RESEARCH ARTICLE

Studying the Effect of Hydroalcoholic Extract of *Teucrium polium* L. Leaves on Antioxidant Activity and Lipid Profile Alterations

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Abstract

Background: Antioxidants are the most important factors preventing oxidative stress and scavenging free radicals. Nowadays, natural antioxidant compounds are highly important in prevention of cardiovascular diseases. In this study, we have investigated the effect of hydroalcoholic extract of *Teucrium polium* L. leaves (HETPL) on enzymes related to antioxidant activity and lipid profile alterations in rats. **Materials and Method:** Male Wistar rats were divided into four equal groups for 8 weeks: Experimental groups of 1 and 2, which had a normal diet and high cholesterol diet (2%) respectively, and experimental groups of 3 and 4, which were treated with high cholesterol diet (2%) with HETPL at doses of 85 and 170 mg/kg, respectively. The levels of malondialdehyde (MDA) and plasma superoxide dismutase (SOD) of red blood cell (RBC) and lipid profile were measured at the beginning and at the end of the study. **Results:** The levels of SOD in RBC and plasma MDA in the experimental Group 2 had a significant increase compared to the experimental Group 1 ($P < 0.001$). The level of these two enzymes in the experimental groups of 3 and 4 was significantly lower than the experimental Group 2 (SOD by $P < 0.05$ and $P < 0.001$ and MDA by $P < 0.01$ and $P < 0.001$, respectively). The mean serum high-density lipoprotein cholesterol level in experimental Groups 1 and 4 at the end of study had no significant alteration compared to the beginning of the study, but in the experimental groups of 2 and 3, a significant decrease was observed at the end of the study than the beginning of the study ($P < 0.001$ and $P < 0.01$, respectively). Mean serum levels of triglyceride (TG), cholesterol, and low-density lipoprotein cholesterol (LDL-c) in experimental Groups 2 (LDL-c and cholesterol: $P < 0.001$ and TG: $P < 0.01$) and 3 ($P < 0.01$) significantly increased compared to the beginning of the study. **Conclusion:** We conclude that *T. polium* can play an important role in preventing cardiovascular diseases caused by oxidative stress through its antioxidant and hypolipidemic activities.

Key words: *Teucrium polium*, antioxidant, lipid profile, malondialdehyde, superoxide dismutase, rat

INTRODUCTION

The human body has several defense mechanisms against free radicals such as reactive oxygen species including enzymatic and non-enzymatic antioxidant system for the protection of cellular molecules. Due to the fact that the body's defense system may not be sufficient for persistent or intense oxidative stress, certain amounts of external antioxidants are permanently required to balance the levels of antioxidants and oxidants in the human body.[1,2]

Increase in the cholesterol levels leads to the production of free radicals of superoxide in the veins, and the synthesis and release of endothelial-derived vasodilators

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decrease.[3] Treatment of lipid profile disorders leads to reduction in cardiovascular disease significantly.[4]

Today, the use of herbal and dietary herbs containing natural antioxidants, which has a high potential for removal of free radicals and reducing oxidative damage, is increasing.[5,6] Considering that plants are considered as the most important natural sources of antioxidants, the number of studies in this area is increasing. The antioxidant effects of herbal compounds are partly attributed to the presence of phenolic and flavonoid compounds, which are found in all parts of the plant including leaves, fruit, seed, root, and skin.[7] Medicinal herbs have a special importance in providing health care for communities for the prevention and treatment of diseases. The largest part of the world's herbal medicine market relates to the production and supplement of secondary metabolites derived from these herbs. Therefore, secondary metabolites usually have a very high added value.[8]

Teucrium polium L. is a medicinal herb from the Lamiaceae family.[9] This herb is a medicinal plant with antioxidant potential.[10] *T. polium* L. leaves have nutritional, and especially, medicinal usages.[11] *T. polium* L. is used in traditional medicine to treat chronic diseases of the stomach and also as diuretic, anti-hypertensive, antibacterial, anti-inflammatory, anti-diarrhea, antidiabetes, and anticonvulsants.[12,13] Phytochemical study of *T. polium* has shown that this herb contains tannin, terpenoid, saponin, sterol, flavonoids, and leuco-anthocyanins.[14-18] Furthermore, the extract of this plant is effective in reducing blood glucose and lipid levels and also treating prostate cancer.[19]

According to the undeniable effect of natural antioxidant in preventing cardiovascular diseases, this study was conducted to evaluate the antioxidant activity of hydroalcoholic extract of *T. polium* leaves (HETPL) and its effect in correction of lipid profiles in rats.

