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Effect of Hydro-Alcoholic Extract of Rosemary on Lipid Profile and Liver Enzymes in Male Wistar Rats Fed with High-Fat Diet

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Running title: Effect of rosemary extract on lipid profile and liver enzymes in rats

Abstract

BACKGROUND: The liver plays a major role in the metabolic regulation of dietary nutrients. As a well-known risk factor, obesity is characterized by accumulating extra fat in the body. Previous studies have shown that high-fat diets result in non-alcoholic hepatic steatosis. The steatosis can lead to serious liver diseases. Because of the adverse effects of the available drugs for obesity, increasing efforts are being made to develop natural product-based safe and effective medicines.

OBJECTIVES: The present study aimed to assess the effects of hydroalcoholic extract of rosemary on lipid profile and liver enzymes in male Wistar rats fed with high-fat diet.

METHODS: The extract was prepared using an ethanol/distilled water (1:1) mixture. Thirty-two rats were randomly divided into four groups: control (standard diet), high-fat (HF) diet, RE (standard diet + rosemary extract), and HF+RE (HF diet + rosemary extract), and treated for 12 weeks. Biochemical parameters were determined using standard methods.

RESULTS: The total cholesterol changed from 54.2 mg/dL in control group to 76.8 mg/dL in HF diet. In the RE group, the high-density lipoprotein (HDL) and low-density lipoprotein (LDL) enhanced and decreased, respectively, compared to the standard regimen. The extract caused 14.4% reduction ($p<0.05$) in triglyceride levels in comparison to the HF rats. Compared to the HF diet, the HF+RE diet caused significant ($p<0.05$) reduction in the aspartate transaminase (AST) and alanine transaminase (ALT).

CONCLUSIONS: Although hydroalcoholic extract of rosemary was relatively effective to diminish the influence of high–fat diet on total cholesterol and liver enzymes, to evaluate the recognized safety and efficacy of the extract, more investigations must be performed.

KEYWORDS: High-fat diet, Lipid profile, Liver, Medicinal plant, Rosemary

Introduction

Obesity increases the risk of other diseases and health problems, e.g., heart disease, diabetes, high blood pressure, and certain cancers. As a complex medical problem, obesity involves excessive quantity of body fat, generally results from a combination of inherited and environmental factors, and can be managed by diet and exercise (Fock and Khoo, 2013). Dietary fat has the most contribution to obesity. Fat is the dietary nutrient with the greatest energy content (Jackson *et al.,* 2020). Therefore, HF diets can lead to obesity and predispose various metabolic changes, e.g., hyperinsulinemia, dyslipidemia, and type 2 diabetes mellitus. Furthermore, it is associated with some other diseases and health problems, such as atherosclerosis, hypertension, immune impairment, insulin resistance, and non-alcoholic steatohepatitis (Lopez *et al.,* 2005; Shiota and Tsuchiya, 2006).

High lipids diet causes hyperlipidemia referring to a decrement in HDL cholesterol and an increment in total cholesterol, triglycerides, and LDL cholesterol (Vafa *et al.,* 2011). Due to the side effects of the current synthetic drugs, the tendency to practice the traditional treatments has been increased recently. In most developing countries, primary health care depends on medicinal herbs (Mohammed *et al.,* 2021). According to the epidemiological observations, consumption of medicinal plants has had healing consequences on hyperlipidemia. When common remedies cannot control the disease, using such plants is particularly important (Bahmani *et al.,* 2015).

The liver plays a special role in protecting the body against chemical components. Increment in environmental and industrial pollutants can cause liver damage (Kadhim *et al.,* 2021). As one of the most chronic liver diseases with a rising prevalence of about 20-30% globally, non-alcoholic fatty liver affects mainly obese people and type 2 diabetes patients. It causes steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (Parsi *et al.,* 2020). Mainly found in the liver, the levels of aspartate transaminase (AST) and alanine transaminase (ALT) enzymes in serum may be a sign for of the liver disease and/or damage. To prevent and treat non-alcoholic fatty liver disease, lifestyle modification has been reported as the best strategy (Hallsworth and Adams, 2019). Based on the studies reported in the literature, diets containing antioxidants and anti-inflammatory agents (e.g., active ingredients in some medicinal plants) can effectively treat the disease (Parsi *et al.,* 2020).

