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Antihyperlipidemic Activity of *Gymenma sylvestre* R. Br. Leaf Extract on Rats Fed with High Cholesterol Diet

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Abstract: Effect of *Gymnema sylvestre* R. Br. Leaf extract on high cholesterol fed diet rats was investigated. Hyperlipidemia was induced in rats by giving high cholesterol diet (2% cholesterol, 1% sodium cholate and 2% coconut oil) for seven days in standard rat chow diet. The hydroalcoholic extract of *Gymnema sylvestre* R. Br. leaves (200 mg kg^{-1} b.wt.) was orally administered once a day to rats fed with a high cholesterol diet for seven days. High cholesterol fed diet rats exhibited significant increase in total serum cholesterol, triglycerides, low density lipoproteins, very low density lipoprotein and significant decrease in high density lipoproteins. Treatment with hydroalcoholic extract of *Gymnema sylvestre* R. Br. leaves significantly decreased total serum cholesterol, triglycerides, low density lipoproteins, very low density lipoprotein and increased the high density lipoproteins in hyperlipidemic rats and was comparable with that of standard atorvastatin. Hence, it was concluded that significant antihyperlipidemic activity of hydroalcholic extract of *Gymnema sylvestre* R. Br. leaves may be due to the presence of acidic compounds, flavonoids, phenols, saponins, tannis (Phenolic compounds) and triterpenoids found in the preliminary phytochemical screening.

Key words: Coronary heart disease, high cholesterol diet, *Gymnema sylvestre*, gymnemic acid, total cholesterol

INTRODUCTION

Coronary arterial diseases are responsible for more deaths than all other associated causes combined (Jain *et al*., 2007). Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis-associated conditions, such as Coronary Heart Disease (CHD), ischemic cerebrovascular disease and peripheral vascular disease (Hardman and Limbird, 2001). Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease (Kumar *et al*., 2008). Reduction in serum cholesterol levels reduces the risk for CHD (Jain *et al*., 2007). The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease (Davey Smith and Pekkanen, 1992). Currently available hypolipidemic drugs have been associated with a number of side effects (Brown, 1996). The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function (Kumar *et al*., 2008). An herbal treatment for hypercholesterolemia has almost no side effects and is relatively cheap, locally available. They are effective in reducing the lipid levels in the system (Berliner and Suzuki, 1996). Medicinal plants play a major role in antihyperlipidemic activity (Kumar *et al*., 2008).

The plant *Gymnema sylvestre* R. Br. (Asclepiadaceae) is a large woody climbing plant found species is through out India, in dry forests upto 600 m, common throughout the district from January to November. Distributed in Asia, Tropical Africa, Malaysia and Srilanka (Gurav *et al*., 2007). It used as a stomachic, diuretic and anti-diabetic remedy. The total saponin fraction of the leaves, commonly known as gymnemic acid, has an anti-sweetening effect (Wen-cai *et al*., 2000). According to recent reports gymnemic acid formulations have also been found useful against obesity (Yoshikawa *et al*., 1997). The triterpenoid saponin contain several acylated (tigloyl, methylbutyroyl etc.) derivatives of deacylgymnemic acid which is 3-O-b-glucuronide of gymnemagenin (3b, 16b, 21b, 22a, 23, 28-hexahydroxy-olean-12-ene). The individual gymnemic acids (saponins) include gymnemic acids I-VII, gymnemosides A-F, gymnemasaponins etc. (Gurav *et al*., 2007).

As far as our literature survey could ascertain, there is no study reported for treating the hyperlipidemia with hydroalcoholic extract *Gymnema sylvestre* R. Br. leaf at

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the dose of 200 mg kg^{-1} using Sprague Dawely female rats fed on a high cholesterol diet. Hence, the present study was undertaken to demonstrate the effect of *Gymnema sylvestre* R. Br. leaf extract on lipid profile of hyperlipidemic rats using standard lipid lowering agent atorvastatin.

