Induced Hyperlipidemia in Adult Male Rats and Treated by using *Rosmarinus officinalis* Aqueous Extract

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Abstract

The current work was goaled to revealed anti-hyperlipidemia activity of *R. officinalis* extract. This work utilized 20 male rats that divided to negative group; positive group was given drinking water containing 0.5% H_2O_2 and (1%) cholesterol for 60 days. Third group: hyperlipidemia rats were treated with (50mg/kg/daily) extract. Fourth group: hyperlipidemia rats were treated with (100mg/kg/daily) extract. The outcomes revealed significant ($P < 0.05$) rise in cholesterol and triglyceride and reduce the HDL in positive group compare with negative group. Malonedialdehyied (MDA) was increased with reduce in glutathione (GSH) compare with negative group. Otherwise, diameters of aorta artery show significant ($P < 0.05$) rise compare with negative group. *R. officinalis* aqueous extract when used in treatment, the outcomes exhibited improvement in lipid profile, MDA, GSH and diameters of aorta artery compare with negative group. It was concluded that *R. officinalis* extract possess anti-hyperlipidemia activity.

Keywords: *Rosmarinus officinalis; hyperlipidemia; lipid profile; malonedialdehyied; glutathione.*

Introduction

R. officinalis, arising from the Mediterranean region is describe as aromatic plant (family: Lamiaceae) [1], and is called rosemary [2]. As well as to utilize in culinary side due to the feature aroma, *R. officinalis* is widely used by indigenous people [3]. The products of *R. officinalis* are utilized in testing resulting in their low toxicity and major medicinal properties [4]. *R. officinalis* extract composed of various types of polyphenols inclusive phenolic acids and flavonoids. These molecules show strong antioxidant properties which decrease lipid peroxidation, prevent reactive oxygen species production and suppress inflammation, and comprise from terpenoids with flavonoids, phenols and volatile oil [5]. *R. officinalis* is important for its therapeutic in folk medicine utilized for example like antidepressant activity, hepatoprotector activity, antidiabetic activity, antiangiogenic activity, anti-inflammatory activity and antitumor activity [6-9]. Hyperlipidemia is defined as a heterogeneous cluster of troubles characterized by an increase of lipids profile in the blood, hyperlipidemia also refers to elevate in concentrations triglycerides, and cholesterol in the serum [10]. Hypercholesterolemia may be occurring through high-fat diet or in persons with physiological defects like decreased of receptors number functioning LDL-cholesterol [11].

Materials and Methods

Animal model

In this work 20 rats, (wt 140-180 gm with age 2-4 month) were used and kept on a standard diet and normal saline until beginning the experiment.

Aqueous extraction

The solution of 20% extract was prepared by utilizing boiling water (100 ml) with leaf powder (10 gm), then the mixture was centrifuged at 10000 rpm for 10 min, then supernatant of mixture was put in oven at 45°C to dry, after that dried powder was collected and stored until use [12].

Experimental design

20 rats were utilized in this work and divided in four groups (each group consist five rats):

v Negative group: received normal saline only.

v Positive group: drinking water containing 0.5% H_2O_2 and (1%) cholesterol for 60 days

v Third group: hyperlipidemia rats were treated with (50mg/kg/daily) extract for 30 days.

v Fourth group: hyperlipidemia rats were treated with (100mg/kg/daily) extract for 30 days.

Biochemical measurements

Total cholesterol, triglycerides and high density lipoprotein (HDL) levels were determined using standard kits of biomereiux kit, France. Malondialdehyde (MDA) is determination based on formation of colored complex upon reaction with thiobarbutyric acid. The detection was recorded at (500 nm). Glutathione was measured according to method of [13].

Histological study

Aorta artery from all studied groups were fixed by using formalin 10% , then embedded by using paraffin. After the routine processing, paraffin sections of aorta tissue were cut into 7 µm and stained by using haematoxylin and eosin [14].

Statistical analysis

All data of present work were collected and analyzed by utilizing the SPSS version 12. Descriptive data was summarized using mean, standard error (SE). P values < 0.05 were considered statistically significant.

Results

Lipid profile

The levels of TC (283.1 ± 11.15), TG (243.83 ± 9.35) and HDL (25.92 ± 3.6) in second group show high significant changes ($P < 0.05$) compare with control group (94.1 ± 5.72; 78.03 ± 4.1 and 32.9 ± 2.62 respectively). The levels of TC (98.2 ± 8.63; 91.61 ± 6.51 respectively), TG (81.4 ± 4.8; 74.3 ± 5.17 respectively) and HDL (30.1 ± 4.2; 34.6 ± 5.39 respectively) in third and fourth groups show non-significant changes ($P < 0.05$) compared with control group as shown in figure (1).

