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The effect of rhubarb extracts on lipid profile and oxidative stress in wistar male rats

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ABSTRACT

Normal blood lipids concentration has a key role in reducing of cardiovascular mortality .The present study is aimed to examine the extract of rhubarb on lipid profile 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. No significant difference was seen in triglyceride, LDL cholesterol, blood urea nitrogen, ureic acid, glucose, alanine transferase, asparat transferase and alkaline phosphatase in treatment group compare to control group. However, the serum concentration of total cholesterol has significant difference in prevention 2 and 3 compared with the positive control group. A decrease in serum levels of MDA and increase in antioxidant activity have shown in the prevention groups compared with the negative control. 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 decreases the total cholesterol in rats fed high cholesterol diet and no effect on other biochemical parameters. A significant increase in antioxidant activity was reported in treatments groups.

Keywords: cholesterol, Lipid profile, 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 are the cause of death in industrial countries). These diseases are diagnosed through natural increasing of lipids (triglycerides and cholesterol) and lipoprotein in blood [1]. 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 high side effects such as myopathy, liver damage and Leukopenia has also been reported. Synthetic drug could be interfere with many biochemical substances in human metabolism and some of them have not much been successful in medicine practice [3]. Therefore, find other alternate from 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 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.

MATERIALS AND METHODS

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 were 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, five ml blood was collected by heart puncture from each animal. Serum was separated using centrifuge at 2000 rpm for 10 min at -4˚C and maintained at -20˚C until test. The total cholesterol and HDL-cholesterol, LDL-cholesterol and triglyceride, blood sugar, blood urea, blood uric acid and aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), measured by auto-analyzer (RA 1000-USA). Analyses were performed by local Pars Azemon local company kits.

The level of lipid peroxidation or Malondialdehyde (MDA) was estimated by the thiobarbituric acid (TBA) method (Benzie et. al .1996). Antioxidant potential of total serum was estimated by ferric reducing antioxidant power (FRAP). The liver of each rat was isolated and store in 10 % formalin and prepared aematoxoylin-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

No significant difference was seen in glucose, blood urea, ureic acid, triglyceride, LDL cholesterol, and HDL cholesterol, alanine transferase, asparat transferase and alkaline phosphatase in treatment group compare to control group. However, the serum concentration of total cholesterol has significant difference in prevention 2 and 3

compared with the positive control group. Serum levels of MDA have shown significant decrease in the prevention groups compared with the negative control group P<0.5. There was a dose depended and significant decrease in treatments group was reported in FRAP activity compare to negative controlP<0.5. 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 fatyy 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 biochemical tests in hypercholesterolemia rats

Groups	Glucose (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
Negative control	110.2±24.90	38.76±2.81	2.38 ±.63
Positive control	118±34.62	39.5±1.64	2.9 ±.76
Prevention 1	109.33±39.96	39.56±3.27	2.46 ±.19
Prevention 2	110.5±34.8	37.05±2.24	2.4 ±.35
Prevention 3	112.16±35.94	37.46±3.87	2.56 ±.34

Values are expressed as mean ± SD of 12 animals per groups

Table 2: Effects of Hydro – alcohol Rheum *ribes* extract on lipids profile tests in hypercholesterolemia rats

Groups	Triglyceride (mg %)	Cholesterol (mg %)	HDL (mg %)	LDL (mg %)
Negative control	53.2±18.19	51.80±7.25	20.80±2.94	11.20±4.76
Positive control	70.80±19.84	66.20±3.34	22.40±2.50	25.15±4.3
Prevention 1	63.83±5.41	59.16±8.06	22.33±6.86	18.83±19.61
Prevention 2	60.83±13.71	* 51.83±7.83	24.83±5.87	*13.16±13.10
Prevention 3	* 56.12±3.2	* 50.66±9.70	26.33±4.17	*15.83±6.43

**Significant difference with positive control group. Values are expressed as mean ± SD of 12 animals per groups*

Table 3: Effects of Hydro – alcohol Rheum *ribes* extract on liver enzyme markers and oxidative stress in hypercholesterolemia rats

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	FRAP(µmol/L)	MDA(nmol/L)
Negative control	66±10.48	65.60±54.98	192.4±76	2390. ±311.54	.12± .32
Positive control	71±19.93	69.60±39.23	60.6±157.37	2347.24±261.09	.66 ±.11
Prevention 1	76.66±12.30	72.83±60.87	52.33±203.72	*2401.71±214.79	*.43 ±.06
Prevention 2	81±34.61	76±72.94	51.50±336.41	*2432.76±102.50	*.39 ±.09
Prevention 3	71.66±8.54	74 ±23.13	59.16±213.28	*2484.86±149.14	*.34 ±.07