MATERIALS AND METHODS

Chemicals: Cholesterol, sodium cholate and coconut oil were all purchased from SD-fine chemicals, India, atorvastatin was procured form Ranbaxy labs. Ltd., Gurgaon, India. All other reagents used were of analytical grade.

Instrument: UV spectrophotometer (Shimadzu-UV-1601), Centrifuge Machine (Eltek-research centrifuge-TC-4100D).

Collection and authentification of plant material: The leaves of *Gymnema sylvestre* R.Br. were collected from locally from the college campus of Modasa-Gujarat (India) and were authentified by Dr. M.S. Jangid, Botany Department from Sir P.T Science College-Modasa, India. Authentication specimen number was PRR/01012008 was submitted at Institute's herbarium department for future reference.

Extraction of plant material: The gymnema leaves are graded, cleaned, dried, grinned and disintegrated to required mesh (20-60) and were used for the extraction. It is then extracted with 55% v/v alcohol using soxhlet apparatus for 6 h. The micella is concentrated under reduced pressure to 30% solids. It is filtered, weighed and used for the study (Rajpal, 2002).

Preliminary phytochemical screening: Preliminary phytochemical screening of the gymnema leaf extract was carried out for the detection of the various plant constituents (Khandelwal, 2004).

Animals: Sprague Dawely female rats weighing 200-250 g were acclimatized to the experimental room having temperature 23±2°C, controlled humidity conditions and 12:12 h light and dark cycle. Animals were caged in polypropylene cages in a group with maximum of three animals per cage. The rats were fed with standard food pellets and water *ad libitum*. The study was approved by Institutional Animal Ethical Committee (IAEC), Shri B. M. Shah College of Pharmaceutical Education and Research, Modasa, Gujarat, India (IAEC/BMCPER/02/2008-09).

Induction of Hyperlipidemia: High Cholesterol diet was prepared by mixing cholesterol 2%, sodium cholate 1% and coconut oil 2%, with standard powdered standard animal food. The diet was placed in the cage carefully and was administered for seven days (Pandya *et al*., 2006).

Dose preparation and administration of standard atorvastatin and gymnema extract: Standard atorvastatin at a dose of 10 mg kg^{-1} was prepared by suspending bulk atorvastatin in aqueous 0.5% methylcellulose (Henck *et al*., 1998). The extract of gymnema leaf was dissolved in distilled water and a dose of 200 mg kg^{-1} was given to the rats once in a day along with the high cholesterol diet orally. Treatment was given daily for seven days.

Protocol for Antihyperlipidemic Activity: The experimental animals were divided into four groups, six animals in each group:

- **Group 1:** Normal
- **Group 2:** High cholesterol diet control
- **Group 3:** Standard atorvastatin [10 mg kg^{-1} body weight (b.wt.), orally (p.o.)]
- **Group 4:** High cholesterol diet treated with gymnema extract [200 mg kg^{-1} b.w., p.o.]

Blood sample collection and analysis: On the 8th day, blood was collected by retro-orbital puncture technique, under mild ether anesthesia after 8 h fasting and allowed to clot for 30 min at room temperature. Blood samples were centrifuged at 3000 rpm for 20 min. Serum was separated and stored at -20°C until biochemical estimations were carried out. Serum samples were analyzed spectrophotometrically for total serum cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL-C) was estimated using diagnostic kits which were procured from Lab-Care Diagnostics Pvt. Ltd., Mumbai, India. Very Low Density Lipoprotein (VLDL), High Density Lipoprotein ratio (HDL-C ratio), Atherogenic Index (AI) and low density lipoprotein cholesterol (LDL-C) were calculated by using the formula of Modi and colleagues (Friedewald *et al*., 1972).

Statistical analysis: Experimental results were Mean±SEM (Standard Error of Mean) of 6 animals. The results were statistically analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's multiple tests to determine level of significance. Data were considered statistically significant only when value of $p<0.05$.

