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THE EFFECT OF RHUBARB EXTRACTS ON OXIDATIVE STRESS IN WISTAR MALE RATS

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ABSTRACT

Antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) are important for human defense and oxidative stress .The present study is aimed to examine the extract of rhubarb on antioxidant enzyme and histological changes of liver in rats received high cholesterol diets. Extraction of fresh leaves of rhubarb was carried by maceration process and ethanol 70% was applied as a solvent and. The crude extract was collected was for study. Doses of 500, 666 and 1000 mg/kg BW was used in animal study. Sixty Wistar male adult rats (180-250 gram) divided in 5 groups. The first group (negative control group) received and distilled water. The second group received high fat diet (%2 cholesterol and 2.5% acid colic) for 8 weeks. The prevention group 1, 2 and 3 received high fat diet and leaf extract at doses of 500, 666 and 1000 mg per kg body weight respectively orally for 8 weeks. In the last day all animals have anesthetized, serum samples were prepared for biochemical tests and tissue samples taken from livers for histology studies. Data analyzed using One-way ANOVA and $P<0.05$ was recognized significant. Tissue levels of SOD have shown significant decrease in the positive group compared with the negative control group $P<0.5$.There was a dose depended increase in treatment groups compare to positive control $P<0.5$. Tissue levels of Catalase have shown significant decrease in the positive group compared with the negative control group $P<0.5$.There

was a dose depended increase in treatment groups compare to positive control P<0.5. Tissue levels of NO have shown significant increase in the positive group compared with the negative control group P<0.5.There was a dose depended decrease in treatments group compare to positive control P<0.5. Furthermore, histological examinations of liver showed the fatty liver in positive control group; however liver histology in treatment groups at maximum dose was normal compare to positive control. The extract of rhubarb at doses of 666 and 1000mg/kg BW increased the antioxidant enzyme in rats fed high cholesterol diet and no effect on other biochemical parameters. A significant decrease NO concentration was reported in treatments groups.

Keywords: Liver, Rat, Rhubarb, Oxidative Stress

INTRODUCTION

Hypercholesterolemia is the main risk factor of cardiovascular diseases such as atherosclerosis, myocardial infarction, stroke and cerebrovascular diseases which some of these diseases is the cause of death in industrial countries by free radical generation **[1]**. Free radicals were induced by different method in life. Normal levels of cholesterol and lipids could reduce the cardiovascular risk and be a key role in reducing the cardiovascular mortality **[2]**. Cholesterol-lowering drugs have been used for many decades but presence of side has also been reported. Synthetic drug could be interfering with many biochemical substances in human metabolism **[3]**. Therefore, a natural source with less than side effects is essential for treatment of disease especially hypercholesterolemia in new societies.

Plants are the best new drug sources and usage of them has a long history for therapeutic practice. In the past, people were largely dependent on plants and even today use of medicinal plants has been generalized which many reports reveals important effects of natural drugs especially medicinal plants on reducing triglyceride and cholesterol levels. The value of medicinal plants depends on their phytochemical compounds and they can affect some specific physiologic actions in human. Some phytochemical substances have potential in health improving. Some of the most important bioactive present in plants is including tannins, alkaloids, coumarin, saponins, flavonoids, steroids, anthraquinone, the sterols and terpenes **[4]**. Rhubarb (Rheum ribesformicum L) is a perennial plant belongs to the Polygonaceae family. It used for treatment of constipation, inflammation and

cancer in many years ago. Culinary rhubarb is used as a vegetable and is applied in pies, tarts and sauces. Rhubarb is rich source of phenols compounds, anthocyanin, anthracene derivatives, anthraquinone, emodin and Cyanidin 3-glucoside with antioxidant potential.

The objectives of this study was to determine the protective effect of Rhubarb against hypercholesterolemia which induced by high level of cholesterol and acid colic diet in rats.

METHODS AND MATERIALS

Fresh leaves of rhubarb were collected form Yasuj Iran. The leaves were dried and ground into a fine powder. Extraction was carried out in room temperature for 3 days by maceration process and ethanol 70% was applied as a solvent. The supernatant which concentrated by rotary evaporator (Hyedolph model 4000; Germany) at 40^0C, the crude extract was kept in a refrigerator for further studies. Doses of 500, 666 and 1000 mg/kg BW was used in animal study **.**Six Wistar male rats (180 – 250 gram) were obtained from the animal house at Shiraz University of Medical Sciences. The rats were divided into 5 groups and each group has 12 rats. The negative control group fed with compressed food and distilled water. The positive control group received high fat diet (%2 cholesterol and 2.5% acid colic) and distilled water for 8 weeks. The prevention group 1 received high fat diet and leaf extract of rhubarb at doses of 500 mg per kg body weight (by oral gavage) for 8 weeks.

