ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Suppl 2, 2014

ISSN - 0974-2441

Research Article

ANTIHYPERLIPIDEMIC EFFECT OF *ASPARAGUS GONOCLADOS* BAKER AGAINST CHOLESTEROL DIET INDUCED HYPERLIPIDEMIA IN RATS.

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Received: 28 February 2014, Revised and Accepted: 3 May 2014

ABSTRACT

Objective: To evaluate the antihyperlipidemic potential of the Ethanolic Extract of Root tubers of *Asparagus gonoclados* (EERAG) in cholesterol diet induced hyperlipidemic rats.

Methods: Wistar albino rats were randomly divided into five groups of six each. Group-I served as normal control. Groups II to V were given 5% cholesterol diet for 3 months to induce hyperlipidemia, and for last 28 days were administered either: 0.5ml water/saline for Group- I; cholesterol diet (5%) for Group-II; Standard drug Rosuvastatin (20mg/kg body weight) for Group-III; *A.gonoclados* extract at 250 mg/kg bodyweight for Group-IV and 500mg/kg body weight for Group-V. The effects of EERAG on serum lipid profile, Body Weight and antioxidant enzymes (Superoxide Dismutase and Catalase) were assessed and compared.

Results: Cholesterol diet induced hyperlipidemic rats showed an significant ($P<0.001$) increase in the plasma concentration of Total Cholesterol (TC), Triglycerides (TG), Low-Density Lipoprotein cholesterol (LDL-c), Very Low-Density Lipoprotein cholesterol (VLDL-c) and body weight. Decrease in High Density Lipoproteins Cholesterol (HDL-c) and antioxidant enzymes were observed when compared to normal control rats. Co-administration of EERAG and standard drug Rosuvastatin with high cholesterol diet caused a significant decrease ($p<0.001$) in the concentration of serum TC, VLDL, TG, body weight and increase in the HDL-c and antioxidant enzymes when compared with cholesterol fed control rats.

Conclusion: The result suggests lipid lowering and antioxidant potential of effect of *A. gonoclados*, which serves as a new potential herbal product for preventing hyperlipidemia.

Keywords: *Asparagus gonoclados*, Cholesterol, Hyperlipidaemia, Lipid profile, Antioxidant enzymes.

INTRODUCTION

Global Prevalence of hyperlipidemia induced cardiovascular diseases is appeared to be a major cause of morbidity and mortality in both developing and developed countries. Although several factors, such as a diet high in saturated fats and cholesterol, age, family history, hypertension and lifestyle play a significant role in causing heart failure. The high levels of cholesterol, particularly TC, TG and LDL cholesterol are mainly responsible for the onset of Coronary Heart Diseases [1]. In hyperlipidemic conditions enzymatic as well as non-enzymatic antioxidative defence systems such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), ascorbic acid and reduced glutathione (GSH) are altered leading to reactive oxygen species (ROS) mediated damage [2]. Logical strategy, to prevent atherosclerosis and reduce the incidence of cardiovascular disease events, is to target hyperlipidemia either by drugs or dietary intervention [3]. Synthetic drugs like statins, which reduce the levels of lipids and cholesterol possesses potentially toxic side effects. Viable alternatives to the synthetic drugs are natural products with high lipid lowering potential with minimal or no side effects. Earlier studies also reveal that the consumption of medicinal plants will reduce the risk of hyperlipidaemia in ayurveda[5-8].

Asparagus gonoclados is commonly known as Pillipichara, (Shakakul in Hindi and Thanneer vittanka zhangu in Tamil) which is official in ayurvedic pharmacopoeia of India [9]. *A. gonoclados* is reported as a substitute for *Asparagus racemosus* Wild [10]. In folklore medicine root tubers of *A. gonoclados* are traditionally used for its diuretic, glactogogue, antiulcer, antioxidant activity, antidiabetic, antipyretic, increases lactation. It used to treat diseases like pyorrhoea, spermatorrhoea and urolithiasis [11-13]. *A. gonoclados* contain apigenin, kaempferol, rutin and chalcone glycoside; Flowers contain anthocyanin, malvin and asparagines; and aerial parts contain 4, 4', 6-trihydroxy auronone [14]. Antihyperlipidemic effect of *A. gonoclados* has not yet been previously investigated.. In the present study, we examined the efficacy of the ethanolic extract of root tubers of *A. gonoclados* against cholesterol diet induced hyperlipidemic rats.

