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Cardioprotective role of salvia rosmarinus (rosemary) leaves against oxidative stress and in balancing lipid profile in mice.

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Abstract

Background: Developing countries encounter a change in lifestyle, which establish novel risk factors for cardiovascular disease, leading to an explosion in cardiovascular disease risk all through the developing nations. Dietary therapies and physical activity are the first lines of treatment in hyperlipidemia, and several dietary components with such effects have been identified. This study is intended to assess the potential of Salvia Rosmarinus (SR) leaves powder consumption on diet-induced hyperlipidemia. The main objective of this study is to show that plant products could be equivalent to the available medicines.

Methodology: In this experimental study, 4 to 5 weeks old mice were used with an average weight of 200 gm. Baseline values of all parameters were observed & animals were administered an atherogenic diet for two weeks. 0.11 gm/day rosemary leaves powder was fed by these hypercholesterolemic rats for another two weeks. At the end of the experimental duration, blood samples were collected and evaluated for alterations in plasma lipid profile, glucose, liver enzymes and kidney biomarkers.

Results: According to the research results, oral supplementation of 0.11 gm/day rosemary effectively reduced dietary hyperlipidemia in experimental mice. Significant ($p<0.05$) reduction in body weights, total plasma cholesterol (TC), glucose, alkaline phosphate (ALP) and urea levels were analysed, and a non-significant decrease in triglyceride (TG), low-density lipoprotein (LDL), uric acid and creatinine was observed.

Conclusion: It is concluded that Rosemary can be utilized beneficially for primary prevention trials of cardiovascular disease, but of course, further investigations are required to find out an effective dose of rosemary.

Keywords

Salvia Rosmarinus, Cardiovascular Disease, Total Cholesterol, Hyperlipidemia, Dietary Therapy.

Introduction

Cardiovascular disease (CVD) is regularly viewed as a burden of well off, industrialized social communities. The main source of deaths on the planet is cardiovascular disease and a significant boundary to maintain human advancement[1]. The United Nations in 2011, officially perceived non-transferable infections, incorporating cardiovascular diseases, which is a significant worry for worldwide wellbeing and carried out an aggressive arrangement that drastically decreases the impact of these infections in every zone of the world[2]. 422.7 million CVD instances were expected (95% vulnerability range: 415.53 to 427.87 million patients) and 17.92 million CVD fatalities (95% vulnerability range: 17.59 to 18.28 million CVD fatalities).

Decreases in the age-institutionalized CVD demise rate happened around in between 1990 to 2015 in both high-income and certain middle-income nations. Ischemic coronary illness was the main source of CVD wellbeing declined all around, just as in every zone of the world, accompanied by stroke[3].

The WHO reports that by 2030, 23.6 million death s will occur per year from CVDs, majorly attributable to stroke and coronary cardiac disease[4]. The evaluated yearly stroke rate is 250/100 000 in Pakistan, meaning 350,000 new victims per annum[5]. The occurrence of modifiable threat factors for stroke within Pakistani natives is particularly worrying. Hypertension distress one in three persons elderly extra than forty-five years and 19% of the people elderly fifteen years and above. Pakistan's national Health analysis confirmed 35% of people above 45 years have diabetes mellitus[6]. The general predominance of obesity is 28% in adult females and 22% in adult males, and the utilization of tobacco is 33% in adult males and 4.7% in adult females[7,8].

At present, there has been an expended global interest in discovering compounds isolated from organic products that are pharmacologically safe and have minimal side effects for utilization in preventive medicine and curing diseases. Vegetable metrics of the Labiatae (Lamiaceae) family, such as rosemary, be an important source of substances with antioxidative properties[9,10]. Salvia Rosmarinus (SR) is a medicinal plant native to the Mediterranean region. It is a small evergreen bush with thick, fragrant leaves and is generally cultivated for medicinal, culinary, ornamental, and perfumery purposes. Additionally, Rosemary has been utilized as a curing herb for its astringent, spasmolytic, antimicrobial, expectorant, carminative, anti-rheumatic, anti-inflammatory pain-relieving, and hypotensive properties[11]. It plays a significant role in the anticipation and therapeutic treatment of diseases related to oxidative stress, incorporating malignant growth, cardiovascular and neurodegenerative diseases[12-14]. The phytochemicals present in rosemary leaves inhibit genotoxicity and provide shielding from cancer-causing agents[15]. It has been appeared to reduce blood glucose in a few in vivo examinations[16]. Investigations Proved that rosemary could stop hepatic fibrosis because of chronic liver injury and slow down cirrhosis progression[17]. Extracted rosemary constituents, Rosmanol and epirosmanol, were stated to reduce LDL oxidation in human blood[18]. In vitro investigations depicted that rosemary compounds inhibit interlukin-β (IL-β) and tumor necrosis factor-α (TNF-α) and raised glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activity in various models[19,20]. In vivo, rosemary extricates doses reduced the oxidative stress in the heart of older mice[21,22]. Moreover, rosmarinic acid has powerful potential to decrease myocardial impaired blood pressure in hypertensive mice fed a high fructose diet[23] and shield the heart against cardiac defectiveness and fibrosis after myocardial infarction in mice[24].