Rosmarinus officinalis L. (rosemary) belongs to the Lamiaceae family, is native to the Mediterranean and South European countries, and is also cultivated in Iran. Rosemary is a shrub-shaped perennial plant with branches full of leaves. It may be used in cooking as a spice and in the food industry as a natural preservative. For pharmaceutical purposes, the main branches and leaves of the plant are collected in the spring and summer (Abadi *et al.,* 2016). Caffeic acid, carnosic acid, chlorogenic acid, monomeric acid, oleanolic acid, rosmarinic acid, ursolic acid, alpha-pinene, camphor, carnosol, eucalyptol, remedial, rosmanol, rosmaquinones A and B, secohinokio, and derivatives of eugenol and luteolin are the most reported phytocompounds representing the pharmacological activities of rosemary. For detailed pharmacological effects of phytocompounds from rosemary, see de Oliveira *et al.* (2019).

Some studies have been reported in the open literature on the hydroalcoholic extract of rosemary regarding the constituent carnosol that inhibits formalin-induced pain and inflammation in mice (Emami et al., 2013) and the influence of the extract on anxiety in mice (Noori Ahmad Abadi et al., 2016). However, there are no investigations on the conservative effects of the hydroalcoholic extract of rosemary on HF diet-induced liver enzymes and abnormal levels of cholesterol and other lipids. Therefore, the main objective of the present study was to investigate the influence of hydroalcoholic extract of rosemary on blood lipid profile and the liver enzymes in HF diet-fed adult male rats.

Materials and Methods

Collection and identification of fresh plant materials

The fresh aerial parts of *R. officinalis* were collected manually in spring 2020 from natural growing sites in Fars province, located in south-central Iran (29° 36′ N; 52° 31′ E; altitude, about 1500 m above sea level). The materials were transported to the laboratory. The plant was identified using the authentic specimens deposited at the Herbarium of the Department of Biology, Shiraz Branch, Islamic Azad University, Shiraz, Iran.

Sample preparation and extraction

After washing the fresh samples thoroughly with water to remove dirt, the leaves were separated from the stems accurately and spread in the shade at room temperature (average temperature of about 24 °C) for 7 days. To ensure uniform drying, the leaves were spread as a thin layer with a thickness of about 20 mm, and a moderate forced airflow was produced using a table fan. Dried leaves were pulverized into a fine powder using a mechanical blender and stored at 4°C in amber-colored bottles until the extraction was started.

Preparation of the hydroalcoholic extract from the plant was conducted using the procedure described by Ghazizadeh *et al.* (2020) with minor modifications. About 250 g of the powder was macerated in 100 mL ethanol/distilled water (50:50) mixture and occasionally shaken for 72 h at room temperature of 25°C. The obtained extract was passed twice through filter paper (Whatman No. 1). After that, the solvent was evaporated using a rotary evaporator at a reduced pressure of 100 psi and controlled temperature of 40°C (Rezaei and Ghasemi Pirbalouti, 2019). The produced dried extract was properly protected in an amber glass bottle and stored in a refrigerator at a temperature of 6±2°C until further tests.

