Antihyperlipidemic Effect of Different Fractions Obtained from Teucrium polium Hydroalcoholic Extract in Rats

Abstract

Background: This study was aimed to screen the antihyperlipidemic effect of different fractions of Teucrium polium to obtain the most efficient herbal fraction for isolation of bioactive constituents responsible for hypolipidemic activity. Methods: Chloroform, butanol, and aqueous fractions were obtained from hydroalcoholic extract of T. polium aerial parts using partitioning process. To induce hyperlipidemia, dexamethasone (Dex) was injected 10 mg/kg/day (s.c.) for 8 days. In the test groups, animals received 50, 100 and 150 mg/kg of T. polium hydroalcoholic extract and different fractions orally simultaneously with Dex. Serum lipid profile and hepatic marker enzymes were evaluated using biochemical kits. Results: All treatments, especially chloroform and aqueous fractions, reversed serum lipid markers in hyperlipidemic rats. Maximum reduction in triglyceride (60.2%, P < 0.001) and maximum elevation in high-density lipoprotein (HDL) (35.0%, P < 0.01) was observed for chloroform fraction. Maximum cholesterol-lowering effect (29.0%, P < 0.001) and maximum reduction in low-density lipoprotein were found for hydroalcoholic extract (72.9%, P < 0.001). Aqueous fraction improved all lipid markers at the highest dose. Butanol fraction decreased triglyceride at the lowest dose (43.9%, P < 0.001) and increased HDL (33%, P < 0.05) at the highest dose. There was a significant increase in alanine aminotransferase and aspartate aminotransferase levels in all tested groups compared to normal group (P < 0.001). Conclusions: This study showed strong antihyperlipidemic effect of various fractions derived from hydroalcoholic extract of T. polium. Chloroform and aqueous fractions may be worthy candidates for isolation of bioactive hypolipidemic constituents. However, possible hepatotoxicity should be considered for clinical application.

Keywords: Dexamethasone, hepatotoxicity, hyperlipidemia, Teucrium polium

Introduction

Hyperlipidemia is a well-known risk factor for atherosclerosis. Atherosclerosis is one of the main causes of death and disability worldwide. The modern anti-hyperlipidemic agents such as statins are widely used for reducing cardiovascular risk; however, they are not devoid of potentially serious side effects. For developing novel hypolipidemic agents, it has been focused on alternative natural products because of their great effectiveness, safety, and well-tolerability.

Teucrium polium L. (Labiatae) is a medicinal plant and a rich source of different bioactive compounds. Studies have established various pharmacological effects such as antioxidant, antidiabetic, anti spasmodic, anti hypertensive, cardiotoxic, anticoagulant, anti gastric ulcer, anti inflammatory, and cytotoxic properties for T. polium. Plants belonging to the genus Teucrium are popular for their hypoglycemic and hypolipidemic activities. Some studies have shown the lipid-lowering activity of aqueous extract of T. polium in hyperlipidemic rats. To obtain the most efficient herbal fraction for isolation and identification of the metabolites responsible for hypolipidemic activity, the present study aimed to investigate the effect of different fractions of T. polium on serum lipid profile in dexamethasone (Dex)-induced hyperlipidemic rats.

Methods

Chemicals

The biochemical kits for evaluation of lipid profile, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were purchased from Pars Azmoon Co. (Iran). Folin-Ciocalteu reagent and all other chemicals were purchased from Merck Co., (Germany).
Plant material and fractionation

Aerial parts of *T. polium* were collected from Shahrekord in Chaharmahal and Bakhtiari Province, Iran in May 2015. It was identified by a botanist (Dr. Mustafa Ghanadian, Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran) and its voucher specimen (no. 2718) was deposited at the Herbarium of Isfahan School of Pharmacy and Pharmaceutical Sciences.