MATERIALS AND METHODS

Collection and authentication of roots

The roots of *Asparagus gonoclados* were collected from Talakona forest and Tirumala hills of Chittoor district, Andhra Pradesh, India. The plant materials were taxonomically authenticated by Taxonomist, Prof. K. Madhavachetty, Department of Botany, Sri Venkateswara University, Tirupati, India.

Chemicals

Cholesterol was purchased from HIMEDIA (Mumbai, India) and Rosuvastatin was obtained from RANBAXY Laboratories, Gurgaon, Haryana. Biochemical kits for lipid profile analysis were obtained from Merck Diagnostics India Ltd. All other solvents and chemicals used for the study were of analytical grade.

Preparation of Plant extract

The collected roots were washed with tap water and dried under shade for 4-5 days. Dried roots were powdered using a mechanical grinder and stored in air tight container. About 200g powder was extracted with 500ml of 70% ethanol using soxhlet apparatus for 12h. The extracts were concentrated to dryness under reduced pressure and controlled temperature using a rotary flash evaporator. The dried extracts obtained were used in the present study.

Phytochemical screening

The extract was qualitatively tested for the presence of phytochemicals by TLC and test tube reactions [15] [16].

Acute toxicity studies

Acute Invivo toxicity study of different doses of EERAG was performed in Wistar albino rats, according to OECD guideline no. 420 given by CPCSEA [17]. No death or adverse effects were detected up to 5000mg/kg body weight. Based on these results, 250, 500 mg/kg of dose were taken for the experiment.

Animals

Wistar albino rats weighing between 120-122g were procured from Sri Venkateswara Enterprises, Bangalore, India. The animals were housed in polypropylene cages under standard environmental conditions (temperature 22±2^{0}C; humidity 60±4%) with a 12 h light/dark cycle. Rats were fed with standard rats chow and water ad libitum. All animal experiments were approved by the Institutional Animal Ethical Committee (1521/PO/a/11/CPCSEA) prior to beginning of research work and all procedures were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals".

Experimental design

Rats were randomly divided into five groups of six each. Initially, all rats were acclimatized by giving normal diet for one week. Animals in group I (Normal control) and group II (High Cholesterol Diet control) were given normal diet and 5% cholesterol diet respectively throughout the course of study. Animals in group III to V were given 5% high cholesterol diet (HCD) for three months to induce hyperlipidemia and then for last 28 days of cholesterol treatment, group-III rats were fed with standard drug Rosuvastatin (20mg/kg), Animals of group IV (250 mg/kg) and group V (500mg/kg) which served as treated groups were fed with EERAG. During the experimental period, the weight gained by rats was recorded on 0th, 14th and the 28th day of EERAG treatment.

Collection of blood

Twenty four hours after experimental period, the animals were anaesthetized and blood samples were collected through cardiac puncture. The collected blood was centrifuged at 2500g for 20mins to separate serum and is preserved in -20^{o}C for various biochemical experiments.

Estimation of serum lipid profile

Serum TC, TG and HDL cholesterol were estimated using commercially available kits (Span Diagnostics Pvt. Ltd., India), VLDL and LDL cholesterol was calculated using Fridewald's equations [18]:

VLDL-c = triglycerides/5

LDL-c = Total cholesterol - (HDL-c) - (VLDL-c)

Antioxidant enzymes

Cardiac Superoxide dismutase (SOD) activity was determined by the method of Sun *et al.* [19]. Cardiac catalase activity (CAT) was determined according to the method of Aebi. [20].

Statistical analysis

Experimental results were expressed as mean ± SEM of six animals. Statistical analysis was carried out by using ANOVA followed by Dunnet's multiple comparison tests using Graph pad prism software version 5.0. P values < 0.05 were considered as statistically significant.

RESULTS

Phytochemical analysis

Phytochemical analysis of EERAG showed the presence of Tannins, Saponins, Alkaloids, Carbohydrates, Glycosides, Flavonoids, Proteins and Gums.

