Research Article

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A COMPARATIVE EVALUATION OF GYMNEMIC ACIDS AND EXTRACT OF GYMNEMA SYLVESTRE FOR ITS HYPOLIPIDEMIC ACTIVITY IN TRITON X-100 INDUCED HYPERLIPIDEMIC RATS

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ABSTRACT:

The present study is designed to evaluate the effect of Gymnema sylvestre leaf extract and gymnemic acids on lipid profiles in Triton X-100 induced hyperlipidemic male wistar rats. The leaves of the plant were dried in hot air oven at 50°C, powdered and were subjected to soxhlet extraction. Gymnemic acid-II and IV significantly increased ($p < 0.0001$) plasma HDL-Cholesterol and decreased plasma TC, LDL and TG levels as compared with hyperlipidemic control rats. The results obtained provide a support for the use of this plant in traditional medicine and its further investigations.

KEYWORDS:

Gymnemic acids, lipid profile, hypolipidemic, Gymnema sylvestre

INTRODUCTION

Hyperlipidemia is an abnormal elevation of plasma lipids including largely cholesterol, triglycerides and phospholipids [1-2]. An elevation of plasma lipids may be caused by a primary genetic defect or secondary to diet, drugs or diseases. Despite of differences in lipoprotein distribution and metabolism between humans and rats, hyperlipidemic rat models were extensively used in lipid research[3]. The solution of Triton X-100 has been successfully used to induce hyperlipidemia in rats. Currently available hypolipidemic drugs were known to have number of side effects.Whereas; herbal treatment for hyperlipidemia has fewer side effects. Vegetables, condiments and spices of Indian food have been identified as hypolipidemic in ayurvedha and have folklore significance[4]. Gymnema Sylvestre (Asclepiadacea) and its four active constituents gymnemic acids (G-I, G-II, G-III and G-IV) were selected for evaluation of anti-hyperlipidemic activity in rats. It is commonly known as Meshashringi in Sanskrit and Gurmar in Hindi. It is a potent antidiabetic in ayurveda and homeopathic systems of medicine. *G. sylvestre* contains triterpene saponins[5], and anthraquinone glycosides[6]. Leaves of *G. sylvestre* also proved for anticipating snake bite[7], hepatoprotective and sweet suppressing agent[8]. Among all the constituents oleane saponins reported for their anti-sweetner[9], anti-inflamatory[10] agents. The present study is designed to investigate the anti-hyperlipidemic activity of crude extract of gymnema sylvestre and its active constituents G-I, G-II, G-III and G-IV in triton-100 X induced hyperlipidemic rats.

PROCUREMENT AND IDENTIFICATION OF PLANT MATERIAL

About 4kg of dry leaves of *G. sylvestre* were collected as gift sample from Amruth Ayurvedic Hospital and Research Center, Hyderabad. The plant material was

identified and authenticated by Dr. Anil Kumar, Physician, Amruth Ayurvedic Hospital and Research Center, Hyderabad, Andhra Pradesh.

MATERIALS AND METHODS

PROCESSING OF PLANT MATERIAL

The leaves were freed from the follicles, coiled twigs and other extraneous matter. About 3kg cleaned leaves were dried over night in hot air oven at 50°C and ground to a powder which was passed through a sieve of mesh number40.The powdered material was again dried at 50°C and stored in air-tight, paper-lined tin. The moisture contents of *G. sylvestre* leaves were determined by drying and toluene determination methods. The ash contents were also determined [(11).]

EXTRACTION OF PLANT MATERIAL

The leaves of *G. sylvestre* were cleaned, dried and made to fine powder. The powdered plant material was extracted with ethanol (95%) by Soxhlet extraction process. The solvent was recovered by distillation after completion of the extraction. The obtained leaf extract was stored at -20^{o}C. The crude concentrated extract was used for isolation of gymnemic acids. The extract was suspended in distilled water and alcohol to give 0.1mg *G. sylvestre* extract per one ml of the suspension and administered orally through orogastric tube.

EXPERIMENTAL ANIMALS

Male Wistar rats, weighing between 180-200gm were obtained from Mahaveer Enterprises, Hyderabad. The selected animals were housed five per each of acrylic cages at 25℃, 45-55% humidity and 12hr light/dark under controlled environment. Rats were fed with standard laboratory diet and purified drinking water *ad libitium* throughout the experimental period. All protocols and experiments used in the present study were approved by the Institutional Animal Ethics Committee (IAEC).

ANTI-HYPERLIPIDEMIC STUDIES

The rats were divided into five groups of eight rats in each group.

Group I: (Normal) Received normal saline (*p.o*).

Group II: (HL) Rats were treated Triton-X-100 (100mg/kg, *i. p*).

