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Evaluation of Hypolipidemic Effects of *LyciumBarbarum* (Goji berry) in a Murine Model

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Abstract

Lycium barbarum L. Solanaceae a traditional medicinal plant of China has been accorded a very high safety value. It has been known to possess various beneficial effects like reduction of blood glucose and serum lipids in alloxan-induced diabetes models, anti-ageing, immuno-modulating, anti-cancer, anti-fatigue, and male fertility-facilitatory actions. The present study is an attempt to explore the hypolipidemic effects of powdered *L. barbarum* in high fat diet-induced model of hyperlipidemia. Powdered L. barbarum fruit extract (250mg/kg and 500mg/kg) was administered to Wistar albino rats fed on a cholesterol-rich high-fat diet for 45 days. The effects were compared with the standard drug atorvastatin (10 mg/kg/day, oral). After 30 days of treatment, the lipid profile of the blood samples of the experimental rodents was evaluated.The data presented as mean±SEM were analysed using one-way ANOVA followed by Tukey's post hoc test. $P<0.05$ was considered as statistically significant. In the present study, L. barbarum powdered extract showed a significant reduction in the total cholesterol, triglycerides and very low density lipoprotein-cholesterol levels at both the doses employed ($p<0.05$). However, reduction in low density lipoprotein-cholesterol levels was significant ($p<0.05$) only at the dose of 500 mg/kg when compared to the standard drug group. The increase in high-density lipoprotein levels was significant only at 250 mg/kg. The data thus suggests the positive anti-hyperlipidemic activity of *L. barbarum* in high fat diet-induced hyperlipidemia in rats.

Keywords: Goji berry, hypolipidemic activity, high-fat diet, *Lyciumbarbarum*

1. Introduction

Hyperlipidemia is a major risk factor for coronary artery disease. Cardiovascular diseases have been reported to be the principle cause of death in advanced countries; recently encroaching into the developing world also [1]. The World Health Organization estimate of mortality reports that 12 million people worldwide die from cardiovascular diseases every year [2]. Atherosclerosis, a progressive multifactorial disease of arterial wall is a common culprit of cardiovascular disease [3, 4]. Deposition of cholesterol in the arterial wall is implicated in the pathogenesis of atherosclerosis [5]. Lipoproteins involved in this process include cholesterol carried by very low density lipoproteins (VLDL), remnant lipoproteins and low density lipoproteins (LDL) [6]. Currently used drugswhich include HMG-CoA Reductase inhibitors, bile acid sequestrants, fibric acid derivatives and nicotinic acid [7] are enlisted with various adverse effects like elevated hepatic transaminases, gastrointestinal disturbances, headache, myalgias and rash [8], and elevated values on liver function tests [9]. Hence the search for newer, safer and better drugs is ongoing. Drugs derived from indigenous plant sources might offer more advantages in terms of safety and efficacy.

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Wolf berries or Goji berries (*Lyciumbarbarum* L. Solanaceae) have a long tradition as food and medicinal plants in China and various other Asian countries. *L. barbarum* L. is a deciduous shrub growing up to one to three metres high. The leaves are lanceolate to ovate. The oblong, orange to dark red berries measure up to 2 cm and possess a bitter to sweet taste. The berries are eaten raw, drunk as a juice, wine or tea. They can also be processed to tinctures, powders and tablets. The recommended dosage of dried berries used as medicine varies between 5 and 12 g [10]. Fruit from *L. barbarum* L. has been claimed to possess a large variety of beneficial effects, such as reduction of blood glucose and serum lipids in alloxan-induced diabetes models, anti-ageing, immuno-modulating, anti-cancer, anti-fatigue and male fertility-facilitatory actions [11–16].

Goji berries contain polysaccharides, vitamin C, vitamin B complex, vitamin E, 18 amino acids including the eight essential amino acids which the body does not produce and around 21 **trace elements** including zinc, iron, copper, calcium, selenium, germanium sesquioxide and phosphorus [17]. Some constituents of *Lyciumbarbarum* fruits have been chemically investigated, especially *Lyciumbarbarum* polysaccharide (LBP) components. Five polysaccharides (glycoconjugates) (LbGp1-LbGp5) were isolated and structurally elucidated [18–20].

The various pharmacological actions of the berry fruit are mainly attributed to the presence of polysaccharides and flavonoids [17]. Though a reduction in serum lipids was reported in alloxan-induced diabetic models treated with fruit extracts of *L. barbarum*, literature research revealed no studies conducted in hyperlipidemia models. Hence this study was designed to evaluate the possible hypolipidemic effects of alcoholic extract of*L. barbarum* in high fat diet-induced murine model of hyperlipidemia.