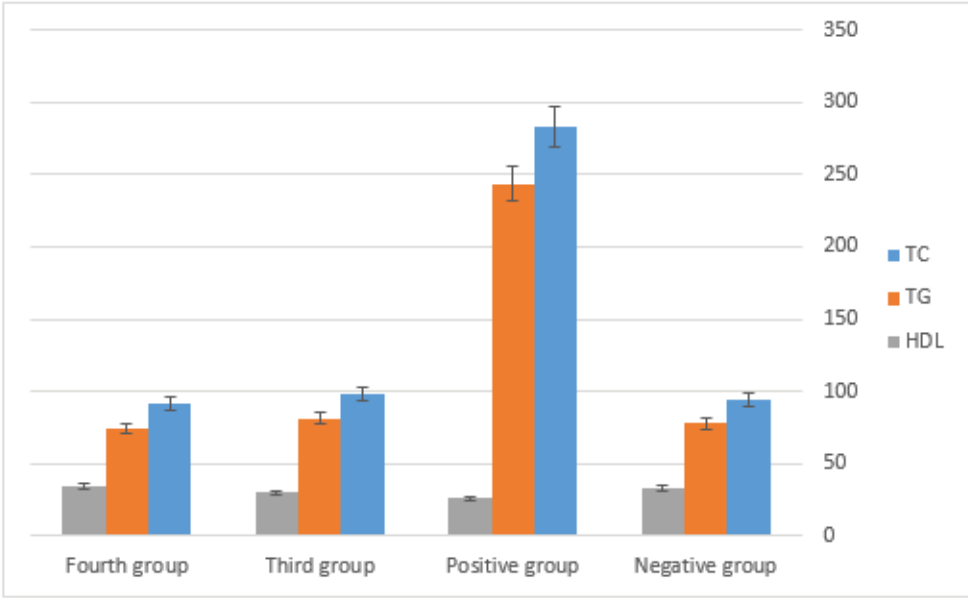

Figure (1): levels of lipid profile in studied groups.

MDA and GSH

The levels of MDA (2.38 ± 0.32) and GSH (0.235 ± 0.028) in positive group show high significant changes (P < 0.05) compare with control group (1.48 ± 0.24 and 0.362 ± 0.038 respectively). The levels of MDA (1.61 ± 0.38; 1.52 ± 0.19 respectively) and GSH (0.378 ± 0.028; 0.366 ± 0.026 respectively) in third and fourth groups show non-significant changes (P < 0.05) compared with control group as shown in figure (2).

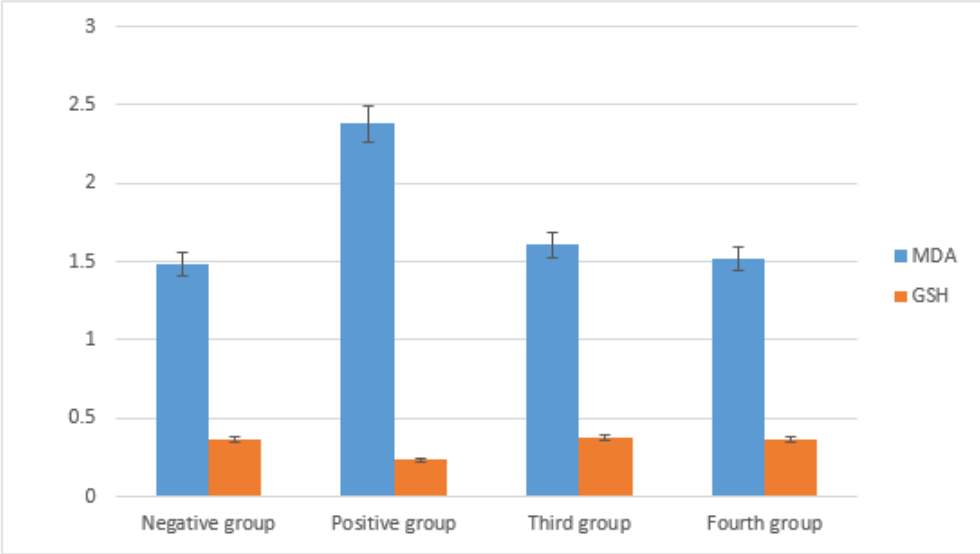

Figure (2): levels of MDA and GSH in studied groups.

Aorta diameters

Aorta diameter (72.2 ± 6.1) in positive group show high significant changes (P < 0.05) compare with control group (34.3 ± 3.5). Aorta diameter (38.17 ± 5.63; 33.28 ± 4.82 respectively) in third and fourth groups show non-significant changes (P < 0.05) compared with control group as shown in figure (3).