MATERIALS AND METHODS

Extraction

T. polium L. plants were collected from the Roumeshgan area, West Lorestan province, and were identified and confirmed in terms of the scientific name. The leaves of this plant were dried in shade and then grinded. 100 g of obtained powder was mixed with a solution of ethanol and distilled water (4–1 ratio, respectively) and placed in a shaker incubator (34°C and 140 rpm) for 3 days. The solution was filtered using a Whatman filter paper, and the ethanol was separated using a rotary machine and a vacuum pump. The extract was placed in the laboratory oven for more concentration for 5 days. Finally, the solution was kept in sterilized Falcon tube at 4°C for later use.

Grouping and treatment

A total of 24 male Wistar rats weighing 100–160 g were purchased from the Tehran Pasteur Institute, kept under laboratory conditions (25°C, 12-h cycles, and adequate water and food), and then randomly divided into four groups with six rats each:

- Experimental Group 1 (positive control) receiving the usual diet.
- Experimental Group 2 (negative control) receiving high-cholesterol diets (2%).
- Experimental Groups 3: Cholesterol diet (2%) for 8 weeks and treated with 85 mg/kg dose of (HETPL) from the 5th week.
- Experimental Group 4 received high-cholesterol diet (2%) for 8 weeks and treated with 170 mg/kg dose of (HETPL) from the 5th week.

The treatment was carried out for 8 weeks through oral gavage.

Biochemical factors and their measurement

Blood samples were collected twice from all rats (at the beginning and the end of the study). After serum isolation, serum levels of malondialdehyde (MDA), superoxide dismutase (SOD), and lipid profiles (high-density lipoprotein cholesterol [HDL-c], low-density lipoprotein cholesterol [LDL-c], cholesterol, and triglyceride [TG]) were measured.

SOD and MDA levels were measured using ZellBio kits of Germany, and lipid profile was measured using the biochemical enzyme kit in Iran and ELISA method using the Hitachi Automatic Analyzer 902.

Statistical analysis

The results were analyzed using SPSS 16 software. *t*-test was used to compare the mean of variables at the beginning and the end of the study, and one-way ANOVA was used to compare the mean of variables of the groups at the end of the study. Mean level of variables for each group was presented as mean ± standard error of the mean. The statistical inference line was considered as $P < 0.05$ for all tests.

RESULTS

Antioxidant activity

The results show that the red blood cell (RBC) levels of SOD at the end of the study in the experimental Group 2 (negative control) receiving high cholesterol diet (2%) were significantly higher than the experimental Group 1 (positive control) with the normal diet ($P < 0.001$). The levels of this enzyme in the experimental Group 3 treated with high cholesterol

diet and treated with HETPL at the dose of 85 mg/kg were significantly higher than the experimental Group 1 ($P < 0.01$) and significantly lower than experimental Group 2 ($P < 0.05$). Finally, in the experimental Group 4, treated with a high cholesterol diet and 170 mg/kg HETPL, there was no significant alteration compared to the experimental Group 1 ($P > 0.05$), but there was a significant decrease compared to the experimental Groups 2 and 3 ($P < 0.001$ and $P < 0.01$, respectively) [Figure 1].

Data have been expressed in means ± standard deviation (SD) ($n = 6$). The P values for the following comparisons are as follows: End level serum of experimental Group 2 versus experimental Group 1 (a = 0.000); end level serum of experimental Group 3 versus experimental Group 1 (b = 0.009); end level serum of experimental Group 3 versus experimental Group 2 (c = 0.036); end level serum of experimental Group 4 versus experimental Group 1 (d = 0.791); end level serum of experimental Group 4 versus experimental Group 2 (e = 0.000); end level serum of experimental Group 4 versus experimental group 3 (f = 0.007) (The mean difference is statistically significant at the 0.05 level).