2. Materials and Methods

2.1 Animals

Wistar albino rats of either sex, weighing 100-200 g, from our breeding stock were used for the study. Animals were housed in standard environmental conditions (22± 3°C, 55 ± 5% humidity and a 12 h light/ dark cycle as per CPCSEA guidelines) and fed with standard rodent diet (containing 4.15% fat, 22.15% protein, 4% carbohydrates (supplied by Amrut laboratory animal feed manufactured by Pranav Agro Industries Ltd., Sangli) and water *ad libitum*. The procedures were carried only after obtaining the approval of the Institutional Ethics Committee.

2.2 Preparation of the Cholesterol-rich High-fat Diet (HFD)

Hyperlipidemia was induced by feeding the rats with cholesterol-rich high-fat diet for 45 days. Deoxycholic acid (5 g) was mixed thoroughly with 700 g of powdered rat chow. Simultaneously cholesterol (5 g) was dissolved in 300 g warm coconut oil. The oil and cholesterol mixture was added slowly into the powdered mixture to obtain a soft, homogenous cake. This cholesterol-rich high-fat diet (HFD) was moulded into pellets of about 3 g each and was used to feed the animals [9].

2.3 Drugs and Chemicals

2.3.1 Preparation of study drug extract

*L. barbarum*fruits were obtained from Goji Berry Private Limited, Mumbai, India, and were dried in sunlight and powdered in an electrical blender. The powder (250 g) was extracted with 50% hydroalcoholic mixture (ethanol and water in 1:1 proportion) at room temperature for 24hr by cold maceration method [21]. It was then filtered with Whatman filter paper no 2. The filtrate was concentrated on heating mantle till a syrupy mass was obtained. This syrupy mass was again dried in hot air oven and kept under refrigeration. The percentage yield was found to be 5.2% w/w with respect to the initial dried fruit material. Thus the prepared fruit extractwas administered at the dose of 10 mg/kg/day and 20 mg/kg/day, per oral, suspended in gum acacia.

2.3.2 Standard drug

Atorvastatin (Lipitor, Pfizer) was administered at the dose of 10 mg/kg/day orally, suspended in gum acacia.

2.4 Study Procedure

The rats were randomly assigned to five groups of six rats each. They were fed on a high-fat diet for 45 days. The animals did not receive any treatment for the first

15 days. During the later 30 days, animals were treated with drug/vehicle. The feeding and treatment schedule was as follows:

Group I: Normal diet (for 45 days)
Group II: High-fat diet (for 45 days)
Group III: High-fat diet (for 45 days) plus atorvostatin 10 mg/kg/day (for the later 30 days)
Group IV: High-fat diet (for 45 days) plus *Lyciumbarbarum fruit* 10 mg/kg/day (for the later 30 days)
Group V: High-fat diet (for 45 days) plus *Lyciumbarbarum fruit* 20 mg /kg/day (for the later 30 days)

After 30 days of treatment, the rats were kept on fast overnight and blood samples were obtained by cardiac puncture of anaesthetised rats (using sodium pentobarbitone 50 mg/kg body weight, i.p. prepared in normal saline) and were sent for complete blood lipid profile.

2.5 Statistical Analysis

The data were presented as mean±SEM and analysed using one-way ANOVA followed byTukey's post hoc test. Statistical Package for the Social Sciences (SPSS) 17.0 version was used for the analysis of the data.

3. Results

As detailed in Table 1, administration of HFD significantly increased the total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and very low density lipoprotein-cholesterol (VLDL-C) levels ($p<0.05$) in the disease control (Group 2) when compared to normal rats.

The standard drug atorvastatin showed a decrease in TC, TG, LDL and VLDL levels, but statistical significance was obtained only with total cholesterol levels. There were no significant changes observed in the HDL-C levels.

When compared to the disease control, the *L.barbarum* extract-treated group showed a significant decrease in the TG levels for both the doses employed. Significant decrease in VLDL-C was observed only at the higher dose used (20 mg/kg) when compared to the disease control. A significant increase in HDL-C was observed for the lower dose. Though there was a dose-dependent decrease in TC and LDL-C levels, it was not statistically significant.

When compared to the standard drug atorvastatin, the test drug extract showed a significant decrease in the TG and VLDL-C levels at 500 mg/kg dose employed, but there was a statistically significant increase in the total cholesterol when compared to atorvastatin. There was a significant increase in HDL-C levels at the lower dose, but this increase was not significant at the higher dose.