Effect of weight gain

An initial body weight of the five groups were not significantly different (120-122g). After high cholesterol diet the weight of rats in Group-II to Group-V was increased significantly. Treatment with standard drug Rosuvastatin decreases the body weight by 23% and 33.84% on 14th and 28th day, respectively when compared to HCD group (Group-II). Administration of different doses of EERAG (250mg/kg, 500mg/kg) significantly reduced the body weight by 15.4%, 22.06% and 25.9%, 30.8% on the 14th and 28th day respectively. The effect of EERAG on body weight was in a dose dependent manner (Table 1).

Table1: Effect of *A. gonoclados* extract on weight gain in hyperlipidemia-induced rats for 12 weeks.

Parameter	Test group	Day 0	14th day	28th day
Weight gain	Group-I	122.7±0.88	125±0.96	128.5±0.76
	Group-II	215.5±1.17***	224.8±0.94***	235.8±0.94***
	Group-III	200.3±1.05***	171.3±1.02**	156±0.96***
	Group-IV	204.5±0.99**	190±1.06***	174.7±0.88***
	Group-V	201.3±0.88***	175.2±0.94***	163±0.96***

Values are expressed as mean ± SEM. Values were significant when compared with the HCD control group. * P<0.05, **P<0.01, ***P<0.001 (one way ANOVA followed by Dunnet's test).

Effect of EERAG on Serum Lipid Profile

In the present study, the levels of serum cholesterol, Triglyceride, LDL & VLDL were significantly increased (p < 0.001) and HDL-c levels were decreased in the animals which received high cholesterol diet feeding when compared to normal control rats. Treatment with EERAG at a dose of 250 and 500mg/kg exhibited a reduction of 19.4% and 29.53% in TC, 17.12% and 26.9% in TG, 26.23% and 41.88% LDL-C, 24.16% and 29.79% in VLDL-C, while HDL-C levels were increased to 17.6% and 34.5% as compared to model group rats. The effect of EERAG on serum lipid profile was in a dose dependent manner (Table 2).

Effect of EERAG on antioxidant studies:

The levels of the antioxidant enzymes viz. SOD, Catalase has significantly decreased in the HCD rats. Treatment with Rosuvastatin significantly increased antioxidant enzymes as compared with hyperlipidemic group (Table 3). Dose dependent increase in the SOD (52%, 65.25%) and CAT (33.2%, 39.9%) levels were observed in EERAG treated groups (250, 500mg/kg).

Table 2: Effect of EERAG on plasma lipid status in control and experimental rats.

Groups	Serum cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Group-I	67.33± 1.62	57.17± 1.79	38.67± 1.14	18.00± 1.06	11.33± 0.88
Group-II	171.00±1.46***	144.8±1.30***	22.67±1.14***	119.3±0.88***	29.67± 1.11***
Group-III	103.5±1.11***	94.83±1.35***	34.83±1.30***	48.90±1.04***	19.83±1.51***
Group-IV	137.8±1.70***	120.00±1.52***	26.67±0.88ns	88.00±1.23***	22.50± 1.11*
Group-V	120.5±1.1***	105.8±1.53***	30.5±1.11**	69.33±1.05***	20.83±1.30***

Values are expressed as mean ± SEM. Values were significant when compared with the HCD control group. * P<0.05, **P<0.01, ***P<0.001 (one way ANOVA followed by Dunnet's test). ns= non significant

Table 3: Effect of EERAG extract on antioxidant enzymes in control and experimental rats.

Groups	SOD	Catalase
Group-I	6.90±0.24	11.38±0.33
Group-II	4.00±0.25***	6.31±0.28***
Group-III	5.37±0.29*	7.36±0.26ns
Group-IV	6.08±0.35**	8.41±0.30***
Group-V	6.61±0.26***	8.83±0.24***

Values are expressed as mean ± SEM. Values were significant when compared with the HCD control group. * P<0.05, **P<0.01, ***P<0.001 (one way ANOVA followed by Dunnet's test). ns= not significant.

DISCUSSION

The present study evaluated the antihyperlipidemic effect of EERAG against cholesterol diet induced hyperlipidemic rats. *Asparagus gonoclados* is a well known medicinal plant in Indian system of folklore medicine with antioxidant potential [12]. However, the cholesterol lowering potential of EERAG in the hyperlipidemic animal model is yet to be elucidated.

