Regulating Effect of Laminaria Japonica Powder on Serum Lipids of Hyperlipidemia in Rats*

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ABSTRACT Objective: To investigate the effect and mechanism of Laminaria japonica (Laminariales, Phaeophyta) on serum lipid of hyperlipidemia in rats. Methods: Forty healthy female Wistar rats were used to establish hyperlipidemia models by feeding fat-rich forage and the kelp powder was applied as raw materials for potential marine drugs. The levels of serum lipid including the triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL) were detected by biochemical assay. The concentrations of lipid perioxide malondialdehyde (MDA) and nitric oxide (NO) were respectively measured by thiobarbituric acid assay and nitrate reductase assay. The activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) were respectively determined by xanthinoxidase assay and chemical colormetry. Results: After treated with kelp feeds, the serum levels of TG, TC and LDL decreased while HDL increased significantly than those in model group rats ($P<0.05$). After treatment with kelp, the levels of MDA and NO in serum and hepatic tissue were lower than those in the model group rats ($P<0.05$), while the activities of SOD and GSH-PX were significantly higher than those in the model group rats ($P<0.05$). Conclusion: L.japonica may interference the metabolism of TG, TC, LDL and HDL and by increasing the activities of SOD and GSH-PX to reduce the levels of MDA and NO to regulate the levels of serum lipids.

Key words: Laminaria japonica; Hyperlipidemia; SOD; GSH-PX; Rats

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Introduction

Hyperlipidemia is one kind of common and frequently-occurring syndromes in metabolic diseases and closely related to cardiovascular and cerebrovascular diseases [1]. Reducing blood lipids levels is an effective methods of preventing and treating cardiovascular and cerebrovascular diseases. Currently, the fibrates[2], stains[3] and methanol extract [4] drugs used clinically could regulate lipid metabolism to reduce serum values of triglyceride (TG) and total cholesterol (TC). As known, superoxide dismutase (SOD) is a enzyme to seize the free radicals and can guard the cell against damaging by getting rid of superoxide anion radical and reducing the production of lipid peroxides (LPO) [5]. Glutathione peroxidase (GSH-PX) is a kind of significant catalyze enzyme which was extensively existed inside of the body, SOD and GSH-Px are a group of important enzyme in cleaning oxygen radicals, when the activity descending can cause the mass accumulation of oxygen radicals metabolites in the body. Malondialdehyde (MDA) is the last metabolite of lipid peroxidation which produced in the metabolization of oxygen radical in organism, which can reflex the content of oxygen radicals' metabolites by the MDA gallery level. Kelp belongs to Laminaria japonica, containing more than 40 kinds of functional components which composed mainly kelp polysaccharide, Laminine, mannitol, vitamin, amino acid and many trace elements[6]. Many physiologic functions of Kelp closely related to the biological activity polysaccharides, Laminaria japonica polysaccharide (LJPS) were abstraction from Laminaria japonica, which mainly composed by alginic acid and brown algae [7], could increase the immunological activity of organism and exhibited anti-aging and anti-tumor effects [8], nonetheless, few research on the reducing blood lipids and antioxidation were reported [9]. This study was to investigate the regulating effects on blood lipids, and explore the mechanism of the powder on hyperlipidemia rats by feeding hyperlipemia rats with the kelp powder.

1 Materials and methods

1.1 Animal models

Forty healthy female Wistar rats, weighted 150-170g, SPF grade (SCXK (LU) 200900100), were purchased from Experiment Animal Center of Qingdao Drug Inspection Institute. The local legislation for ethics of experiment on animals and guidelines for the care and use of laboratory animals were followed in all animal procedures. All animals were acclimatized for 7 days and allowed free access to food and water in a temperature and humidity-controlled housing with natural illumination. The room temperature was maintained at (23± 2) ℃. The blood sample (0.5ml) was collected from tail vein and separated serum for determining the normal values of blood lipids. Ten (n=10) of these experimental animals were randomized as control group fed basically with general forage, and the rest 30 rats were fed with fat-rich forage (which