Animals and experimental design

The current research was conducted using 32 male Wistar rats weighing between 220 and 250 g taken from the Shiraz Islamic Azad University. The rats were kept in individual cages at the ambient temperature of around 22°C and relative indoor humidity of about 50% under a 12 hours dark/light cycle. Before the experiments, all the animal care principles were correctly applied. The laboratory animal care and the experiments were confirmed to be in accordance with the Guidelines of the Animal Ethics Committee of the Kazeroon Azad University. The male Wistar rats were randomly divided into 4 uniform experimental groups as follows:

Group I (Control): Standard diet for 12 weeks,

Group II (HF): HF diet containing 45% wheat flour, 35% corn starch, 7% egg yolk, 6.7% wheat bran, 4% fat, 1% sodium chloride, 1% cholesterol, 0.2% cholic acid, and 0.1% methyl thiouracil for 12 weeks,

Group III (RE): Standard diet period for 12 weeks and treating with 400 mg⁄kg of the extract in the last 4 weeks by oral gavage once daily, and

Group IV (HF+RE): HF diet for 12 weeks and treated with 400 mg⁄ kg of the extract in the last 4 weeks by oral gavage once daily.

In the end, after killing all the rats under ether anesthesia, the blood samples were directly taken by cardiac puncture from the rats' hearts. For biochemical analysis, serum was prepared using a centrifuge machine at 3000 RPM for 15 min and stored at -20°C for further analyses.

Biochemical parameters

Biochemical parameters, including total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and triglyceride (TG), were determined using Pars Azmoon kits (Pars Azmoon Inc., Tehran, Iran). In addition, the serum liver enzymes, including aspartate aminotransferase (AST) and alanine transaminase (ALT), were determined using an enzymatic colorimetric method of Reitman and Frankel (1957). The values were expressed as U/L.

Statistical Analysis

All data were analyzed using SPSS-21 software. The data were expressed as mean ± the standard error of the mean (SEM). A one-way analysis of variance (ANOVA) was performed to compare differences between groups. Data were considered statistically significant at $P < 0.05$.

Results

Lipid profile

Experimentally measured values for total cholesterol, triglyceride, LDL, and HDL in the blood serum of the rats were presented in Table 1. According to the lipid profile, the normal range of the total cholesterol varied between 54.2 and 76.8 mg/dL. For the TC, the obtained results (Table 1) revealed considerable differences among the treatment groups where, compared to the control group, an increment was observed for HF and HF+RE diet groups. The highest cholesterol levels (76.8±11.7 mg/dL) were obtained for the rats fed with the high-fat diet. Furthermore, the average LDL and HDL values were obtained to be in the range of 7.0–11.2 and 27.4–36.1 mg/dL, respectively.

Serum markers of liver damage

The influence of the different treatments on serum aspartate aminotransferase (AST) and alanine transaminase (ALT) levels of the rats were presented in Table 2. As shown in Table 2, the AST and ALT ranged from 134.8 to 224.8 U/L and from 68.4 to 87.2 U/L, respectively.

Discussion

Based on the results obtained in the present research, the extract utilization incorporated with the high-fat diet significantly mitigated the diet influence on TC. The observation is in agreement with the findings reported by Kumar et al. (2013), Naderi *et al.* (2019), and Shahraki *et al.* (2020). Compared to the control group, oral gavage with a hydroalcoholic extract from *O. Rosmarinus* caused a slight decrement in the TC level. Naderi *et al.* (2019) have investigated the influence of hydroalcoholic extract of *Foeniculum vulgare* on the serum lipid profile of male rats fed with a high-cholesterol regimen and found that TC reduces in extract-treated animals. Furthermore, in another experimental study, Hsieh *et al.* (2020) have assessed the serum cholesterol-lowering capability of water extract from *Gynura bicolor* in the Syrian hamster. They found that the TC level of the high-fat-fed hamsters (226 mg/dL) was about 182% higher than the value for the control group. They also reported that incorporating the extract with the high-fat diet caused a significant reduction in total cholesterol level compared to the high-fat diet.