The prevention group received high fat diet and leaf extract of rhubarb at doses 666 mg per kg body weight (by oral gavage) for 8 weeks. The prevention group 3 received high fat diet, with leaf extract of rhubarb at doses of 1000 mg per kg body weight (by oral gavage) for 8 weeks. After the last treatment animals were anesthetized with ether, liver was isolated, weighed and divided two portion one for histopathological study and another for antioxidant determination. For enzymatic bioassay homogenized of liver with phosphate buffered saline (PBS, pH 7.4) (1:5 W/V) in an Ultra-Turrax homogenizer (IKA Labortechnik, Staufen, Germany) was prepared. The homogenate was then centrifuged at 5,000 rpm for 20 min. at 4°C and supernatants were colleted for assessment of oxidative stress.

Assay of catalase (CAT) activity

CAT activity was estimated as the decomposition rate of hydrogen peroxide (H2O2) according to Wang et.al, 2001 **[5]**. Assay of SOD activity was based on the inhibition of pyrogallol auto-oxidation **[6]**.

Measurement of nitrite (an index of nitric oxide, NO)

NO is quickly oxidized to nitrite and nitrate, therefore, nitrite values can reveal NO synthesis, and may be measured colorimetrically using Griess reagent **[7]**. For histopathological study the liver of each rat was isolated and store in 10 % formalin and prepared hemaematoxoylin-eosin slide for histopathology study with light microscope.

Data Analysis

Statistical analysis was carried out by One-way ANOVA followed by Tukey's multiple comparison. Data analyzed using SPSS version 13. All results are expressed as mean ± standard deviation (SD). p <0.05 was considered as the significance level.

RESULTS AND DISCUSSION

Results

Tissue levels of NO have shown significant increase in the positive group compared with the negative control group P<0.5.There was a dose depended decrease in treatments group compare to positive control P<0.5. There was no significant difference between last treatment and negative control. Tissue levels of SOD have shown significant decrease in the positive group compared with the negative control group P<0.5.There was a dose depended increase in treatment groups compare to positive control P<0.5. There was no significant difference between last treatment and negative control. Tissue levels of Catalase have shown significant decrease in the positive group compared with the negative control group P<0.5.There was a dose depended increase in treatment groups compare to positive control P<0.5.

There was no significant difference between last treatment and negative control. Histological examinations of liver showed the fatty liver in positive control group; however liver histology in treatment groups at maximum dose was normal compare to positive control. The microscopic investigation showed that hypercholesterolemia caused histopathological changes in liver of rats. It observed vacuolization in hepatocytes cytoplasm, necrotic changes in parenchyma, and fatty liver cells were evident in rats exposed to hypercholesteroleemia alone (group II), and these lesions were either absent or negligible in rats which received leaves of rhubarb concomitantly (group treatment 4 and 5).

Table 1: Effects of Hydro – alcohol *Rheum ribes* extract on Antioxidant enzymes in hypercholesterolemia rats

Groups	Cat	SOD	NO
Negative control	**5.9 ±0.2**	**4.2 ±0.14**	**44 ±2.5**
Positive control	**2.9 ±0.15** [a]	**1.9 ±0.14** [a]	**75 ±3.3** [a]

Prevention 1	**3.6 ±0.21**	**2.3 ±0.21**	**69 ±3.1**
Prevention 2	**4.2 ±0.12**	**3.4 ±0.22**	**61 ±3.4**
Prevention 3	**4.8 ±0.34 [b]**	**3.9 ±031 [b]**	**53 ±2.9 [b]**

Units: NO nmol/ mg protein, SOD U/ mg protein and CAT U/ mg protein
[a] Significantly different from Negative control group; [b] Significantly different from Positive control; **P < 0.001; Values are expressed as mean ± SD of 12 animals per groups