In addition, plasma MDA levels in the experimental Group 2 (high cholesterol diet) showed a significant increase compared to the experimental Group 1 (normal diet) ($P < 0.001$). In experimental Group 3 (high cholesterol diet plus 85 mg/kg dose of HETPL), there was a significant increase compared to the experimental Group 1 ($P < 0.01$) and a significant decrease ($P < 0.05$) than experimental Group 2 (high cholesterol diet) in plasma levels of the MDA enzyme. In the experimental Group 4, this enzyme showed no significant alteration compared to the experimental Group 1 (high cholesterol diet and 170 mg/kg dose of HETPL) ($P > 0.05$), but it showed a significant decrease compared to the experimental groups of 2 and 3 ($P < 0.001$ and $P < 0.05$, respectively) [Figure 2].

Data have been expressed in means ± SD ($n = 6$). The P values for the following comparisons are as follows: End level serum of experimental Group 2 versus experimental Group 1 (a=0.000); end level serum of experimental Group 3 versus experimental Group 1 (b = 0.011); end level serum of experimental Group 3 versus experimental Group 2 (c = 0.009); end level serum of experimental Group 4 versus experimental Group 1 (d = 0.086); end level serum of experimental Group 4 versus experimental Group 2 (e = 0.000); end level serum of experimental Group 4 versus experimental Group 3 (f = 0.041) (The mean difference is statistically significant at the 0.05 level).

Lipid profile

According to Figure 3, in the experimental groups of 1 and 4, the mean serum HDL-c level at the end of the study was not significantly different from the beginning of the study.

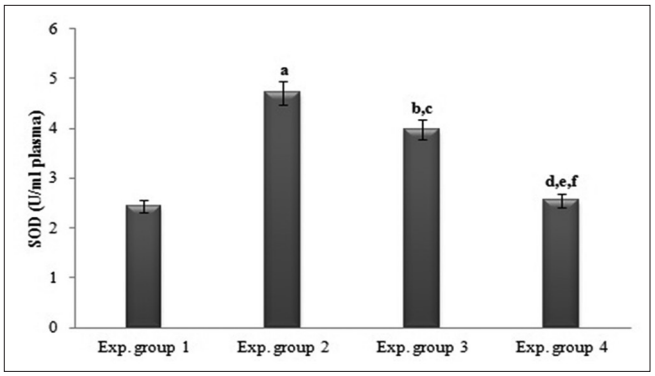

Figure 1: Effect of hydroalcoholic extract of *Teucrium polium* leaves on red blood cell superoxide dismutase level in different groups

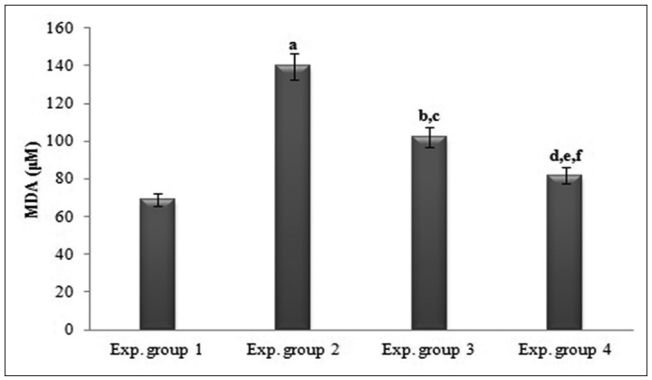

Figure 2: Effect of hydroalcoholic extract of *Teucrium polium* leaves on plasma malondialdehyde level in different groups

However, the levels of this index were significantly decrease in experimental Group 2 (negative control) treated with cholesterol diet and experimental Group 3 compared to the beginning of the study ($P < 0.001$ and $P < 0.01$, respectively). Mean serum levels of TG, cholesterol, and LDL-c in the experimental Group 2 treated with high cholesterol diet (2%) (LDL-c and cholesterol: $P < 0.001$, TG: $P < 0.01$) as well as the experimental Group 3 treated with a dose of 85 mg/kg in addition to high cholesterol diet ($P < 0.01$) significantly increased compared with the beginning of the study. However, the serum levels of these three factors in the experimental Group 1 (positive control) with the normal diet and the experimental Group 4 treated with cholesterol diet at a dose of 170 mg/kg had no significant alteration compared to the beginning of the study [Figure 3].

The P values for comparisons between mean rates of initial and end of study of different groups are as follows: a and d = $P > 0.05$; b (HDL-c, LDL-c and Cholesterol) = $P < 0.001$, b (TG) = $P < 0.01$, and c = $P < 0.01$.