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was composed of general forage 59%, sucrose 20%, pig fat oil 10%, egg yolk powder 10% and cholic acid sodium 1%, well-mixed and made up granular-like forage at room temperature) for 4 weeks to established hyperlipidemia models [10]. At the end of the fourth week, the rat blood was sampled (0.5mL) from tail vein and was separated serum for determination of blood lipid levels. If the triglyceride (TG) >1.5mmol·L^{-1}, total cholesterol (TC) >1.5mmol·L^{-1}, low-density lipoprotein (LDL)>1.0mmol·L^{-1} and high-density lipoprotein (HDL) <0.2 mmol·L^{-1}, the experimental rats were considered as successful hyperlipidemia rat models. Of the 30 rats, 10 rats were excluded due to unsuccessful reaching in the required blood lipids standard; Therefore, the remaining 20 rat models were applied and divided randomly into model (hyperlipidemia) group and treatment (L.japonica) group, each consisting 10 rats respectively

1.2 Interfering methods

The good variety L.japonica powder "Zhongke No.1" were used as feeder for the rats, which was originally selected and harvested from Rongcheng, Shandong. The main components are dietary fiber 26.1%, protein 8.5%, lipid 0.39%, the total amino acid 10.49 mg·$100g^{-1}$, Vitamin A 273 μg·$100g^{-1}$, Vitamin C 3 μg·$100g^{-1}$. The kelp was cut into granular powder, the general forage 90% and the kelp powder 10% were blend and mixed into lump forage, and then aired for the feed. The control group and model (hyperlipidemia) group were fed forage for two weeks. The treat group were fed with the kelp forage for two weeks, each rats consumed about 2g of kelp (equal to 10g·kg^{-1} body weight) per day.

1.3 Sample collection

Serum: At the end of this experiment, all rats were forbid food for 12 h, and then 4 ml blood was collected from eye artery. The blood sample was centrifugalize for 10 minutes at 4000 rpm to separate the serum and was stored at 4 ℃ before the used.

Hepatic tissue: At the end of this experiment, cervical dislocation was use to sacrifice animals and 0.2 g hepatic tissue was collected immediately. The rudimental blood was washed with normal saline and grinded fully on -4℃ ice bath. The hepatic tissue sample was centrifugalize for 10 minutes at 12000 rpm to separate the supernatant and were stored at -20℃before the used.

1.4 Examination indexes

The levels of TG, TC, LDL and HDL was detected by the biochemical assay (the kits were provided by DiaSys Co. Ltd.). Before the determination, samples were redissolved at room temperature and were centrifugalize for the collection of supernatant. Usually, about 100 μl of serum was collected and putting into an automatic chemistry analyzer (Beckman CX-7, USA) for the detection. The sensitivity is mmol·L^{-1}.

The values of malondialdehyde (MDA) and nitric oxide (NO) were detected respectively by thiobarbituric acid and nitratase reduase method with the kits (Jiancheng Institute of Biomedical Technology, Nanjing China). Standardization were conducted on the ultraviolet spectrophotometer (Bechmann DU640, USA) and the selected wavelength were 532nm (MDA) and 550nm (NO) respectively. The sensitivity is nmol·L^{-1}(MDA) and μmol·L^{-1} (NO).

The activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) were detected respectively by xanthinoxidase method and chemical colorimetry with the kits (same as MDA and NO). Standardization were conducted on the ultraviolet spectrophotometer and the selected wavelength were 550nm (SOD) and 412nm (GSH-PX) respectively. The sensitivity is U·ml^{-1}.

1.5 Statistical Analysis

SPSS17.0 software was used for statistical analysis. Data were expressed as mean ± standard difference ($\bar{x}$± s). Multi-group comparison was made by analysis of variance (ANOVA) and Student's test, and two-group comparison by t-test. Values were considered to be significant when P is less than 0.05.