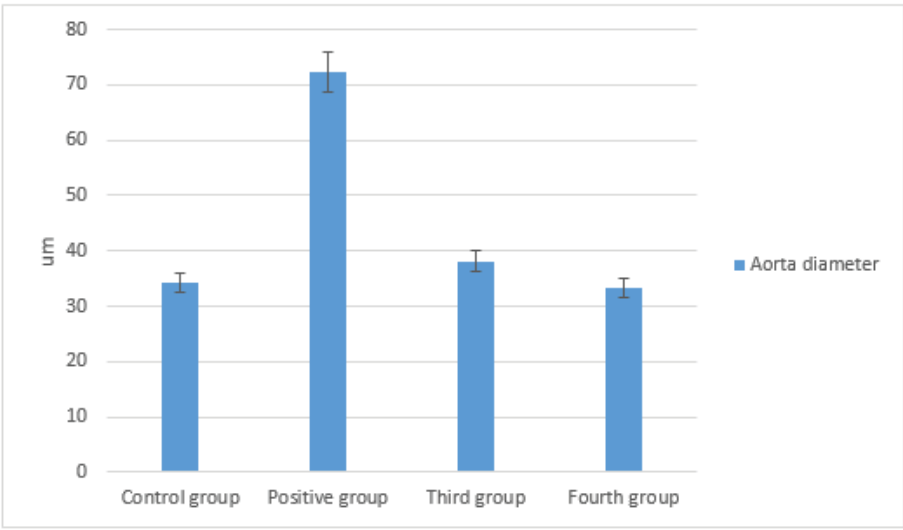

Figure (3): Aorta diameter in studied groups.

Discussion

The current work exhibit the role of *R. officinalis* extract to reduce the levels of cholestrel and triglyceride, aorta diameters and oxidative/antioxidant factors. In study carried out by [15] referred that *R. officinalis* extract lead to reduce the elevated cholesterol in rats that feeding on the high-fat diet (HFD) that is in agreement with outcomes of present work. Al-Sheyab et al. [16] demonstrated clearly the hypolipidemic activity of *R. officinalis* species. The lipid profile (TC, HDL, LDL and TG) showed reduction in rosemary-fed mice as compared to the HC mice. Otherwise, significant elevation of the HDL was observed in rosemary-fed mice as compared to the HC mice. Otherwise, *R. officinalis* extracts have the ability to decompose free radicals by quenching active singlet oxygen and by trapping and quenching radicals before they reach a cellular target [17] that explains the role of *R. officinalis* extract to regulate the oxidative/ antioxidant factors. Also, Rosemary is also capable preventing of lipid peroxidation process that is caused by oxidative stress [18]. In addition to reducing the amount of reactive species in the body, rosemary has been found to increase the activity of antioxidant enzymes [19-20]. About the thickness of aorta and the role of *R. officinalis* extract, rosemary extract prevented weight gain by limiting lipid absorption process in the intestine. This was made possible through the prevent of pancreatic lipase activity [21]. Finally, the third study found rosemary extract to inhibit and prevent lipid synthesis via the suppression of diacylglycerol acyltransferase (DGAT) [22] that may explain the ability of rosemary extract to prevent the accumulation of lipid in tissues.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Reference