4. Discussion

Lyciumbarbarum, a well-known traditional Chinese medicinal herb, possesses diverse biological activities and pharmacological functions including reducing blood glucose and serum lipids. It has long been used to treat diabetes mellitus and related hyperlipidemia [11, 22]. However, its pharmacological and chemical bases are not well understood. The purpose of this study was to investigate and evaluate the anti-hyperlipidemic

Table 1: Plasma lipid profile of rats fed on high-fat diet and treated with atorvastatin and Goji berry extract

Groups	TC	TG	HDL	LDL	VLDL
Normal diet	70.50±0.96	87.33±6.23	21.67±0.49	31.16±1.71	17.67±1.16
High-fat diet (HFD)	130.67±2.93*	187.00±6.98*	30.50±3.88	59.16±1.63*	36.83±1.34*
HFD+Atorvastatin	105.83±2.57*[a]	164.67±6.13*	30.00±1.18	43.13±2.88	32.70±1.27*
HFD+*L. barbarum* (10 mg/kg/day)	124.67±3.29*[b]	159.00±6.06*[a]	41.50±2.46*[a,b]	47.50±8.95	31.80±1.21*
HFD+*L. barbarum* (20 mg/kg/day)	126.50±4.39*[b]	125.50±7.58*[a,b]	34.50±1.34*	61.63±4.07*	25.10±1.52*[a,b]

All the values are expressed as mean±SD, where *$p<0.05$ when compared with normal diet,[a]($p<0.05$) when compared with high fat diet, [b]($p<0.05$) when compared with standard (atorvastatin)

properties of *L. barbarum* fruit extract in a high-fat diet-induced model to provide scientific evidence for the development of *Lyciumbarbarum* as a potential natural oral hypolipidemic agent.

In the present study, hyperlipidemia in rats was well established by feeding them with HFD [9]. There was a significant increase in all the lipoproteins as well as triglyceride levels (Table 1). However, absurdly, even HDL-C levels were elevated in the entire group that was fed HFD. The confounding factor could possibly be the coconut oil component of HFD. There is evidence of coconut oil raising HDL-C levels in rodents [23], non human primates [24] and in humans also [25]. Coconut oil has a high content of lauric acid, which is known to increase cholesterol [26]. It increases both total cholesterol and HDL-C. Apart from this, coconut oil is also known to have antioxidant property. This could probably be the reason for the rise in HDL-C observed in our study.

The results of the current study are concordant with a previous study which evaluated the hypoglycemic and hypolipidemic effects of *Lyciumbarbarum* fruit water decoction, crude polysaccharides, and its purified polysaccharide fractions in alloxan-induced diabetic rabbits and healthy mice [17]. After the treatments of *Lyciumbarbarum* fruit water decoction, crude polysaccharides (glycoconjugates) and purified polysaccharide fractions in hyperlipidemic rabbits for 10 consecutive days, there was a significant decrease ($p<0.01$) in serum lipids (TC and TG) while there was a marked increase in HDL-C ($p<0.05$). The serum lipid profiles before and after treatment showed that all the three *Lyciumbarbarum* extracts/fractions could produce significant hypolipidemic effect in tested rabbits.

Previous studies exploring the role of antioxidants in improving hyperlipidemia have shown that antioxidant (vitamin C) administration in hypocholesterolemic rats improves endothelial function of coronary and peripheral vessels [27]. As described earlier, a previous study has shown that both polysaccharides and vitamin antioxidants from *Lyciumbarbarum* fruits are possible bioactive components of hypolipidemic effect in an alloxan-induced rabbit model [14]. Botanical polysaccharides occur naturally mainly as a glycoconjugate, i.e. a conjugate of glycan with peptides or proteins. Polysaccharides from *Lyciumbarbarum* are such a type of polysaccharide and are major bioactive constituents in *Lyciumbarbarum* fruits. Four polysaccharide fractions were isolated and purified previously [18–20]. In addition, *Lyciumbarbarum* fruits contain other bioactive components like carotene, riboflavin, ascorbic acid, thiamine, nicotinic acid, betaine, coumarin (scopoletin), zeaxanthin, cryptoxanthin, etc., most of which are antioxidants and are responsible for antioxidant properties of *Lyciumbarbarum* [17]. Among these antioxidant components, there may be some interactions and synergistic effects for antioxidant properties that contribute to its hypolipidemic effect.

To conclude, the present study substantiates the hypolipidemic effect of *L. barbarum* in high-fat diet-induced model of hyperlipidemia. However, further studies exploring the effects on individual lipid profiles at higher doses and mechanistic evaluation to identify the mechanisms of action are warranted.

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