DISCUSSION

In our study, high cholesterol diet increased serum levels of plasma MDA and RBC SOD. However, administration of

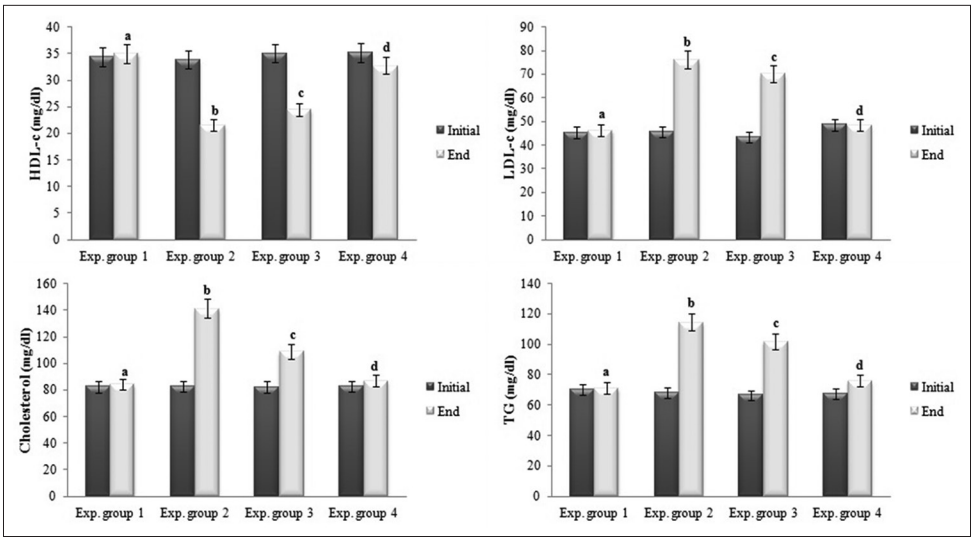

Figure 3: Comparison of the average lipid profile (high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, cholesterol, and triglyceride) in different groups at the beginning and the end of the study

hydroalcoholic extract of *T. polium* almost prevents these alterations and the level of these enzymes within the normal range.

Antioxidants scavenge free radicals by playing their physiological role.[20] This effect is related to the presence of flavonoids.[21] Phenolic compounds probably apply antioxidant endogenesis in the bodies of organisms through induction of defense system.[21-23] Reducing oxidative stresses results in preventing the progression of atherosclerosis.[24] During the process of lipid peroxidation, free oxygen radicals, ketones, ethers, aldehydes, and foam cells are produced, which interrupt the attachment of endothelial cells and the formation of plaque.[25] Sharififar *et al*. stated that alcoholic extract of *T. polium* inhibits lipid peroxidation.[26] Another study conducted by Suboh *et al*. indicated that *T. polium* has antioxidant effects.[27]

In the present study, alterations in lipid profiles caused by a high cholesterol diet were corrected by treatment with *T. polium*. These effects are consistent with previous studies. For example, Mousavi *et al*. showed that treatment with hydroalcoholic extract of *T. polium* caused a significant decrease in total serum lipids, triglycerides, and very-LDL-c, as well as liver triglyceride levels.[28] In another study by Haraguchi *et al*., it was shown that the aqueous extract of *T. polium* reduced the levels of total cholesterol in the animal model.[29] Various reports from several studies all emphasized on the fact that this herb has hypoclostrolic properties.[30,31] Mousavi *et al*. stated that triglyceride reduction in the liver after treatment with hydroalcoholic extract of *T. polium* may indicate the preventive effect of its flavonoids on the synthesis of triglycerides in the liver and their secretion into the blood flow.[28] In another study, Reinner *et al*. found that this herb has lipid reduction effects through lowering the level of cholesterol in the blood, which reduces the cholesterol effect by inhibiting its absorption in the small intestine and induction of its hepatic diffusion.[32] However, some studies have reported poor or none effect of flavonoids on plasma lipids and lipoprotein levels.[33,34]

On the other hand, there is a wide range of active agents including alkaloids, glycosides, terpenoids, sterols, triterpenes, and flavonoids in *T. polium* herbs.[35,36] Some types of its flavonoids may eliminate lipid synthesis and liver secretion.[37]

CONCLUSION

It can be concluded that the HETPL has a protective effect on prevention of cardiovascular disease by reducing lipid content and inhibiting lipid peroxidation. Considering the more positive effect of 170 mg/kg *T. polium* dose of extract, the effect of this extract seems to be dose dependent.

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