Methodology

The study was conducted on healthy male mice weighing 200 to 220 gm and 4 to 5 weeks old that was procured by the animal laboratory of the International center for chemical and biological sciences, Karachi. The animals were housed in a well ventilated 12 hours light and dark cycle. The animal was classified into three categories (n = 6) and treated orally for 15 days. The first category was served as the control that received normal rat commercial pellets & distilled water, second one Hyperlipidemic animals fed on a high-fat diet, i.e. 1 gm fat (butterfat)/100 gm of the daily diet, the third category got 0.11 gm of rosemary leaves powder/kg of body weight along with hyperlipidemic diet.

Plant Material

Undried Salvia Rosmarinus leaves were purchased from a local market in Karachi, Pakistan, in October 2019. Cleaned with water and air-dried for two days, then placed in the oven for 24 hours until completely dried. To make powder, dried leaves were crushed in an electric grinder then stored in a clean and dried covered plastic container at ambient temperature.

Blood Sampling

Blood specimens were obtained via cardiac puncture technique from unconscious animals who had inhaled chloroform and collected in centrifuge tubes (containing EDTA as an anticoagulant) and centrifuged at 3000 rpm 10 min to isolate plasma [25]. Plasma was separated and kept in the freezer till the time of assay.

Tissue Sampling

After dissection heart, liver and kidneys were separated, cleansed with freezing cold saline, eliminate sticky fat and connective tissue from the organ and soaked in 10% buffered formalin solution for fixation. All the tissues were rinsed with (80%, 90% and 100%) alcohol for two hours, respectively. Submerge the tissues in acetone for an hour to finish the dehydration process. Tissues were then impregnating in melted paraffin wax for 1 hour. Wax was then poured into an L-shaped glass container with tissues and left it for some minutes until it settled and became firm. The paraffin blocks were placed in the microtome, and 5-10 mm thick pieces were cut up from the tissues. Deparaffinize with Xylene and rehydrate the tissue pieces after immersing in (100%, 90%, 80%) isopropyl alcohol. The tissue pieces were then coated with Hematoxylin / Eosin dye, and a histopathological examination was analyzed using a light microscope.

Biochemical Analysis

The lipid profile was done using the enzymatic method, cholesterol oxidase/peroxidase aminophena-zone (CHOD-PAP) on Landwind C 100 plus auto analyzer, LDL was calculated using the Friedewald formula. Plasma glucose concentration was identified by enzymatic Trinder, glucose oxidase-peroxidase (GOD-POD) procedure. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) concentration and kidney biomarkers were assayed in plasma using enzymatic Biomed diagnostic kits.

Statistical Analysis

Collected data were tabulated, and needed statistical analyses were done utilizing the SPSS version 20 software. A p-value$<$0.05 was considered to be statistically significant. The data are represented as mean and standard deviation (SD), and a comparison of means was performed using the student t-test for skewed data.

Results

The study demonstrated the hypolipidemic activity of rosemary leaves. The oral administration of a high-fat diet to mice produced a significant increase in lipid profile as compared to control group animals, and significant reduction was found in plasma high-density lipoprotein (HDL) concentration while the oral administration of rosemary leaves powder significantly decrease total cholesterol (TC) concentration and body weights and the non-significantly decline was seen in triglyceride (TG) and low-density lipoprotein (LDL) (Table 1) whereas non-significant elevation in HDL-C was observed in rosemary ingested mice.