RESULTS

The rats fed with high cholesterol diet for seven days exhibited significant increase in TC, TG, LDL-C and VLDL and significant decrease in HDL-C, HDL-C ratio as

Table 1: Effect of gymnema extract on lipid profile of high cholesterol diet rats

Parameter (mg dL^{-1})	Normal	High cholesterol diet control	High cholesterol diet treated with atorvastain	High cholesterol diet treated with gymnema extract
TC	85.19±8.08	261.27±17.99*	107.21±15.78**	172.15±18.73**
TG	63.08±2.20	120.00±02.95*	74.23±03.35**	87.44±03.59**
HDL-C	46.69±1.60	21.23±01.08*	36.29±02.35**	30.82±02.69**
LDL	25.88±6.07	216.03±16.32*	56.07±12.76**	123.84±15.32**
VLDL	12.62±0.41	24.00±00.59*	14.84±00.67**	17.49±00.72**
HDL-C ratio	121.27±24.74	8.85±6.39*	51.17±17.50	21.81±16.80
AI	1.31±1.26	5.65±2.73	2.05±1.43	2.84±1.33

Each value is Mean±SEM (n = 6). *Significantly different from normal groups ($p<0.05$). **Significantly different from high cholesterol diet control groups ($p<0.05$)

compared to the normal animals. Treatment with atorvastatin (10 mg kg^{-1} b.wt., p.o.) showed significant decrease in elevated TC, TG, LDL-C and VLDL, with significant increase in HDL-C ($p<0.05$) as compared to the high cholesterol diet control. Whereas treatment with hydroalcoholic extract of gymnema leaves at a dose of 200 mg kg^{-1} b.wt., p.o. showed significant decrease in the elevated TC, TG, LDL-C and VLDL, with significant increase in the HDL-C ($p<0.05$) as compared to the high cholesterol diet control (Table 1).

DISCUSSION

Despite significant medical advances, heart attacks due to coronary artery disease and stroke are responsible for more deaths than all other causes combined. A 1% drop in serum cholesterol reduces the risk for CHD by 2% (Jain *et al*., 2007). Gymnema has been used in Indian traditional medicine, Ayurvedic medicine, from ancient times and is said to be effective in promoting urination, digestion, antiviral, diuretic, antiallergic, hypoglycemic, hypolipidemic and antiobesity agent for the treatment of diabetes, obesity and dental caries (Anonymous, 2006). Hence, based on traditional claims the present study has been undertaken to demonstrate the effect of *Gymenma sylvestre* R. Br. Leaf on Rats Fed with High Cholesterol Diet. Modern lipid lowering agents i.e., statins (atro-vastatin, simvastatin, rosuvastatin etc.) are expensive. The most important adverse effects of statins are liver and muscle toxicity. Other risk factors are: hepatic dysfunction, renal insuffici-ency, hypothyroidism, advanced age and serious infections (Moosa *et al*., 2006). Water soluble fraction of alcoholic extract of gymnema lowered the glycogen content of the tissue significantly on isolated rat hemi diaphragm in normal and glucose fed hyperglycemic rats (Chattopadhyay, 1998). Leaves of gymnema are effective in treatment of diabetes present investigation was done to evaluate the effect of gymnema leaves on lipid profile of rats because hyperglycaemia is also accompanied with hypertriglyceridemia and hypercholesterolemia (Qureshi *et al*., 2009). In this study parameter of lipid profile were evaluated for all normal and hyperlipidemic rats. It was found that there was a significant ($p<0.05$) reduction in TC, TG, VLDL, LDL and significant ($p<0.05$) increase in HDL-C which proves that the leaf extract of gymnema can be used for the treatment of hyperlipidemia. Hence, apart from the wide usage of gymnema leaf as antidiabetic it can also be used for the treatment of Hyperlipidemia. The gymnema extract at a dose of 200 mg kg^{-1} b.wt. orally showed significant Antihyperlipidemic activity which may be due to presence of flavonoids, phenols, tannis (Phenolic compounds) and triterpenoids found in the preliminary phytochemical screening. Hence, we conclude that further clinical studies are needed to evaluate the antihyperlipidemic potential of the Gymnema leaf.

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