Group III: Rats were treated Triton-X-100 (100mg/kg, *i. p*) + G-I (50 mg/kg, *p.o*).

Group IV: Rats were treated Triton-X-100 (100mg/kg, *i. p*) + G-II (50 mg/kg, *p.o*).

Group V: Rats were treated Triton-X-100 (100mg/kg, *i. p*) + G-III (50 mg/kg, *p.o*).

Group VI: Rats were treated Triton-X-100 (100mg/kg, *i. p*) + G-IV (50 mg/kg, *p.o*).

Group VII: (Extract) Treated with Triton and Extract (100 mg/kg, *p.o*).

Group VIII: (AT) Treated with Triton and Atorvastatin (10 mg/kg, *p.o*).

This study was carried out for seven days. On 8th day of the treatment, the blood was collected by retro orbital sinus puncture, under mild ether anesthesia in heparinized tubes. Serum was obtained by immediate centrifugation of blood samples using remi ultra cooling centrifuge at 3000 rpm for 5 mins at room temperature and was used for estimating serum lipid profiles (serum TC, TG, LDL-C and HDL-C). All collected samples were stored at 4°C [(11)].

BIOCHEMICAL ANALYSIS

Plasma lipid levels include TC, TG and HDL-C were carried out using respective diagnostic commercial kits from Qualigens diagnostics, Mumbai, India and LDL-C in plasma was calculated as per Friedewald estimation[(12)],

LDL-C= TC-HDL-TG/5.

STATISTICAL ANALYSIS

The results were expressed as mean ± SD. The Triton control was compared with normal and the experimental results were compared with Triton control. Statistical analysis was carried out using paired t-test and one-way analysis of variance (ANOVA) followed by Bonferroni's test. Differences below $P<0.05$ implied statistically significance.

RESULTS AND DISCUSSION

In the present study, *G. sylvestre* extracts and its constituents were selected to screen for their antihyperlipidemic activity in Triton X-100 (100 mg/kg) induced hyperlipidemic rats[4]. Triton X-100 (100 mg/kg) has successfully induced hyperlipidemia in rats by increasing the serum TC, TG and LDL-C levels [13]. The effect of ethanolic extracts of *G. sylvestre* and gymnemic acids I, II, III and IV on serum lipid profile levels was shown in Table-I. Treatment with ethanolic extracts of *G. sylvestre* at the doses of 100mg/kg and G-I, G-II, G-III and G-IV 50mg/kg respectively reduced the serum TC, TG and LDL-C levels significantly ($P < 0.0001$) and increased the serum HDL-C levels when compared to the hyperlipidemic control group. The change in lipid levels in groups of II, III, IV, V, VI and VII were comparable with group of Atrovastatin treated rats. Among 4 constituents, G-II reduced the elevated lipid levels more significantly than the others. Oral administration of *G. sylvestre* extract and gymnemic acids significantly reduced the elevated lipid levels in rats possibly by controlling the hydrolysis of certain lipoproteins and their selective uptake and metabolism by different tissues.

It is widely accepted that reduction in plasma HDL is a risk factor for developing atherosclerosis. HDL facilitates the translocation of cholesterol from the peripheral tissues to liver for catabolism. The increase in HDL, a cardio protective lipid slows down the atherosclerotic process[14]. Increased levels of HDL after administration of *G. sylvestre* extract concluded that it is a potent cardio protective agent. Several studies showed that an increase in HDL-C is associated with a decrease in coronary risk. High levels of TC and LDL-C are major coronary risk factors[15,16]. Administration of *G. sylvestre* extract and gymnemic acids lowered both TC and LDL cholesterol in hyperlipidemic rats. This lowering of TC and LDL-cholesterol would reduce the incidence of coronary events [17].

ATHEROGENIC INDEX (AI)