Table 1: Alteration detected in body weight, lipid profile, liver enzymes & kidney biomarkers in all group of animals

Parameters	Control (G1)	Hyperlipidemic (G2)	SR treated (G3)
Body weight (gm)	129.33±8.49	212.16±5.51	160.8±3.89
Total Cholesterol (mg/dl)	100.43±3.58	161.43±27.12	120.98±9.70
Triglyceride (mg/dl)	88.61±3.41	131.04±17.99	120.51±14.3
HDL (mg/dl)	33.22±0.94	24.45±1.167	31.1±6.34
LDL (mg/dl)	49.48±2.27	110.76±27.05	82.75±13.52
Glucose (mg/dl)	124.55±6.21	153.91±3.55	122.86±13.8
AST (U/L)	34.52±2.30	74.04±2.35	28.79±0.04
ALT (U/L)	25.8±1.39	61.65±2.06	58.87±13.19
ALP	55.22±2.69	93.12±2.58	62.36±9.68
Urea (mg/dl)	33.2±0.74	52.85±1.48	40.46±2.64
Uric Acid (mg/dl)	2.77±0.15	3.57±0.08	2.22±0.77
Creatinine (mg/dl)	0.49±0.02	1.007±0.09	0.72±0.34
Total protein (g/dl)	6.13±0.192	6.69±0.036	6.16±0.31

Values are presented as mean ± SEM. Significance difference between experimental groups is by t-test*($p<0.05$),**($p<0.01$),***($p<0.005$).

Liver enzymes showed a remarkable decrease in G3 mice when compared with G2 mice indicating that rosemary possesses hepatoprotective potential. To discover the rosemary's biological properties, we performed a further test that showed a significant change in glucose and distinguishable variation in Urea, Uric acid, creatinine and total protein concentrations, which elucidate the tremendous antioxidant property of rosemary herb (Table 1). The histopathological observations in the current study are in agreement with the biochemical findings. Scoring of the morphological and functional findings of heart, liver and kidney are described and summarized in control, hyperlipidemia, and SR treated rats.

Table 2: Comparison in Microscopic Examination of Heart, Liver & Kidney among all experimental groups

.

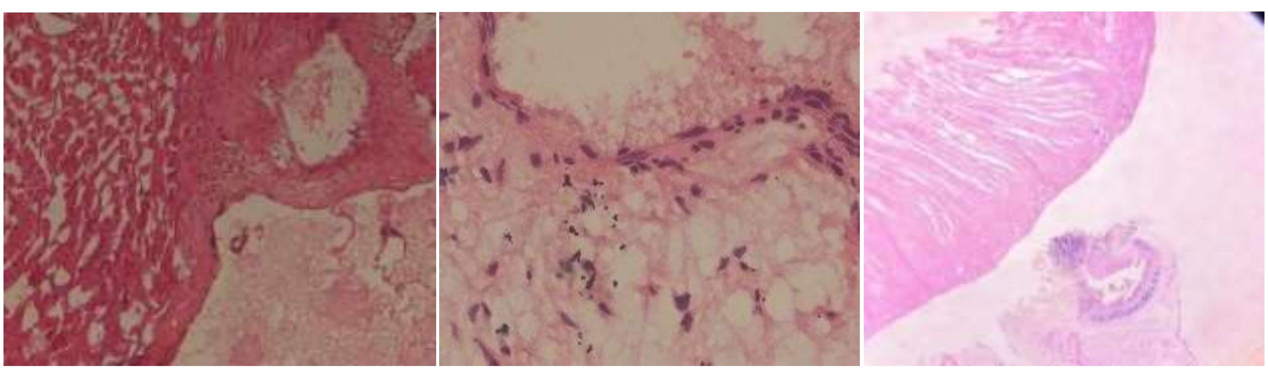
	Description	Control	Hyperlipidemic	SR treated
Heart	Colour	Brownish Red	Brownish Red	Brownish Red
	Texture	Smooth	Smooth	Smooth
	Inflammation	-	+4	+2
	Foam Cells	-	-	-
	Myocardial hypertrophy	-	+3	+1
Liver	Colour	reddish-brown	yellowish-brown	slightly reddish-brown
	Texture	smooth	greasy	slightly smooth
	Enlargement	-	+4	+1
	Lobular Inflammation	-	+2	+1
	polymorphic infiltration	-	+2	+1
	Periportal fibrosis	-	-	-

Kidney	Colour	brown	brown	Brown
	Texture	smooth	smooth	Smooth
	Enlargement	-	-	-
	Mesengial matrix proliferation	-	+2	+1
	Interstitial inflammation	-	+3	+1
	Tubular atrophy	-	+1	-

Scale: (-) normal, (+1) mild, (+2) moderate, (+3) moderate to severe, (+4) severe
Histopathological alterations in cardiomyocytes, hepatocytes and kidney cells were rated with a scale from zero to four [26]. 0 = no distinguishable impairment, 1 = mild impairment less than 25, 2 = moderate impairment 25-50%, 3 = moderate to severe impairment 75%, 4 = severe impairment 75-100% based on the severity of structural alteration.