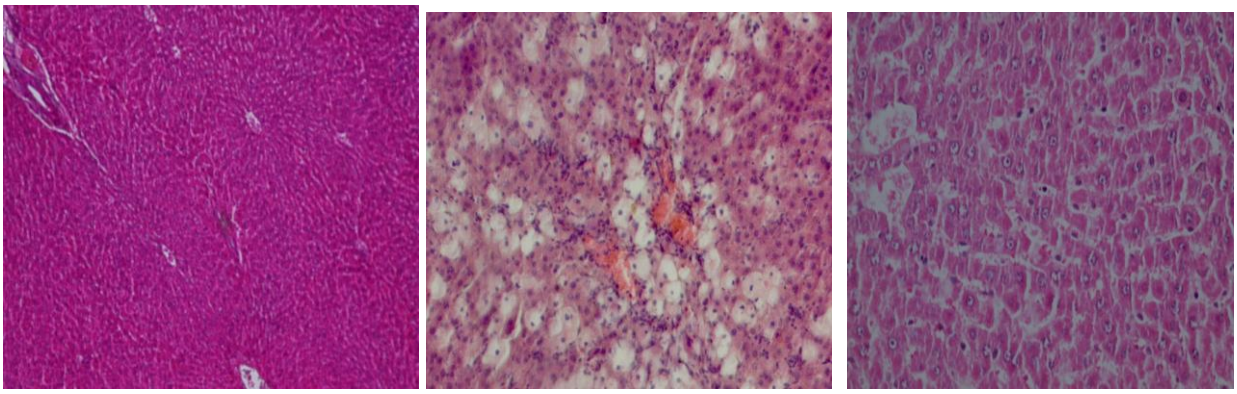

Figure 1: Light microscopy of liver microscopic tissue in different groups. (A) Normal architecture of liver (40x); (B) After hypercholesterolemia (high fat diet) vacuolization in hepatocytes, necrotic changes in parenchyma, (40x); and (C) hypercholesterolemia induced histopathologic changes in rat liver modified by Hydro – alcohol Rheum *ribes* treatment (40x)

DISCUSSION

Nutrition plays a key role in hypercholesterolemia disease. According to several studies hypercholesterolemic was induced by of saturated fatty acids and cholesterol which induce free radical generation **[8, 9]**. In the present study for inducing hypercholesterolemia, cholesterol (%2), colic acid (%0.25), wheat white flour (%0.10), sucrose (%0.10) and pig oil (%0.15) were added to rat foods. All biochemical enzymatic antioxidant activity increased follow hyperlipidemia. The results showed that lipid profile effected antioxidant enzyme such as CAT and SOD. This was demonstrated by significant reduction of liver tissues content of SOD activity and CAT activity as antioxidants parameters.

Super oxide dismutase (SOD) is an antioxidant enzyme which protect the cell and tissues from injuries that induced by Superoxide radicals. It cause decreased superoxide radicals via conversion to hydrogen peroxide. Superoxide scavenging property of plant extract might be due to the existence of secondary metabolites such as total phenol and flavonoid compounds **[10]**. In the present study, high lipidemia made significant damage on liver tissue, which is evidenced by histopathology architecture. Catalase is an antioxidant enzyme which converts hydrogen peroxide to water. Levels

of SOD and CAT enzymes were decreased by high fat diet, thus transforming the tissue to the peroxidative damage.

In the present research, the tissuue nitrate level was significantly increased by inoculation of rats with high dose of fat. These results were in concurrence with a previous study reported that of the activity iNOS is unregulated in high lipiemia related with extreme creation of NO. Additionally another study confirmed that the increase nitrate level is marker for the inflammation **[11]**. However treatment with extract induced the level of antioxidant enzyme in prevention 2 and 3 groups compare with positive control group. Therefore, the minimum dose of plant extract (prevention 1 group) could not significantly increase of antioxidant enzyme and NO level.

The histopathology examination was confirmed the antioxidant activities in positive and high dose of plant extract. In high dose of plant extract the damage in hepatocellular which induced by hyperlipidemia was decreased that reveals the plant extract protect liver from hyperlipidemia. Many studies have shown **[12]** the positive effect of antioxidants and also medicinal plants with antioxidant properties in reduction of oxidative stress. Rhubarb extracts rich in flavonoid compounds such as quercetin is a new alternate for hyperlipidemia treatment **[13]**. In a study, the effect of rhubarb stalk fiber on hypercholesterolemia in human was examined. In this study, administering rhubarb stalk powder daily for 4 weeks could significantly reduce oxidative stress **[14]**. In another study, hydro alcoholic extract of rhubarb has reduced cholesterol in hypercholesterolemic rabbits compared with nicotinic acid. Another research has shown that rhubarb reduces serum cholesterol and cholesterol esters in liver and increases bile secretion in mice **[14]**. Another study has shown that rhubarb extracts reduced cholesterol and triglyceride induced by hypothyroidism. The present research results revealed that all treatments have demonstrated antioxidant activity by SOD and CAT assay. Antioxidant potential probably due to the presence of total phenol and flavonoid compounds. The antioxidant activity of medicinal plants could be associated to the level of their phenolic compounds.