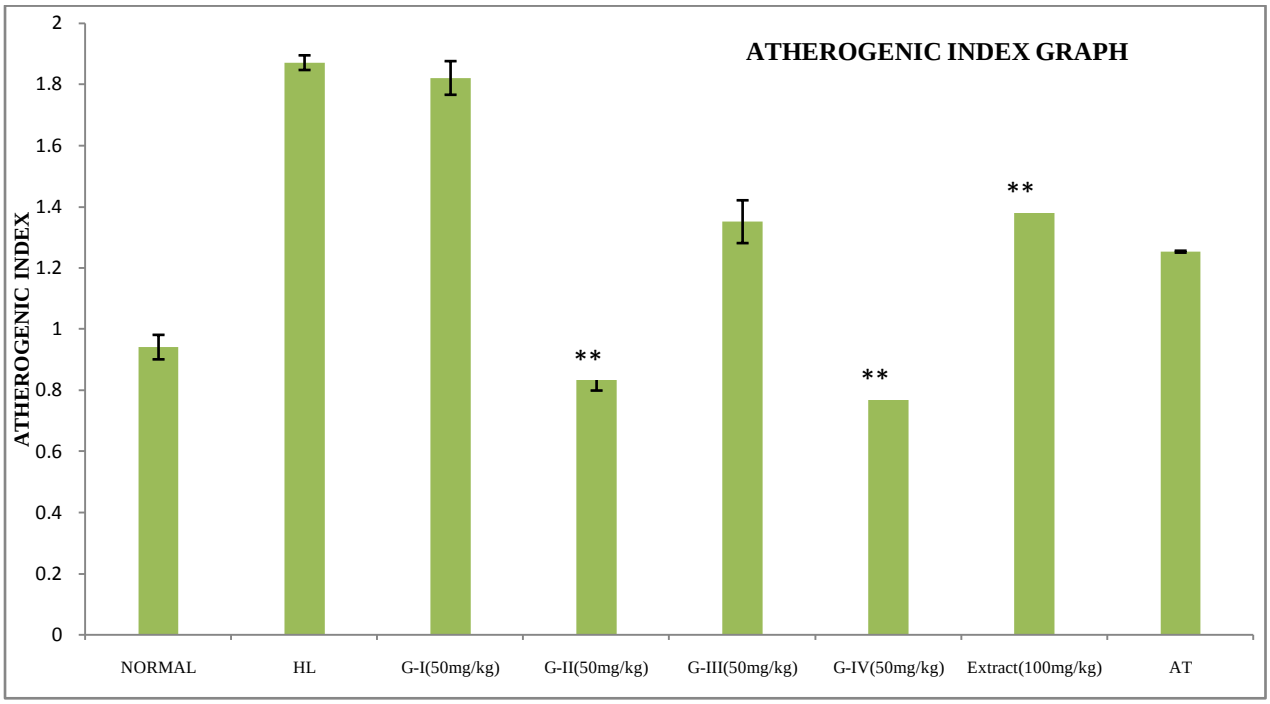
Atherogenic Index was calculated by the equation: (total cholesterol-HDL-cholesterol)/ HDL-cholesterol [18]. The values of AI in hyperlipidemic and gymnema treated groups were shown in Figure I. The ratio was significantly increased in Triton induced hyperlipidemic rats compared with normal group. These elevated ratios returned to near normal levels in groups of rats treated with ethanolic extracts of gymnema and Atorvastatin. The rise in AI in hyperlipidemic rats enhanced the probability of cardiovascular pathogenesis and endothelial dysfunction. A significant decrease in AI value was observed in herbal supplemented animals, suggesting the cardio protective potential of this herb.

Table-I: **Effect of ethanolic extracts of *G. sylvestre* on serum lipid profile levels in Triton induced hyperlipidemic rats.**

Group	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Group I: Normal	80.88±1.13	65.38±3.46	41.50±1.31	26.30±1.89
Group II: HL(Triton)	141.88±1.46	160.75±1.28	49.38±1.19	60.38±2.23
Group III: Triton + G-I (10 mg/kg)	139.63±1.92	149.38±1.51	49.38±1.19	60.38±2.23
Group IV: Triton + G-II (10 mg/kg)	112.25±2.05	96.88±1.55	59.88±0.83	32.60±1.39
Group V: Triton + G-III (10 mg/kg)	131.38±1.30	147.00±1.31	55.75±1.04	46.23±1.33
Group VI: Triton + G-IV (10 mg/kg)	112.63±2.26	70.75±1.16	60.88±1.13	37.53±1.97
Group VII: Triton +Gymnema extract	111.00±1.20	91.38±1.06	45.75±1.04	46.98±1.73
GroupVIII:Triton+ Atorvastatin (AT) (10mg/kg)	101.38±1.06	91.13±1.13	45.00±0.93	38.10±1.20

*Values are in mean ± SD; n=8; *= p < 0.0001 Vs Group II*

Figure-I: Atherogenic index values in hyperlipidemic, gymnema extract and gymnemic acids treated group of rats.



*Values are in mean ± SD; n=8; *= p < 0.0001 Vs Group II*

CONCLUSION

All the fractions obtained from the ethanolic extracts of *G. sylvestre* and gymnemic acids significantly reduced the Triton-X-100 induced hyperlipidemic rats. G-II reduced more significantly the elevated lipid levels as compared to other groups used in the study. In conclusion, the study results infer that gymnemic acids and *G. sylvestre* extracts show significant hypolipidemic activity.

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Conflict of Interest:

Conflict of interest declared none.

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