2 Results

2.1 The values of TG, TC, LDL and HDL

Before modeled, the normal values of TG, TC, LDL and HDL in serum were 0.97 ± 0.22, 1.29 ± 021, 1.01 ± 0.07 and 0.30± 0.03 mmol·L^{-1}, respectively in 40 rats. After modeled, the values of TG, TC, LDL and HDL in serum were 2.20 ± 0.17, 2.25± 0.19, 1.26± 0.13 and 0.28± 0.04 ·L^{-1}, respectively in 20 successful model rats, which were significantly different with those before modeled and those of control group (P<0.05). After treatment, the levels of TG, TC and LDL in serum of model group were significantly higher while HDL was lower than those of control group (P<0.05). The values of TG, TC and LDL in serum of treatment group were significantly lower, but HDL higher than those of model group (P<0.05, Table 1).

Table 1 The levels of TG, TC, LDL and HDL in serum after treatment (mmol ·L^{-1})

Groups	n	TG	TC	LDL	HDL
Control group	10	0.89± 0.18	1.43± 0.10	0.93± 0.19	0.30± 0.08
Model group	10	1.83± 0.29[a]	1.93± 0.20[a]	1.21± 0.12[a]	0.17± 0.09[a]
Treated group	10	0.97± 0.26[b]	1.43± 0.18[b]	0.93± 0.17[b]	0.30± 0.13[b]

a Compared with control group, P<0.05; b Compared with model group, P<0.05

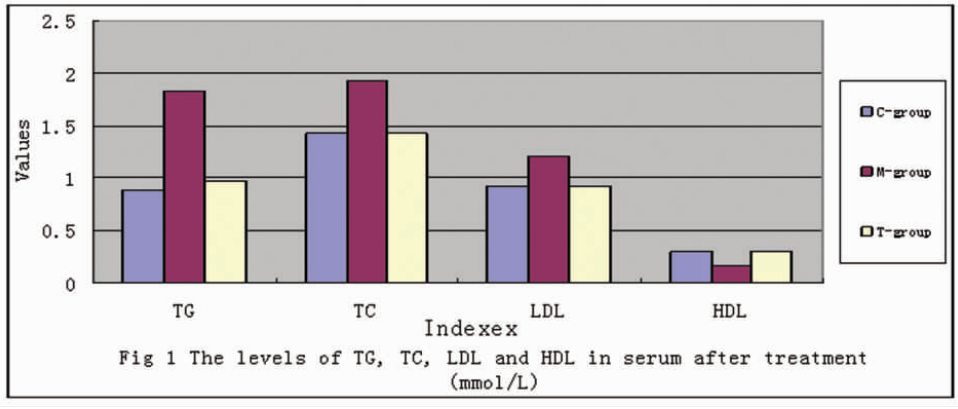

Fig.1 The levels of TG, TC, LDL and HDL in serum after treatment (mmol·L^{-1})

2.2 The values of MDA and NO

It is showed that the values of MDA and NO in serum of model group were significantly higher than those of control group (P<0.05). The values of MDA and NO in serum of treatment group were significantly lower than those of model group (P<0.05). The variations of MDA and NO activities in hepatic tissue are similar to that in the serum (Table 2).

Table 2 The levels of MDA and NO after treatment

Groups	n	MDA (nmol·L^{-1})		NO (μmol·L^{-1})	
		Serum	Hepatic tissue	Serum	Hepatic tissue
Control group	10	6.73± 0.83	5.58± 0.37	20.94± 2.91	21.45± 3.68
Model group	10	9.69± 0.89[a]	6.81± 0.78[a]	26.10± 2.33[a]	26.14± 3.72[a]
Treated group	10	6.19± 0.82[b]	5.71± 0.35[b]	21.28± 2.49[b]	21.46± 3.28[b]