For preparation of hydroalcoholic extract, the air-dried plant material (3 kg) was soaked in percolation tank using ethanol: water (70:30) for 3 days and then extracted with a flow of 2 mL/min for 5 days. It was filtered and concentrated by rotary evaporator at 45°C to yield a green viscous residue (257 g). This extract was dissolved in methanol: water (70:30) and partitioned with hexane in a separation funnel to give a biphasic solution. Upper solution (hexanoic part) rich in chlorophyll and fats was discarded and methanolic part was concentrated, suspended in water, and then successively partitioned between equal volumes of chloroform and butanol to give chloroform, butanol, and aqueous fractions. Each fraction was evaporated under vacuum condition and kept in refrigerator at −20°C until use.

**Total phenolic assay**

Determination of total phenolic content was performed using colorimetric Folin–Ciocalteu procedure. Briefly, each plant sample (20 μL) was mixed with diluted Folin–Ciocalteu reagent and sodium carbonate (20%). After 120 min, absorbance was read at 765 nm by an ultraviolet-visible spectrophotometer. Total phenols' amount was estimated using a standard curve of gallic acid and was expressed as milligram of gallic acid equivalents (GAEs)/100 mg of the dried plant extract in three independent experiments.

**Animals**

Male Wistar albino rats weighing 200–220 g were obtained from the animal house of Isfahan School of Pharmacy and Pharmaceutical Sciences. Animals had free access to tap water and standard rat pellet and were kept under standard laboratory condition including a 12 h light/12 h dark cycle and 20°C ± 2°C temperature. The experiment was conducted according to the international guidelines for laboratory animal use and care.

**Experimental protocol**

Rats were randomly divided into 15 groups of 6 rats in each. To induce hyperlipidemia, animals received subcutaneously (s.c.) injection of Dex (10 mg/kg/day) for 7 days. In the test groups, rats received Dex and simultaneously treated orally with 50, 100, or 150 mg/kg of *T. polium* hydroalcoholic extract or different fractions using an intragastric tube. Negative control group received daily injection of saline (1 mL/kg, s.c.) and orally administration of the vehicle (carboxymethyl cellulose 1% in water). Atorvastatin (40 mg/kg, orally) was administered as a reference treatment in positive control group. At the end of the experiment on day 8, blood samples of overnight fasted rats were collected from retroorbital plexus with heparinized capillary tubes under light ether anesthesia. Serum was collected for biochemical analysis and animals were sacrificed by an overdose of anesthetic.

**Biochemical analysis**

For estimation of hepatic marker enzymes (ALT, AST) and lipid profile including triglycerides, total cholesterol, and high-density lipoprotein-cholesterol (HDL-C), the respective biochemical kits were used while low-density lipoprotein-cholesterol (LDL-C) and atherogenic index were calculated using the following formulas: LDL-C = cholesterol − (triglyceride/5) − HDL-C and atherogenic index = (total cholesterol − HDL-C)/HDL-C. All experiments were repeated at least three times.

**Statistical analysis**

The results were reported as mean ± standard error of mean. For statistical evaluation, one-way analysis of variance followed by Tukey post hoc test was performed using SPSS software version 18.0 (SPSS Ltd., Quarry Bay, Hong Kong). *P* < 0.05 was considered statistically significant.

**Results**

**Total phenolic content**

*T. polium* was standardized based on its polyphenolic contents. The hydroalcoholic extract, chloroform, butanol, and aqueous fractions were contained %7.53 ± 0.55, %2.64 ± 0.47, %11.51 ± 0.46, and %13.48 ± 0.33 GAE (mg/100 mg), respectively.