*Values are expressed as mean ± SD of 12 animals per groups. Aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), ferric reducing antioxidant power (FRAP) and Malondialdehyde (MDA. * Significant difference with positive control group.*

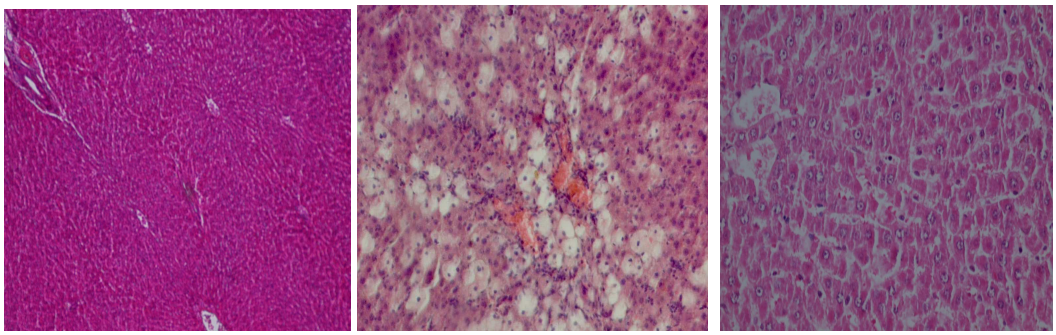

Fig. 1: Light microscopy of liver microscopic tissue in different groups. (A) Normal architecture of liver (40x); (B) After hypercholesterolemia (high fat diet) vacuolization in hepatocytes, necrotic changes in parenchyma, (40x); and (C) hypercholesterolemia induced histopathologic changes in rat liver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

Today it has been proved that nutrition plays a key role in etiology of hypercholesterolemia. Several animal and human studies have approved hypercholesterolemic properties of saturated fatty acids and cholesterol [5]. High cholesterol diet is often used to increase tissue or serum cholesterol in order to measure metabolic disorders associated with hypercholesterolemia in animal models. 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. Most of biochemical tests except lipid, antioxidant activity and MDA in treatment groups were not different to control groups. The results showed that total cholesterol, TG and LDL cholesterol had significant differences in prevention 2 and 3 groups with positive control group. Therefore, the minimum dose of plant extract (prevention 1 group) could not significantly reduce total cholesterol and TG and LDL cholesterol in rat fed by high cholesterol diet. Many studies [6] have shown the positive effect of antioxidants and also medicinal plants with antioxidant properties in reduction of blood sugar and lipid. Rhubarb extracts rich in flavonoid compounds such as quercetin is a new alternate for hyperlipidemia treatment [7].

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 total cholesterol and LDL cholesterol (Goel et. al. 1997). In another study, hydro alcoholic extract of rhubarb has reduced cholesterol in hypercholesterolemic rabbits compared with nicotinic acid. Also there has not known mechanism for effect of rhubarb hydroalcoholic extract on reduction of blood cholesterol in exprimental animals. Another research has shown that rhubarb reduces serum cholesterol and cholesterol esters in liver and increases bile secretion in mice [8]. Another study has shown that rhubarb extracts reduced cholesterol and triglyceride induced by hypothyroidism and possibly could stop the side effects of hypercholesterolemia and hypertriglyceridemia and other cardiovascular disorders in hypothyroid patients. But in prevention 1 group, the minimum dose could not significantly reduce serum cholesterol which may be due to lower levels of effective compounds in the extract. The level of serum MDA has shown significant difference between the positive control group and prevention groups while they had not significant difference with negative control group. MDA is lipid peroxidation product and could inactivate membrane carriers by forming internal and intermolecular bridges. Cholesterol-rich diets make extensive changes in antioxidant defensive mechanisms. Reported that plasma MDA has been increased in rabbits fed with cholesterol-rich diets. Also it was reported similar results in their studies.

However, MDA in rats received cholesterol-rich diets are two time higher than negative control and it shows that hypercholesterolemia increases lipid peroxidation [9]. In the present study, there was a significant difference between serum MDA in positive control (receiving cholesterol-rich food) and negative control groups. Therefore, the results show that leaf extract of rhubarb at doses of 666 and 1000 mg/kg body weight reduce total cholesterol and LDL cholesterol in rats received cholesterol diet. However at high doses of rhubarb extract (1000 g/kg body weight) significantly reduce TG serum in these animals. The present research results revealed that all treatments have demonstrated antioxidant activity by FRAP 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 decreases the total cholesterol, LDLcholestero and TG in rats fed high cholesterol diet and no effect on other biochemical parameters. A significant decrease in MDA and significant increase antioxidant activity and was reported in treatments groups

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