The HF diet caused significant increments in both LDL and HDL levels compared to the control group. The RE regimen resulted in enhancement in HDL while LDL was significantly decreased. In addition, RE+HF caused a significant decrease in LDL compared to high-fat-fed rats. Dietary fat increases HDL levels both by increasing the transport rates and decreasing the fractional catabolic rates of HDL cholesterol ester and apolipoprotein (Hayek et al., 1993). Previous studies have shown that increases in LDL-cholesterol resulting from substitution of dietary saturated fat for carbohydrate or unsaturated fat are due primarily to increases in large cholesterol-enriched LDL, dense LDL particles and apolipoprotein B (Chiu et al., 2017). According to epidemiological studies, the concentration of antioxidants (e.g., vitamin C, lutein, and carotenoids) in the blood is enhanced due to augmented consumption of fruits and vegetables.

Furthermore, as stated by Zern and Fernandez (2005), dietary polyphenols (often found in vegetables and fruits) moderate the influence of cardiovascular disease. Hsu *et al.* (2008) have studied the lipid-lowering effects of tomato paste in Syrian hamsters. They reported that the administration of the HF diet with tomato paste to the hamsters for 8 weeks decreased their serum TC and LDL levels by 14.3% and 11.3%, respectively, compared to those found in the animals fed with the HF diet alone.

The TG level was significantly increased in the rats fed with the HF diet compared to the other groups. The statistical analysis revealed that the TG difference between the control and RE groups was insignificant. Treating the HF group with the rosemary extract caused about a 14.4% reduction in TG level. The observation may originate from probable intestinal peristalsis and inhibited enzyme lipase under the extract treatment, which has resulted in decreased lipid absorption in the intestine. Furthermore, the extract perhaps has increased the activity of Apolipoprotein C-II cofactor, resulting in enhanced activity of lipoprotein lipase, and consequently, the hydrolysis of triglycerides. Conducting experimental research to evaluate the efficacy of extract of *Ginkgo Biloba* seeds in the HF diet in mice, Kang (2017) found that the TG level increased in HF-fed animals while the level in extract-treated samples was lower than those obtained for low-fat and HF regimens. According to Schwingshackl and Hoffmann (2014), in animal models, the induction of hyperlipidemia is affected by the fat content, cholesterol amount, and/or experimental time periods.

The toxicity of several compounds mainly affects the liver since the substances go mostly through first-pass metabolism. Therefore, the liver becomes the key organ for evaluation of the influences of different chemicals into the body (Tousson *et al.*, 2020).

Hepatocytes are the chief functional cells of the liver and contain large amounts of useful enzymes to detect and determine liver damages. AST is a microsomal enzyme released in the blood in large amounts. Furthermore, AST could be found in large amounts in the liver and with destructive liver tissue. As a liver-specific enzyme in the cytosol, ALT is a sensitive and specific criterion for liver cell damage (Hosseini *et al.,* 2013).

The values obtained in the present study for AST and ALT are in line with other studies reported for rats. The AST (134.8 U/L) and ALT (68.4 U/L) obtained in this study for control animals are comparable with the reported results by Al-Attar et al. (2015) for Wistar rats under standard diet (98.50 and 49.17 U/L, respectively). The researchers also found the AST and ALT values to be 94.17 and 49.11 U/L for the rats treated with rosemary leaves. Robert et al. (2021) reported the AST and ALT for Wistar rats fed with normal diet and water to be 130.4 and 131.2 U/L, respectively. Ramezanyfard Darabi and Hemayatkhah Jahromi (2018) reported the AST and ALT concentration for adult male Wistar rats fed with standard diet to be 113.14 and 34.14 U/L, respectively. Furthermore, evaluating the liver parameters in Wistar rats following repeated oral administration of different green tea extracts, Bun et al. (2006) found the AST and ALT to be in the ranges of 91.9–147.3 U/L and 31.4–87.6 U/L, respectively. Taheri *et al.* (2012) have studied the influence of hydroalcoholic extract of *Berberis vulgaris* root on the liver enzymes activity in male hypercholesterolemic rats and reported that AST and ALT vary from 177.4 to 300.4 U/L and from 41.7 to 80.4 U/L, respectively for the studied rats.