**Effect on triglyceride levels**

Administration of Dex resulted in a significant increase in serum triglyceride level (*P* < 0.001). Atorvastatin and chloroform fraction (at all doses) significantly reduced triglyceride level compared to Dex control group (47.8% and 60.2%, respectively). However, there was no significant difference between 3 doses of chloroform fraction. Hydroalcoholic extract and butanol fraction at the dose of 50 mg/kg (*P* < 0.001) and aqueous fraction at the dose of 150 mg/kg (*P* < 0.05) were found to be effective in decreasing triglyceride level (27.0%, 43.9% and 19.9%, respectively). Maximum reduction of 60.2% was observed for chloroform fraction at the dose of 150 mg/kg [Figure 1]. The post hoc analysis of results also showed significant differences between the chloroform fraction at the dose of 150 mg/kg with other fractions at their highest dose (*P* < 0.001).
Effect on cholesterol levels
Dex caused a significant elevation in cholesterol level ($P < 0.001$). As shown in Figure 1, atorvastatin significantly reduced cholesterol level (21.3%). Hydroalcoholic extract reduced cholesterol concentration at all doses; however, it was more effective at the lowest dose (29.0%). Aqueous and chloroform fractions showed lowering effect at the dose of 150 mg/kg (21.2% and 17.96% reduction, respectively). More analysis of groups also revealed significant differences between the hydroalcoholic extract at the dose of 50 mg/kg with other fractions at their lowest dose ($P < 0.001$). No cholesterol-lowering effect was observed for butanol fraction.

Effect on high-density lipoprotein levels
Significant decrease in HDL level was observed in Dex-induced hyperlipidemic rats ($P < 0.05$). Treatment with atorvastatin, hydroalcoholic extract, and butanol fraction (at the highest dose) significantly increased HDL level (37%, 28% and 33%, respectively). Administration of doses of 100 and 150 mg/kg from aqueous fraction was also effective in increasing HDL (28% and 30%, respectively). However, maximum elevation of 35% in HDL level was found for chloroform fraction at the highest dose [Figure 2]. Multiple analyses showed no significant difference between various doses of different fractions.

Effect on low-density lipoprotein levels
In Dex-induced hyperlipidemic rats, LDL level significantly increased compared to normal rats ($P < 0.001$). Treatment with atorvastatin and aqueous fraction (at highest dose) significantly reduced LDL level (30.1% and 50.0%, respectively). Maximum reduction of 72.9% was observed for hydroalcoholic extract. However, no lowering effect was observed on LDL concentration for butanol and chloroform fractions [Figure 2]. The post hoc analysis of results also exhibited significant differences between various doses of hydroalcoholic extract with different doses of other fractions ($P < 0.001$) except for aqueous fraction at its highest dose ($P = 0.85$).

Effect on atherogenic index
All treatments significantly ($P < 0.001$) decreased atherogenic index as a noteworthy predictor of atherosclerosis in hyperlipidemic rats [Figure 3]. More analysis of groups also showed significant differences between the hydroalcoholic extract at the dose of 50 mg/kg with other fractions at their lowest dose ($P < 0.01$). However, there was a significant difference at the dose of 100 mg/kg ($P < 0.001$) and 150 mg/kg ($P < 0.01$) only with butanol fraction.

Effect on alanine aminotransferase and aspartate aminotransferase levels
As exhibited in Figure 4, administration of hydroalcoholic extract of $T$. polium and its fractions at all doses significantly increased serum level of ALT and AST in hyperlipidemic rats in a dose-dependent manner ($P < 0.001$). The less toxic effect on serum level of ALT was observed
It is important to mention that several forms of hyperlipidemia appear to be more potent in hypolipidemic activity of Teucrium polium fractions. However, further studies are needed to determine possible mechanisms of action of each constituent for the management of hyperlipidemia.

Unfortunately, despite strong hypolipidemic activity, hepatotoxic effect was observed for all fractions of T. polium mainly for chloroform fraction. Highly elevation of ALT and AST enzymes may be related to neo-clerodane diterpenes, especially teucrin A and teuchamaedryn A in this fraction. Their furan and hydrofuran rings polyoxidized by CYP3A4 and produced ROS responsible for liver damages. In conclusion, this study showed strong hypolipidemic effect of chloroform, butanol, and aqueous fractions of hydroalcoholic extract of T. polium in an animal model of hyperlipidemia. Chloroform and aqueous fractions may be potential candidates for isolation of bioactive anthyperlipidemic constituents; however, identification and separation of hepatotoxic compounds and taking low doses should be considered for clinical application.

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**Conflicts of interest**

There are no conflicts of interest.

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