinaster and Pinus roxburghii (25-27). The branches extract of Pinus roxburghii has also shown the strong α-glucosidase inhibitory activity in vitro (28).

The bark extract of Pinus pinaster has displayed antidyshlipidemic effect by reducing LDL and increasing HDL in animal and clinical studies (29,30). Further, Kim et al., reported that the essential oil obtained from the extract of Pinus koraiensis leaves inhibits acyl-CoA enzyme A: cholesterol acyltransferase and up-regulates LDL receptor representing hypolipidemic activity (31).

Concerning Pinus eldarica, Fallahhuseini et al., have defined the antihyperglycemic effect for the nut extract of this plant in alloxan-induced diabetic rats without any effect on triglyceride and cholesterol levels (11). However, in their recent study, Pinus eldarica nut extract resulted in a significant decrease in blood cholesterol level in hypercholesterolemic rabbits (12).

The presence of bioactive constituents in the bark of Pinus eldarica including polyphenolics such as catechin, taxifolin, caffeic acid and ferulic acid and some monoterpens and sesquiterpenes such as α-pinene and β-caryophyllene may be responsible for the hypoglycemic and antihyperlipidemic activities of this plant (15). The beneficial effects on serum glucose and lipid profile have been recognized for catechins by controlling hemoglobin A1c and lowering cholesterol and LDL levels (32). Taxifolin modulates the production and secretion of hepatic lipoproteins (33). Further, it protects diabetic animals by decreasing blood glucose and serum insulin level and improving the pathological alterations of kidneys via NF-κB pathway (34). Caffeic acid reduces the blood glucose level by elevating the glucose uptake and stimulating the insulin receptor signaling cascade, and inhibits hyperlipidemia by controlling the lipogenic enzymes in the liver (35,36). Ferulic acid recovers glucose and lipid dysregulation through regulating the expression of lipogenic and gluconeogenic genes in the liver (37).

The major limitation of the present study was the lack of evaluating the mechanisms of antihyperglycemic and antihyperlipidemic effects of the Pinus eldarica bark extract because of financial budget restriction.

Conclusion

The present study indicated the in vivo antioxidant, hypoglycemic and hypolipidemic properties of hydroalcoholic extract of Pinus eldarica bark in dexamethasone-induced dyslipidemia. The observed effects are probably due to bioactive constituents especially the high amounts of phenolic compounds in the bark extract of this plant.

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Conflict of Interest

Authors declared no conflict of interest.

References

4. Schulz E, Anter E, Keaney J, John F. Oxidative stress, antioxidants, and endothelial function. Curr...


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