The minimum values of both enzymes belonged to the control rats, while the maximum value for both AST and ALT were observed in the animals fed with HF diet. Accordingly, hepatic dysfunction may be induced in the rats. In general, increased AST and ALT levels serves as a reliable index for the injured parenchymatous cells of the heart and liver, respectively (Tousson *et al.,* 2020). Consequently, the observed significant increments in these enzymes' activity mainly refer to the HF diet lesions in heart and liver tissues.

The results revealed that the HF+RE diet caused a significant reduction in the AST and ALT concentrations compared to the HF diet. In addition, the concentration of both enzymes in the experimental group that received RE was significantly lower than those obtained for the HF group. The best results of liver function belonged to the animals fed with the hydroalcoholic extract from rosemary due to the lowest difference with the control diet. Similar findings have been reported by Hosseini *et al.* (2013) for the influence of barley extract on AST and ALT enzymes in rat liver. The researchers observed no significant differences for the enzymes between the control and the treated rats. Besides, the AST and ALT concentrations were found to be significantly decreased in the experimental group fed with the extract compared to the cholesterol group. Al-Attar and Shawush (2015) studied the influence of rosemary leaf extract on liver cirrhosis induced by thioacetamide (TAA) in male Wistar rats. They observed hepatic cirrhosis with many histopathological alterations in the rats, and also reported significant increases in the serum ALT and AST levels. The researchers found that treating the rats with rosemary leaf extract decreased the histopathological alterations induced by TAA intoxication. By evaluating the ALT and AST activities, Rašković *et al.* (2014) reported that the hepatoprotective effects of rosemary essential oil on Wistar rats could ameliorate liver injury induced by prooxidant agent CCl4.

Conclusion

It could be concluded that the influence of a high-fat diet on total cholesterol and liver enzymes may be diminished using a hydroalcoholic extract from rosemary. To evaluate the safety and efficacy of the extract, more investigations must be done.

Conflict of Interest

The authors declare that they have no conflict of interest. The authors alone are responsible for the content of the paper.

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Table 1. Serum lipid profile for male Wistar rats fed with different regimes

Diet	LDL (mg/dL)	HDL (mg/dL)	Total cholesterol (mg/dL)	Triglyceride (mg/dL)
Control	8.1±2.0^{b}	27.4±6.7^{c}	54.2±13.2^{c}	77.4±15.1^{c}
HF	11.2±1.4^{a}	34.8±8.1^{ab}	76.8±11.7^{a}	122.2±17.7^{a}
RE	7.0±1.7^{c}	32.2±7.6^{b}	51.8±9.0^{b}	75.2±13.5^{c}
HF+RE	8.4±0.9^{b}	36.1±9.9^{a}	62.4±12.6^{b}	104.6±14.3^{b}

*Groups with different letters indicate a statistically significant difference (($P < 0.05$)).

Control: Standard diet for 12 weeks,
HF: High-fat diet containing 45% wheat flour, 35% corn starch, 7% egg yolk, 6.7% wheat bran, 4% fat, 1% sodium chloride, 1% cholesterol, 0.2% cholic acid, and 0.1% methyl thiouracil for 12 weeks,
RE: Standard diet period for 12 weeks and treating with 400 mg/kg of the rosemary extract in the last 4 weeks by oral gavage once daily
HF+RE: High-fat diet for 12 weeks and treated with 400 mg/ kg of the rosemary extract in the last 4 weeks by oral gavage once daily

Table 2. Serum AST and ALT of male Wistar rats fed with different regimens.

Diet	AST (U/L)	ALT (U/L)
Control	134.8±3.2^{c}	68.4±2.6^{c}
HF	224.8±4.1^{a}	87.2±3.3^{a}
RE	139.6±2.7^{c}	70.4±2.4^{c}
HF+RE	212.8±4.9^{b}	79.6±3.1^{b}

*Groups with different letters indicate a statistically significant difference (($P < 0.05$)).