CONCLUSION

The extract of rhubarb at doses of 666 and 1000 mg /kg BW decrease antioxidant activity in rats fed high cholesterol diet. A significant decrease in NO and significant increase antioxidant activity and was reported in treatments groups. Enzyme markers and

histopathological results of liver tissue revealed that *plant extract* had beneficial effects on the liver with hyper lipidemia.

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REFERENCES

[1] Maruthappan VG, Shree KS. Blood cholesterol lowering effect of adenantherapavonina seed extract on atherogenic diet induced hyperlipidemia rats. *Ijpsr*, 2010; 1(7).

[2] Elsik M, Krum H. Hypertension: How long to go? Australian Prescriber, 2007; 30(1): 6.

[3] Gholamhoseinian A, Shahouzehi B, et al. "Inhibitory Activity of Some Plant Methanol Extracts on 3-Hydroxy-3-Methylglutaryl Coenzyme a Reductase." International Journal of Pharmacology, 2010; 6(5): 705-711.

[4] Hanna K. Examination of antibacterial and antifungal activity of selected non-antibiotic products. Acta Pol Drug Res, 2008; 65: 779-782.

[5] Wang Y, Oberley LW, Murhhammer DW. Evidence of oxidative stress following the viral infection of two Lepidopteran insect cell lines. Free Rad Biol Med 2001; 31: 1448-1455.

[6] Marklund S.L. Product of extracellularsuperoxide dismutase catalysis, FEBS Lett. 1985, 184(2): 237-239.

[7] Kleinbongard P, Rasaf T, Dejam A, et al. Griess method for nitrite measurement of aqueous and protein containing sample. Meth Enzymol 2002, 359: 158-68. 17.

[8] Caggiula A W, Mustad V A. Effects of dietary fat and fatty acids on coronary artery disease risk and total and lipoprotein cholesterol concentrations: epidemiology studies. Am. J. Clin. Nutr. 1997; 65: 1597S–1610S.

[9] Khan J, Mohamad M, Anisah Froemming G R, Islam M N. High Fat Diet Increases Uptake of Particles by the Peyer's Patches of Ileum in Rats. Inte. Medical J., 2013; 20(4): 432-34.

[10] Mirzaei A, Mirzaei M, Khosravani S A, Salehpour Z. Radical Scavenging Potential of Iranian Quercus Brantii and Juglans Regia. Life Science Journal 2013; 10(7s): 1246-50.

[11] Lundberg, J.O, M, Herulf, M, Olesen J, Bohr C, Tysk N.P Wiklund E, Morcos,

P.M, Hellstrom E, Weitzberg and G, Jarnerot. Increased nitric oxide production in collagenous and lymphocytic colitis, Eur. J. Clin. Invest. 27(10): 869-871.

[12] Fallah Huseini H, Larijani B, Fakhrzadeh H, et al. The efficacy of silymarin onhypercholrsterolemic type II diabetic patients. Iranian Journal of Diabetes and Lipid Disorders, 2004; 3: 2, 201-206.

[13] Fatma T, Çiğdem A K. Anthraquinones and Flavonoids from Rheum ribes. Ankara Ecz. Fak. Derg, 2003; 32 (1): 31-35.

[14] Goel V, Cheema SK, Agellon LB, Orikul B, Basu TK. Dietary rhubarb (Rheum rhaponticum) stalk fiber stimulates cholesterol7alpha-hydroxylase gene expression and bile acid excertion in cholesterol-fed C57BL/6J mice, Br J Nutr, 1999; 81: 65-71.

[15] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem, 1979; 95: 351-358.

[16] Benzie I F, Strain J.J. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power the FRAP Assay. Anal Biochem, 1996; 239: 70-76.

[17] AL-Khateeb A.R, Al-Talib H, Mohd M S, Yusof Z, Zilfalil B A. The Role of Lipid Lowering Therapy in the Achievement of Therapeutic Goal among Malaysian Hyperlipidemic Patients. International Medical Journal, 2013; 20(3): 272-75.

[18] Manal F. I; Mohamed Z G, Mohamed A H. Study of the hypolipidemic properties of pectin, garlic and ginseng in hypercholesterolemic rabbits. Pharmacol. Res., 1999; 39: 157-166.