Any altered myocardial histological changes were absent in the heart tissues of the control group. Severe inflammation and moderate to severe Myocardial hypertrophy can be seen in the hyperlipidemic Group of mice. Our experimental outcomes revealed that SR treated group endeavour to reduce the myocardiocyte's enlargement and reversed the progression of inflammation from severe to mild impairment, thus preventing serious myocardial damage (Table 2, Figure 1). High-fat diet-induced hepatotoxicity in G2 mice confirmed by lobular inflammation with progressive fibrosis in the microscopic study, although rosemary administration has partially inhibited hepatotoxic changes in G3 mice and also overturned the greasy layer of liver back to smooth texture (Table 2, Figure 2). Report and figure of stain section of the kidney tissue elucidate the effects of a high-fat diet in G2 mice by presenting moderate mesangial matrix proliferation with evidently severe interstitial inflammation-causing tubular injury whereas mild changes were examined in SR treated Group of mice that confirmed renodefensive property of rosemary (Table 2, Figure 3).

Figure 1: Histopathological Examination of Heart Tissue

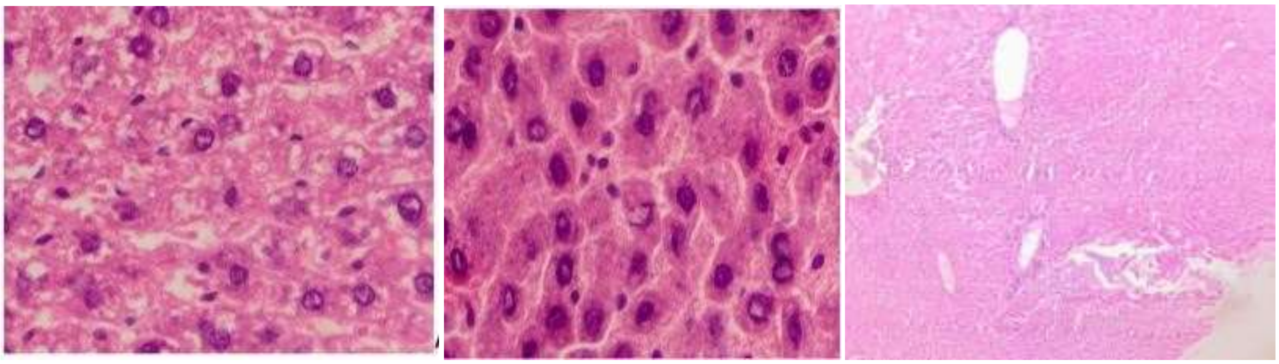


A — Histology of control group rats

B — Histopathological features of hyperlipidemic rat

C — Histology of SR treated rat

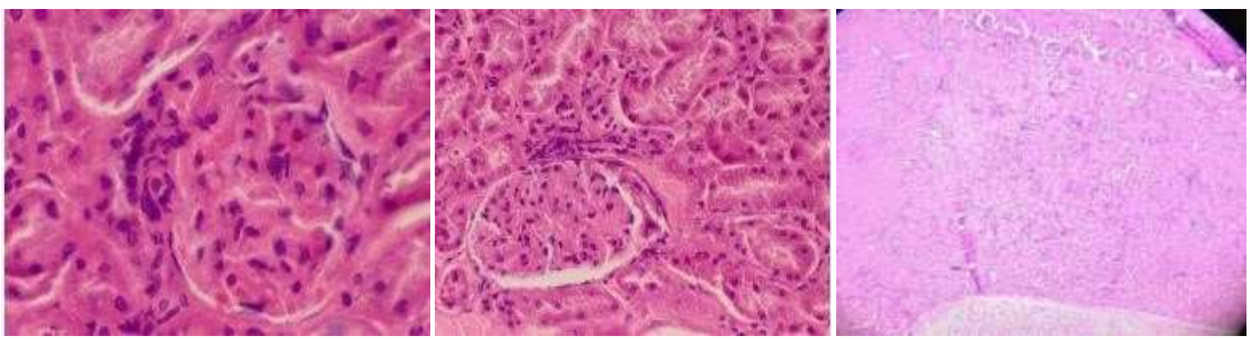
Figure 2: Histological Examination of Liver Tissue



