

Phytochemical evaluation and hypocholesterolemic activity of *Enicostemma axillare*

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Abstract

Hypercholesterolemia is the leading cause for the development of various diseases made pharmaceutical companies to turn towards the herbal products with fewer side effects. In the present research, the hypocholesterolemic activity of *Enicostemma axillare* (EA) along with their phytochemical evaluation has been done. Preliminary phytochemical analysis was carried out in different extracts of EA. The hypocholesterolemic effect of 85 % methanolic extract of EA was evaluated in fructose induced hyperlipidemic animals. Antioxidant enzymes such as catalase, TBARS, GSH, GST and lipid profile such as cholesterol, LDL, VLDL, HDL and Triglycerides were analyzed in heart and plasma samples. Administration of EA decreases the lipid profile and TBARS significantly ($p < 0.05$). Likewise, EA administration increases the antioxidant and HDL significantly ($p < 0.05$). The results reveal that EA is a rich source for phytoconstituents and can be used as a potent hypocholesterolemic and antioxidant agent in pharmaceutical industry.

Key words

Fructose, Lipid profile, Phytoconstituents.

Introduction

Heart disease is the leading cause of death. Many of the risk factors like smoking, lack of exercise and consumption of a high fat diet are responsible for causing heart disease. A healthy diet is important for both prevention and treatment of cardiovascular disease¹. Hypercholesterolemia is a well recognized risk factor for coronary artery disease². "Atherosclerosis" is the principle underlying cause of coronary heart disease which is the commonest cause of death in industrialized world and of stroke and peripheral vascular disease, which is also a major cause of morbidity and mortality³. Increased intake of food may be related to oxidative stress⁴. Pamplona⁵ has observed that increased caloric intake is an important factor decreasing the mitochondrial membrane fluidity and increasing the reactive oxygen species generation. Antioxidant substances are believed to suppress the onset and development of atherosclerosis. Compounds such as probucol have shown effect to reduce the progression of atherosclerosis lesions in hyperlipidemic rabbits⁶.

In addition, flavonoids and phenolic compound have also seen to have antioxidant effect⁷. Plant polyphenol exert cardiovascular benefits by altering concentrations of blood lipid components and a high intake of polyphenols can significantly reduce the risk of mortality from cardiovascular disease⁸. Plants are the source of medicinal agents since time immemorial from the dawn of civilization. Even today plants are the most exclusive source of drugs for the majority of the world population and plant products constitute about 25% of prescribed medicine⁹. *Enicostemma axillare* (EA) is used as a laxative, anti-inflammatory and also as liver tonic. The antitumor activity of methanolic extract of EA has been evaluated against Dalton's ascetic lymphoma in swiss albino mice¹⁰. EA is slightly effective against malaria¹¹. The aqueous extract of the plant reduce the blood sugar of diabetic animals¹². In the present study, we have made an attempt to quantify the phytoconstituents in different extracts of EA along with the evaluation of hypocholesterolemic and antioxidant activity of phytoconstituents rich extract.

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Materials and Methods

Plant Materials

The root of EA was collected from Madurai district, Tamilnadu, India, dried under shade and coarsely powdered. The plant material was identified by the Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Thanjavur, and Tamilnadu, India

Extraction

The plant material was soaked in different solvents like hexane, chloroform, ethyl acetate and 85 % methanol. The extract was concentrated *In-vacuo*. The concentrated extract was stored in a dessicator until used for experiments. The yield of extract was calculated as 1.86 %, 2.52 %, 1.56 % and 3.34 % respectively.

Preliminary phytochemical analysis Quantitative and Qualitative estimation of Phytoconstituents

The concentration of total phenolic content¹³, tannin¹⁴, carbohydrate¹⁵, Vitamin C¹⁶ and Vitamin E¹⁷ were estimated in raw plant of EA. The EA extract was tested for the presence of various phytoconstituents like flavonoids, tannin, alkaloids by following the method of Trease¹⁸.

Evaluation of EA on hypocholesterolemic activity Experimental animals

Albino wistar rats of 150-200 g were obtained from Central animal house, Centre for Advanced Research in Indian System of Medicine, SASTRA University, Tamilnadu, India. They were housed under standard environmental conditions of temperature (22±2°C) and relative humidity of 30-70 %. A 12:12 h light/dark cycle was followed. All animals had free access to water and standard pelleted laboratory animal diet. This study was reviewed and approved by the Institutional animal ethical committee (Reg.No. 817/04/ac/cpcsea).

Experimental protocol for Fructose induced hyperlipidemia

Rats were divided into 4 groups as follows. Each group consists of 6 animals. 10 % fructose was used as inducing agent for hyperlipidemia. The animals were treated with fructose along with extract for 21 days.