1. Nieto, G.; Gaspar R.ID and Julián C. Antioxidant and Antimicrobial Properties of Rosemary (Rosmarinus officinalis, L.): A Review. J. Medicines. (2018). 5(98): 1-13.
2. Andrade, J. M.; Celia F.; Catarina G.; Diogo L.; Catarina P. R. and Patrıcia R. *Rosmarinus officinalis* L.: an update review of its phytochemistry and biological activity. J. *Future Sci. OA* (2018). *1-11.*
3. Jardak M, Elloumi-Mseddi J, Aifa S, Mnif S. Chemical composition, anti-biofilm activity and potential cytotoxic effect on cancer cells of *Rosmarinus officinalis* L. essential oil from Tunisia. *Lipids Health Dis.* (2017). 16(1): 190-197.
4. Elmhdwi, M. F.; Mariam M A.; Naema M.; Yusra F L. and Wejdan K. Biochemical Studies on the Effect of Fixed Oil Extracted from Rosmarinus officinalis on Blood Lipid Level in Male Albino Mice. J Biomed Res Rev. (2018). 1(1): 43-49.
5. Colica, C.; Laura D. R.; Vincenzo A.; Antonino D. L. and Ludovico A. Rosmarinic Acid as Potential Anti-Inflammatory Agent*J. Rev. Rec. Cln. Tri.* (2018). *13:* 240-242.
6. Zhang Y.; Smuts J.P. and Dodbiba E. Degradation study of carnosic acid, rosmarinic acid and rosemary extract (Rosmarinus officinalis L.) assessed using HPLC. J Agric Food Chem. (2012). 60(3):9305–9314.
7. Lazalde-Ramos B.P.; Zamora-Perez A.L. and Gutiérrez-Hernández R. DNA protective effect of rosmarinus officinalis total extract in mouse peripheral blood. *MOJ Toxicol.* (2018). 4(2):75–79.
8. Amaral GP.; De Carvalho N.R.; Barcelos R.P.; Dobrachinski F. and Portella RD. Protective action of ethanolic extract of Rosmarinus officinalis L. in gastric ulcer prevention induced by thanol in rats. Food ChemToxicol (2013) 55: 48-55.
9. Ayoob I. New semi-synthetic rosmarinic acid-based amide derivatives as effective antioxidants". *Chemistry Select* (2017). 2.31 (2017):10153-10156.
10. Karam I.; Ma N.; Yang Y.J. and Li J.Y. Induce Hyperlipidemia in Rats Using High Fat Diet Investigating Blood Lipid and Histopathology. J Hematol Blood Disord (2018). 4(1):104-109.
11. Musial, D. C.; Tânia C. A. B.; Aline P. I.; Lívia B. and Ana C. B. B. Hypercholesterolemia and hepatic steatosis in mice fed on low-cost high-fat diet. J.

Acta. Scien. Health Sci. (2013). 35(1): 23-27.

12. Atala, M. L. and Muhammed A. A. Antibacterial activity of Rosmarinus officinalis and Dodonaea viscosa leaves extracts against Escherichia coli and Staphylococcus aureus. Iraqi J. Sci. (2017). 58(3B): 1393-1397.
13. Burtis C B. and Ashwood E.R. Tietz textbook of clinical chemistry**,** 3rd edition, W. B. Sauders Comp. (1999). Pp: 790.
14. Humason, G.L. Animal tissue techniques. Freeman, W.H. (3th ed.) , San Francisco press. UAS. (1972). PP.641.
15. Ibarra, A.; Julien C.; Marc R.; Amparo C. B.; Aurelie C. and Christophe R. Carnosic acid-rich rosemary (Rosmarinus officinalis L.) leaf extract limits weight gain and improves cholesterol levels and glycaemia in mice on a high-fat diet. British J. Nutr. (2011). 106: 1182–1189.
16. Al Sheyab, F. M.; Nizar A.; Lina S. and Rehan B. The Effect of Rosemary (Rosmarinus officinalis. L) Plant Extracts on the Immune Response and Lipid Profile in Mice. J. Bio. Life Sci. (2012). 3(1): 37-58.
17. Moreira M.R.; Ponce A.G.; dell Valle C.E. and Roura SI. (Inhibitory parameters of essential oils to reduce a foodborne pathogen. LWT, 2005). 38: 565-570.
18. Bulbul A.; Bulbul T.; Biricik H.; Yesilbag D. and Gezen S.S. effect of various levels of rosemary and oregano volatile oil mixture on oxidative stress parameters in quails. African Journal of Biotechnology (2012). 11: 1800-1805.
19. Afonso M.S.; De O Silva AM.; Carvalho E.B.; Rivelli D.P. and Barros S.B. Phenolic compounds from Rosemary (Rosmarinus *officinalis* L) attenuate oxidative stress and reduce blood cholesterol concentration diet-induced hypercholesterolemic rats. J. Nutr. Meta. (2013). 10: 10-19.
20. Rafie H.; Soheila H. and Grant E. Rosmarinus *officinalis* (Rosemary): A Novel therapeutic Agent for antioxidant, antimicrobial, anti-cancer, antidiabetic, antidepressant, neuroprotective anti-inflammatory and anti-obesity Treatment. J. Herb Med. (2017). 3(2): 8-14.
21. Ibarra A.; Cases J.; Roller M.; Chiralt B.A. and Coussaert A. Carnosic acid-rich rosemary (Rosmarinus *officinalis* L.) leaf extract limits weight gain and improves cholesterol levels and glycaemia in mice on a high-fat diet. British J. Nutr. (2011). 106: 1182-1189.
22. Cui L.; Kim MO.; Seo J.H.; Kim IS. and Kim N.Y. (Abietane diterpenoids of Rosmarinus *officinalis* and their diacylglycerol acyltransferase-inhibitory activity Food Chemistry 2012). 132: 1775-1780.