A B C

Histology of control group rats | Histopathological features of hyperlipidemic rat | Histology of SR treated rat

Figure 3: Histological Examination of Kidney Tissue



A B C

Histology of control group rats | Histopathological features of hyperlipidemic rat | Histology of SR treated rat

Discussion

Herbs are opulent in antioxidants. A scientific report proposes that herbs are additional intense injury-related inhibitors to tissue and inflammation by elevated glucose levels and circulating lipids. Their phenolic content can hinder the development of compounds that promote damage produced by metabolic disorders. Since herbs have exceptionally low-calorie substances and are a sound source of antioxidants and other potential bioactive alloys in diet[27]. The phenolic component in rosemary has been demonstrated to be fruitful in reducing hypercholesterolemia[28-30]. As our experimental results inferred that ingestion of rosemary in G3 mice remarkably balanced dyslipidemia, which initiates cardiovascular disturbance because phenolic substances are characterized by their capacity to strengthen antioxidant enzyme activity and minimized free radical formation. Quing-Feng reported that the high LDL-C level in the hyperlipidemic Group of animals (110.76±27.0) might be due to changes in hepatic LDL receptor that contribute to the elevation in blood cholesterol levels induced by a high-fat diet. It has been established that cholesterol-rich diets increased LDL-C levels in the blood and caused oxidative stress that increased oxidized LDL levels[31], so the excess cholesterol is harmful to health and raised risk of cardiovascular diseases and strokes.

LDL-C control has been demonstrated to be an important factor for diminishing cardiovascular events both in patients with atherosclerotic infections and in those with risk factors for progressing atherosclerotic ailment [32]. we obtained the low values of LDL (82.75±13) in G3 mice when compared with G2 (110.76±27) animals. We found a promising effect of rosemary administration in the lipid profile of G3 mice that corroborates by another study that extraction of rosemary with essential oil reduce malondialdehyde (MDA) levels (a marker of lipid peroxidation), increase SOD (superoxide dismutase) activity, serum HDL-C and enhanced antioxidant capacity[33]. Our result regarding glucose level is encouraged by a researcher Koga, who stated that constituents of rosemary exert hypoglycemic effects as it increases the insulin level by regenerating the β-cells of the pancreas and it potentiates insulin secretion that might inhibit the intestinal absorption of glucose via inactivation of the intestinal alpha-amylase enzyme[34]. Our outcomes are supported by research that declared that SR extracts prominently decreased plasmatic glucose, TG, TC and LDL while increasing HDL and erythrocytes levels[35]. We found that HDL-C level was ameliorated in G3 SR treated mice encouraged by study of[36,37] two scientist Barter & Cockerill who stated that HDL hinders vascular inflammation and promotes endothelial function[38]. However, HDL-C alone isn't the concern factor in reducing cardiovascular diseases. There is a need to reconsider the HDL metabolism with an alert focus on the capacity, synthesis, size and number of moving HDL particles in the circulating system, instead of the cholesterol transferred by HDL particles, which makes up a generally small extent on the cardiovascular burden[39,40]. Extensive evidence revealed that rosemary chiefly maintained glycemic blood level. Our results regarding blood glucose were also agreed with the research of Al-Attar[41], who reported that rosemary contains a hypoglycemic effect due to α-pinene, Camphor, cineole and borneol present in it. Many studies have corroborated rosemary's positive health effects, which we have also observed in our experimental outcomes.

Histological results obtained from a curative treatment group of animals indicate that rosemary exerts a restoring effect on cardiac, hepatic and renotic structural disorganization. Hepato alterations detected by us exactly resembles with hepatohistopathological outcomes discovered by scientist Altunkaynak[42]. Experimental evidence has also expressed that the whole plant powder or extract possesses better pharmacological activity than an isolated ingredient due to synergistic interchanges between the discrete components[43].

Conclusion

Scientific investigations and current research undoubtedly show that the consumption of SR was effective in reducing diet-induced hypercholesterolemia in experimental animals and helps control and modify other risk factors of cardiovascular diseases, which is not possible only with the available pharmacological therapies of dyslipidemia. This analysis's findings indicate that the effects of SR in lowering lipid profile were possibly due to the antioxidant properties of its natural chemical constituents. SR, therefore, can be used in the primary prevention of metabolic disorders. More physiological, biochemical and histopathological research is required to explore the possible usage of specific doses of SR and its constituents as potential natural therapeutic agents in limiting cardiovascular diseases' escalation.

Conflicts of Interest

None.

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