Group 1- Water

Group 2 - 10 % Fructose in distilled water/kg b.wt.

Group3- 10 % Fructose in distilled water/kg b.wt.+150 mg/kg b.wt. of extract

Group 4- 10 % Fructose in distilled water/kg b.wt +250 mg/kg b.wt. of extract

On 21st day all the animals were allowed for overnight fasting and were sacrificed by decapitation in anesthetized condition. The heart was excised, washed in saline and homogenized in Tris buffer (0.1 M, pH 7.4). Lipid was extracted from the portion of the heart by following the method of Folch¹⁹. Plasma was separated by centrifugation at 2000 rpm for 10 minutes. Various antioxidants like Lipid peroxidation (TBARS)²⁰, Glutathione peroxidase (GPx)²¹, Reduced Glutathione (GSH)²², catalase²³ were analyzed. Plasma total cholesterol and High Density Lipoprotein – Cholesterol (HDL-C) concentrations were determined using enzymatic kits from Randox Laboratories Ltd., United Kingdom²⁴. HDL-C was analyzed after precipitation of apo B-containing lipoproteins with dextran sulfate²⁵. In heart homogenate, total cholesterol and HDL concentration were estimated by following the method of Zak²⁶. Triglycerides concentration of Plasma and heart homogenate (TGL) concentrations were determined by Foster²⁷. Very Low Density Lipoprotein (VLDL) is TGL/5. Low Density Lipoprotein - Cholesterol (LDL-C) concentrations were then determined using the Friedewald equation²⁸.

Statistical analysis

Values are Mean ± Standard Error mean (SE) (n=6). Significant difference has been observed using One Way Analysis of Variance (ANOVA) using Duncan Multiple Range Test (DMRT). Values not sharing common alphabets (a,b,ab,c,d) are differ significantly at p<0.05.

Results

The EA is observed to be a rich source of phytoconstituents like phenol, tannin, carbohydrate and vitamins like Vitamin C and Vitamin E. The % yield of all the Phytoconstituents in 85% methanolic extract was found to be lower than that of whole raw plant (Table1).

Qualitative analysis of EA extract showed the presence of various phytoconstituents. Alkaloids and phytosterol are eluted in hexane and chloroform extract. Flavonoids, polyphenols and phytosterol are eluted in ethyl acetate extract. 85 % methanolic extract has shown the positive result for flavonoids, polyphenols, phytosterol and carbohydrate (Table 2).

Table 1: Concentration of phytoconstituents in raw herb and 85 % methanolic extract of *Enicostemma axillare*

S.No.	Sample	Concentration of raw herb	Concentration of 85 % methanolic extract
1.	Phenol	3.63 ± 0.56	0.0073±0.0003
2	Tannin	3.94± 0.78	0.0207±0.0004
3	Carbohydrate	4.4 ±0.31	0.0002±0.0003
4	Vitamin C	1.97±1.23	0.0232±0.0003
5	Vitamin E	1.98 ±0.23	0.0139±0.0002

Table 2: Qualitative analysis of phytoconstituents in different extracts of *Enicostemma axillare*

Phytoconstituents	Hexane	Chloroform	Ethylacetate	85% Methanol
Alkaloids	+	+	-	-
Flavonoids	-	-	+	+
Polyphenolics	-	-	+	+
Phytosterol	+	+	+	+
Saponins	-	-	-	-
Fixed oils and fats	-	-	-	-
Carbohydrates	-	-	-	+
Amino acids and proteins	-	-	-	+

Note – (-) refers absent, (+) refers present

Table 3: Effect of EA extract in plasma lipid profile of fructose induced hyperlipidemic rats

Sample	Experimental model	Cholesterol (mg/dl)	TGL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Plasma	Group 1	65.3 ± 1.2 a	70.8 ± 1.3 a	21.8 ± 0.2 b	14.16 ± 0.1 a	29.3 ± 0.2 a
	Group 2	175.2± 10.5 c	175.3±9.5 b	13.3±0.1 a	35.06±0.3 b	126.8±0.1 d
	Group 3	125.3±8.3 b	85.4±6.5 a	18.3±0.2 ab	17.08±0.1 a	89.92±0.3 c
	Group 4	80.2±5.5 ab	70.2±5.2 a	19.2±0.3 b	14.04±0.1a	52.96±0.2 b
Heart	Group 1	4.2±0.2 a	8.8±0.3 a	2.9±0.2 b	1.76±0.1 a	0.46±0.03 a
	Group 2	12.1±0.5 c	19.3±1.5 b	2.1±0.1 a	3.86±0.3 b	6.14±0.5 c
	Group 3	7.3±0.3 b	12.4±1.0 ab	1.9±0.2 a	2.48±0.2 ab	2.92±0.3 b
	Group 4	6.2±0.5 ab	17.3±0.2 ab	2.8±0.3 b	3.46±0.2 ab	0.6 ± 0.02 a