In developing countries, the risk of cardiovascular diseases may increase with intake of high cholesterol diet food. High levels of cholesterol in the circulating blood forms sticky plaque along the artery walls. This blocks the flow of blood to the vital organs and finally leads to heart stroke. Synthetic drugs like Rosuvastatin, competitive inhibitor of HMG-CoA reductase enzyme is known to reduces blood levels of triglycerides and slightly increases levels of HDL-cholesterol[21, 22]. Recently, the use of natural products or drugs with cholesterol lowering and free radical scavenging capacity had gained much attention than the synthetic drugs. This is due to their less or no adverse side effects.

In the present study, Serum lipid profile of cholesterol diet fed rats showed increased levels of serum total cholesterol (TC), triglycerides (TG), LDL, VLDL levels and decline in HDL-c level. Elevated levels of serum LDL-c and VLDL-c are the presumptive marker for premature atherosclerosis and other cardiovascular diseases. A low level of HDL-c and high level of TG is also an important risk factor for cardiovascular disease [23]. Co-administration of EERAG (250 mg/kg and 500 mg/kg b.w. p.o.) for 28 days modulated the lipid metabolism by decreasing the serum TC, VLDL and LDL cholesterol and TG level. This indicates reduced cholesterol biosynthesis and increased rate of LDL uptake by the liver. Interestingly, EERAG showed significant (p< 0.0001) increase in the HDL cholesterol level. This is due to increased reverse cholesterol transport from peripheral organs to the liver [24]. The results were consistent with standard drug Rosuvastatin. Phytochemical investigation of this plant showed the presences of Tannins, Saponins, Alkaloids, Carbohydrates, Glycosides, Flavonoids, Proteins and Gums. It has been reported that flavonoids, saponins and tannins play a role in hypolipidemic effect [25]. Saponins precipitate cholesterol, from micelles and interfere with hepatic circulation of bile acids making it unavailable for intestinal absorption, this forces liver to produce more bile from cholesterol (serum). This leads to decline in serum cholesterol level. Besides, saponins also lower the plasma LDL-c levels through an increased turnover of LDL-c to hepatic tissue, which is then converted to bile acids [26]. It also reported to lower triglycerides by inhibiting pancreatic lipoprotein lipase [27] and the subsequent decline in VLDL-C levels could be directly correlated to a decline in TG levels [28]. Flavonoids can increase HDL-C and also decreases oxidation of LDL- cholesterol [29]. The data of the present study clearly suggest that EERAG maintain cholesterol homeostasis by its antihyperlipidemic effect.

Hyperlipidemia causes oxidative stress and reduces the antioxidant defence system; there by elevating lipid peroxides [30]. Reactive oxygen species cause cell mutations, damage immune cells and wipe out cytokine pathways, wrinkles by oxidative damage of DNA, proteins, Lipids [31]. SOD and CAT are the two major scavenging enzymes that remove the toxic free radicals. SOD is the first enzyme in antioxidant defence system that protects tissues against oxygen free radicals by catalyzing the removal of superoxide radical (0^{2-}), which damages the membrane and biological structures [32]. CAT has been shown to be responsible for the detoxification of significant amounts of H_20_2 [33]. Plants are a natural source of biologically active compounds known as phytoconstituents[34]. There was a reduction in the activity serum SOD and CAT in HCD induced rats when compared to control rats, this may be due to the enhanced production of Reactive Oxygen Species (ROS) by hyperlipidemia. This free radical affects the antioxidant activity. Treatment with EERAG restores the HCD induced alteration in the activity of SOD and CAT to near control due to its free radical scavenging activity.

CONCLUSION

In conclusion, the current study reveals that EERAG can be used as effective antihyperlipidemic therapeutic agent. Administration of EERAG to HCD animals might have beneficial effects in reducing body weight, lipid level in serum and in enhancing antioxidant enzyme activity. This may contribute to its Cardioprotective potential with low or no adverse side effect. In comparison to standard drug Rosuvastatin, hypolipidemic effect of EERAG was found to be low but comparable. Further study of individual phytoconstituents of the EERAG responsible for hyperlipidemic effect and their mechanisms of action are current under investigation in our laboratory.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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