Note: a P<0.05 vs control group; b P<0.05 vs model group

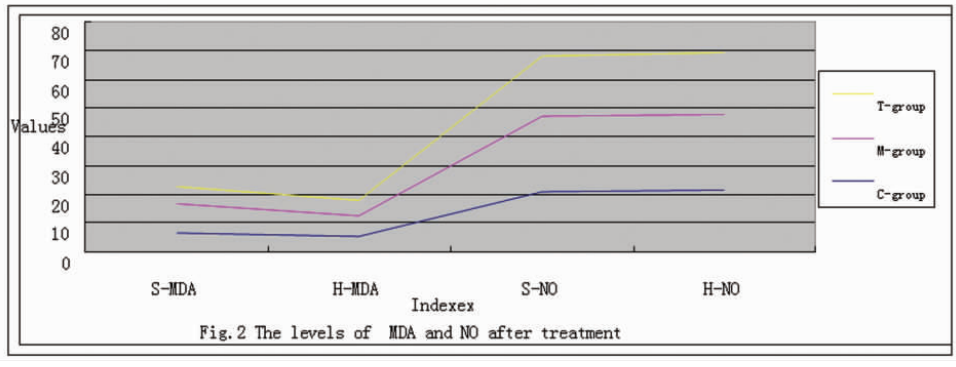

Fig.2 The levels of MDA and NO after treatment

2.3 The activities of SOD and GSH-PX

It was indicated that the activities of SOD and GSH-PX in serum of model group were significantly lower than those of control group (P<0.05). The activities of SOD and GSH-PX in serum of treatment group were significantly higher than those of model group (P<0.05). The variations of SOD and GSH-PX activities in hepatic tissue are similar to that in the serum.(Table 3).

Table 3 The activities of SOD and GSH-PX after treatment

Groups	n	SOD (U·ml^{-1})		GSH-PX (U·ml^{-1})	
		Serum	Hepatic tissue	Serum	Hepatic tissue
Control group	10	241.95± 9.95	609.89± 49.30	291.54± 8.26	429.15± 28.12
Model group	10	219.28± 7.79[a]	502.28± 53.48[a]	255.37± 7.95[a]	345.77± 22.56[a]
Treated group	10	247.36± 7.70[b]	588.86± 40.35[b]	286.96± 9.32[b]	394.65± 34.13[b]

Note:a P<0.05 vs control group; b P<0.05 vs model group

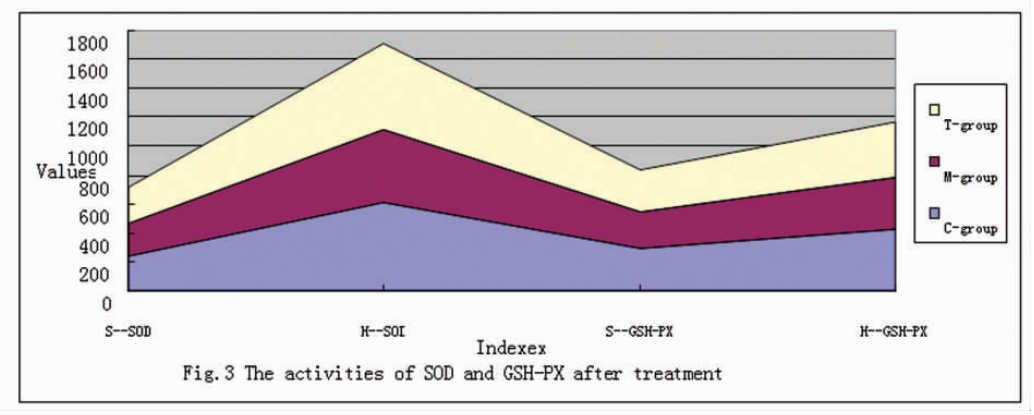

Fig.3 The activities of SOD and GSH-PX after treatment

3 Discussion

3.1 The regulating effect of L.japonica on blood lipids

Lipid metabolic disorders were generally considered as a crucial risk factor to the occurrence of hyperlipidemia [11]. It is therefore need to conduct the treatment of reducing TG, TC, LDL and increasing HDL to patients with lipid metabolic disorder patients [12], and control actively the other risk factors to reducing cardiovascular diseases caused by hyperlipidemia [13]. Clinical trials indicated that statins drugs could signficantly decrease the serum values of LDL and redcuce about 30% for the occurrence and mortality of cardiovascular diseases, while cause remarkably side effect as muscle pain with a lot of dosage [14-15].