Discussion

EA is a rich source of phytoconstituents like tannin, Vitamin C and Vitamin E. Different extracts of EA point out the presence of different phyto-constituents (Table 1). Among the different extracts of EA, 85 % methanolic extract contains higher concentration of the Phytoconstituents like flavonoids, tannins etc. is taken for further pharmacological evaluation. Hyperlipidemia is observed in patients with NIDDM, obesity, hypertension, etc. Treatment with natural herbs is likely to be fraught with lesser side effects compared to the presently used synthetic oral hypolipidemic agents. In this present research work, we have made an attempt to evaluate the hypolipidemic and antioxidant activity of EA in fructose induced hyperlipidemia. Though the exact mechanism for the hypoglycaemic effect of EA has been reported earlier¹², the hypoglycaemic effect might be related with insulin resistance. The hypocholesterolemic effect of EA has not been reported earlier. This made us to select an animal model in which hyperglycaemia and hyperlipidemia are involved. Research on findings the molecular mechanism for the fructose induced hyperlipidemia is underway. Some mechanisms of high fructose diet induced hypertriglyceridemia are insulin resistance in rats²⁹. Although fructose in the diet alters the activity of several enzymes and regulates hepatic carbohydrate metabolism, leading to hepatic insulin resistance³⁰ and hypertriglyceridemia³¹, the mechanisms by which an excess of fructose produces these effects are unknown. Feeding rats with high dosage of fructose (>60 % of total calories in diet) affects lipid metabolism and causes hyperlipidemia³², insulin resistance, hyperinsulinemia and mild hypertension, which are features associated with obesity-related hypertension. Fructose feeding evokes significant alterations particularly in liver TGL metabolism and is reported to be atherogenic due to induction of lipogenic enzymes in liver³³. The use of 10% fructose in drinking water for a period of 1 week or longer is equivalent to a diet containing 48-57% by calories, and has been found to be most suitable for the production of insulin resistance in rats³⁴. In our study, administration of fructose for 21 days significantly increased the glucose, insulin and triglyceride levels similar to an earlier study¹⁵. Administering EA (150 mg/kg) has prevented the development of hyperlipidemia.

Lipid changes observed in fructose-treated rats are noted to have elevated levels of cholesterol, TGL, LDL, VLDL and HDL-C was decreased. Accumulation of cholesterol, TGL, VLDL and LDL is observed in tissues. These findings are consistent with the results of Michaelis³⁵. The increased conversion of Carbon from fructose in to glycerol-3-phosphate might be responsible for the elevated level of TGL in fructose administered animals³⁶. Feeding a high fructose diet to diabetic rats produces an increase in activity of HMG-CoA reductase and addition of fructose to cultured rat hepatocytes increases HMG-CoA reductase by approximately 3-fold³⁷. The EA extract might be an inhibitor of HMG CoA reductase which is responsible for the observed decrease in the level of lipid profile. The ascorbic acid concentration of EA is reported in the Table I. Vitamin C can inhibit the formation of ox-LDL in *In-vitro* condition. Even if ascorbic acid is water soluble and is not incorporated in LDL particles, it has been proposed that this Vitamin may prevent LDL particle oxidation by scavenging free radicals and other reactive species in aqueous milieu³⁸. The concentration of flavonoids and polyphenolic compound of EA are mentioned in Table I. It has noted that the intake of flavonoids inversely correlates with the plasma total cholesterol and LDL cholesterol concentrations³⁹. Polyphenols reduce the susceptibility of LDL to oxidation in *In-vitro*⁴⁰.

Conclusion

EA extract is a rich source of phytoconstituents. Among the different extracts of EA, 85 % methanolic extract is a rich in phytoconstituents. The pharmacological evaluation of EA has proved that, 85 % methanolic extract of EA exhibits hypolipidemic activity by decreasing the level of total cholesterol, LDL, VLDL and TGL increases the level of HDL. Likewise, the same extract also displays the antioxidant activity by increasing the activity of enzymatic antioxidants like GPx and Catalase, non-enzymatic antioxidants like GSH along with the decrement of TBARS. In pharmaceutical companies, 85 % methanolic extract of EA can be used as a potent hypolipidemic and antioxidant activity. In future, the mechanism of action and isolation of active compound responsible for the hypocholesterolemic effect of EA should be carried out.

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