Control: Standard diet for 12 weeks,
HF: High-fat diet containing 45% wheat flour, 35% corn starch, 7% egg yolk, 6.7% wheat bran, 4% fat, 1% sodium chloride, 1% cholesterol, 0.2% cholic acid, and 0.1% methyl thiouracil for 12 weeks,
RE: Standard diet period for 12 weeks and treating with 400 mg⁄kg of the rosemary extract in the last 4 weeks by oral gavage once daily
HF+RE: High-fat diet for 12 weeks and treated with 400 mg⁄ kg of the rosemary extract in the last 4 weeks by oral gavage once daily

تاثیر عصاره هیدروالکلی رزماری بر پروفایل لیپیدی و آنزیم‌های کبدی در موش‌های صحرایی نر ویستارتغذیه شده با رژیم غذایی پرچرب

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چکیده

زمینه مطالعه: کبد نقش مهمی در تنظیم متابولیک مواد مغذی رژیم غذایی دارد. به عنوان یک عامل خطر شناخته شده، چاقی با تجمع چربی اضافی در بدن مشخص می‌شود. مطالعات قبلی نشان داده‌اند که رژیم های غذایی پرچرب منجر به استئاتوز کبدی غیر الکلی می‌شود. استئاتوز می تواند منجر به بیماری های جدی کبدی شود. داروهای شیمیایی کمی برای درمان چاقی در دسترس است. علاوه بر این، به دلیل عوارض جانبی نامطلوب این داروها، تلاش‌های فزاینده‌ای برای تولید داروهای ایمن و موثر مبتنی بر محصول طبیعی در حال انجام است. اخیراً، به دلیل تأثیرات منفی و سمیت نسبتاً کم ، در دسترس بودن، مقرون به صرفه بودن و کارایی قابل قبول، استفاده از گیاهان دارویی افزایش یافته است.

هدف: مطالعه حاضر با هدف ارزیابی تاثیر عصاره هیدروالکلی رزماری بر پروفایل لیپیدی و همچنین آنزیم‌های کبدی در موش‌های صحرایی نر نژاد ویستار با رژیم غذایی پرچرب انجام شد.

روش کار: عصاره با استفاده از مخلوط اتانول/آب مقطر (1:1) تهیه شد. سی و دو سر موش صحرایی به طور تصادفی به چهار گروه شامل کنترل (رژیم غذایی استاندارد)، رژیم غذایی پرچرب (HF)، رژیم استاندارد + عصاره رزماری (RE) و رژیم غذایی پرچرب + عصاره رزماری (HF+RE) تقسیم و به مدت 12 هفته تیمار شدند. پارامترهای بیوشیمیایی با استفاده از روش‌های استاندارد اندازه‌گیری شدند.

نتایج: کلسترول کل از 54/2 (برای گروه کنترل) به 76/8 میلی‌گرم در دسی‌لیتر (برای رژیم غذایی پرچرب) تغییر کرد. در مقایسه با رژیم استاندارد، تغذیه RE به ترتیب منجر به افزایش و کاهش HDL و LDL شد. تیمار گروه HF با عصاره باعث کاهش 14/4 درصدی سطح تری‌گلیسیرید شد. در مقایسه با رژیم HF، رژیم غذایی HF+RE باعث کاهش معنی‌دار در AST و ALT شد.

نتیجه گیری نهایی: اگر چه عصاره هیدروالکلی رزماری نقش نسبتاً موثری در کاهش تأثیر رژیم غذایی پرچرب بر کلسترول تام و آنزیم‌های کبدی ایفا کرد، برای ارزیابی ایمنی و اثرات واقعی و اثربخشی عصاره، تحقیقات تکمیلی باید انجام شود.

کلمات کلیدی: رژیم غذایی پرچرب، کبد، گیاهان دارویی، رزماری، پروفایل لیپیدی