In this experiment, after treatment by kelp, the serum levels of TG, TC and LDL declined remarkably while the HDL higher than those in the model group. The results indicated the kelp could also reduce the TG, TC and LDL values and increase HDL values. It is suggested that the kelp may influence TG, TC, LDL and HDL matebolism, and adjust blood lipid values by activating enzymatic regulation on lipoprotein in hepatic tissue.

3.2 The anti-oxidant effect of L.japonica

It is reported that L. japonica polysaccharide had obviously effects on anti-coagulation, spasmolysis, depolymerization, hypotensive, hypolipemic, reducing blood viscosity, expanding vessels and improving microcirculation in connection with cardiovascular and cerebrovascular disease [9]. Animal experiment indicated that Laminarina Japonica polysaccharides could directly get rid of peroxide ion free radicals (O_2-) and hydroxy free radicals (OH-) in vitro and ex vitro, and coul enhance antioxidant enzymes activities of serum and tissue in vitro[16].

Malondialdehyde (MDA) was the last substance during the lipid peroxidation, which could yield oxygen radical in organisms and accelerate oxygen radical generation [17]. Nitric oxide (NO) is a important signal and effective molecule of organism[18]. If NO was exceptional or the homeostasis in the system oxidize/ antioxidation, it would resulted in the consistency of oxygen radical abnormally high, aggravate oxygen radical pathologically and accelerate the cell senescence, finaly produced a premium on disease. This study found that the MDA and NO values in model control group increased than those in normal control groups. It is indicated that hyperlipermia could generate lipid peroxidation in rats, while the values of MDA and NO in simvastatin and the kelp treated groups were obviously decreased. It can be concluded that the simvastatin and the kelp exhibited effects of antioxidation to lighten lipid peroxidation and regulating lipoprotein metabolism caused by the hyperlipidemia.

Superoxide dismutase (SOD), a natural antioxidant enzyme catching free radical, could get rid of O_2- free radicals and guard the cell against demaging[19]. Glutathione peroxidase (GSH-PX) is eitherly a catalyze enzyme which was extensively existed, and protect the structure and function of epicyte, then interdiction the chain reaction of lipid peroxidation [20]. In the present experiment, the activities of SOD and GSH-PX in simvastatin and the kelp treatement groups were obviously rasised up than those in simvastatin group. It could be concluded that both the simvastatin and Laminaria can enhance antioxidase enzyme activities, and then reduce lipid peroxidation. GSH-PX could also reducted oxide decreased lipid peroxide injury to reduce MDA level. Therefore, Kelp could lighten various injury mediated by active oxygen species due to the lower activity of antioxidant enzymes.

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昆布(海带)对高脂血症大鼠血脂的调节作用和机制*

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摘要 **目的**:探讨昆布(海带)在实验性高脂血症大鼠中的降血脂作用和机制。**方法**:健康雌性 Wistar 大鼠 40 只,应用高脂饲料喂养方法建立高脂血症动物模型,海带粉饲料喂养干预治疗。生化法检测大鼠血清甘油三酯(TG)、总胆固醇(TC)、低密度脂蛋白(LDL)和高密度脂蛋白(HDL)水平。硫代巴比妥酸法和硝酸还原酶法分别检测脂质过氧化物丙二醛(MDA)和一氧化氮(NO)含量,黄嘌呤氧化酶法和化学比色法分别测定超氧化物歧化酶(SOD)和谷胱甘肽过氧化物酶(GSH-PX)活性。**结果**:经海带干预治疗后,动物血清 TG、TC 和 LDL 水平较模型组显著降低、HDL 水平显著升高($P<0.05$)。治疗组动物血清和肝组织 MDA 和 NO 水平显著低于、而 SOD 和 GSH-PX 活性均显著高于模型组($P<0.05$)。**结论**:海带可能影响 TG、TC、LDL 和 HDL 等组分的代谢,通过增强抗氧化 SOD 和 GSH-PX 的活性,降低体内 MDA 和 NO 的水平,发挥调节血脂水平的作用。

关键词:昆布(海带);高脂血症;超氧化物歧化酶;谷胱甘肽过氧化物酶;大鼠

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