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ENICOSTEMMA AXILLARE- A HYPOLIPIDAEMIC HERB

K. Gayathri*[1], R. Dhevi[2], M. Mohamed Shabi[3] and G. Victor Rajamanickam[4]

[1]Assistant Professor, Department of Biochemistry, School of Life Sciences, Vels University, Chennai, Tamilnadu, India.

[2] Research Scholar, Department of Siddha Medicine, Tamil University, Thanjavur, Tamilnadu, India.

[3]Lecturer, Department of Pharmacology, Asia Metropoliton University, Malaysia.

[4]Director, Centre for Disaster Management, PRIST University, Thanjavur, Tamilnadu, India.

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***Correspondence for Author**
Dr. K. Gayathri
Assistant Professor, Department of Biochemistry, School of Life Sciences, Vels University, Chennai, Tamilnadu, India.

ABSTRACT

Hypercholesterolemia 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 hypocholesterolaemic 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 hypocholesterolaemic 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 analysed 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.

KEYWORDS: fructose, lipid profile, antioxidants, 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 disease.[1]

Hypercholesterolemia is a well recognized risk factor for coronary artery disease.[2] "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 mortality.[3]

Increased intake of food may be related to oxidative stress[4] Pamplona[5] 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 rabbits. [6] In addition, flavonoid and phenolic compound have also seen to have antioxidant effect.[7] 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 disease.[1]

Plants are the source of medicinal agents since time immemorial from the dawn of civilization. People are utilizing the important biological properties of various plants for the treatment of different diseases. 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 medicine.[9]

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 Dolton's ascetic lymphoma in swiss albino mice.[10] *E.axillare* is slightly effective against malaria.[11] The aqueous extract of the plant reduce the blood sugar of diabetic animals.[12]

In the present study, we have made an attempt to quantify the phytoconstituents in different extracts of EA along with the the evaluation of hypocholesteroaemic and antioxidant activcity of phyto-consituents rich extract.

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, Tamilnadu, India.

Extraction

The plant material was soaked in different solvents like hexane, chloroform, ethyl acetate and 85 % methanol. The extract was concentrated *In-vaccuo*. 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 phytoconsitutents

The concentration of total phenolic content,[13] tannin, [14] carbohydrate, [15] Vitamin C, [16] and Vitamin E [17] 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 Trease.[18]

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.

Group 3 - 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. The animals were sacrificed by decapitation in anesthesed condition. Volatile anesthetic agent was used used for anesthesia. 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 Folch.[19] Plasma was separated by centrifuged at 2000 rpm for 10 minutes. Various

antioxidants like Lipid peroxidation (TBARS),[20] Glutathione peroxidase (GPx), [21] Reduced Glutathione (GSH), [22] catalase [23] were analyzed. Plasma total cholesterol and High Density Lipoprotein – Cholesterol (HDL-C) concentrations were determined using enzymatic kits from Randox Laboratories Ltd., United Kingdom. [24] HDL-C was analyzed after precipitation of apo B–containing lipoproteins with dextran sulfate.[25] In heart homogenate, total cholesterol and HDL concentration were estimated by following the method of Zak. [26] Triglycerides concentration of Plasma and heart homogenate (TGL) concentrations were determined by Foster. [27] Very Low Denstiy Lipoprotein (VLDL) is TGL/5. Low Density Lipoprotein - Cholesterol (LDL-C) concentrations were then determined using the Friedewald equation. [28]

Statistical analysis

Values are Mean ± Standard Error (SE) (n=6). Significant difference has been observed using one Way Analaysis 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 phyto-consituents in 85 % methanolic extract was found to be lower than that of whole raw plant (Table 1).

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

Fructose administration is observed to increase the level of lipid profile like total cholesterol, LDL, VLDL and TGL in both plasma and heart homogenate ($p<0.05$, Table 3). On treating animals with EA extract at the dose of 150 and 250 mg/kg b.wt. all the above mentioned lipid profile are found to be decreased significantly ($p<0.05$). Likewise, the HDL level is seen to be decreased in diseased animals and increased in treatment ($p<0.05$, Table 3).

TBARS, the reflection of oxidative stress is observed to be increased in both plasma and heart homogenate of diseased animals and return back to normal level in treatment ($p<0.05$, Table 4, 5). The antioxidants like GSH, GPX and catalase is noticed to be decreased in

plasma and heart homogenate of diseased animals ($p<0.05$, Table 4, 5). Pretreating animals with extract is noted to increase the level of antioxidants.

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

S.No.	Sample	Concentration (%)	Concentration (%)
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±0.00003
4	Vitamin C	1.97±1.23	0.0232±0.0003
5	Vitamin E	1.98 ±0.23	0.0139±0.0002

Values are Mean ± SD.

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

Phyto constituents	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

Values are Mean ± SE. (n=6). Significant difference has been observed using one Way ANOVA (DMRT). Values not sharing common alphabets are differ significantly at $p<0.05$.

Table 4: Effect of EA extract in plasma antioxidants of fructose induced hyperlipidemic rats

S.No	Experimental model	TBARS (nMol of Malondialdehyde/ mg of protein)	GSH (µg of GSH/mg of protein)	GST (nMol of CDNB-GSH conjugate formed/min/mg of protein)
1.	Group 1	0.02±0.006 ab	2.2±0.05 b	1.3±0.01 c
2.	Group 2	0.05±0.01 b	1.4±.0.01 a	0.58±0.02 a
3.	Group 3	0.02±0.01 ab	1.75±0.02 ab	0.89±0.02 b
4.	Group 4	0.01±0.008 a	2.0±0.01b	1.12±0.01 c

Values are Mean ± SE. (n=6). Significant difference has been observed using one Way ANOVA (DMRT). Values not sharing common alphabets are differ significantly at $p<0.05$.

Table 5: Effect of EA extract in heart antioxidants of fructose induced hyperlipidemic rats

S.No	Experimental model	TBARS (nMol of Malondialdehyde/ mg of protein)	Catalase (µMol of H_2O_2 used/min/mg of protein)	GSH (µg of GSH/mg of protein)	GST (nMol of CDNB-GSH conjugate formed/min/mg of protein)
1.	Group 1	0.34±0.15 ab	33.39±3.35 c	2.3 ± 0.02 d	1.2 ± 0.01 c
2.	Group 2	1.16±0.01 b	1.34 ± 0.01 a	1.5 ± 0.01 a	0.6 ± 0.03 a
3.	Group 3	0.49±0.03 ab	12.56 ± 9.65 b	1.8 ± 0.02 b	0.9 ± 0.01 b
4.	Group 4	0.25±0.061 a	42.90 ± 4.05 d	2.1 ± 0.03 c	1.1 ± 0.02 c

Values are Mean ± SE. (n=6). Significant difference has been observed using one Way ANOVA (DMRT). Values not sharing common alphabets are differ significantly at $p<0.05$.

DISCUSSION

EA is a rich source of phytoconstituents like tannin, Vitamin C, 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 most of the phytoconstituenst like flavonoids, tannin etc. Based on the presence of higher concentration of phyto-constiteunts, 85 % methanolic extract of EA alone 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 hypoglyceamic effect of EA has been reported earlier, [12] the hypoglycaemic effect might be related with insulin resistance. The

hypocholesterolaemic 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 hyperlidemia is underway. Some mechanisms of high fructose diet induced hypertriglyceridemia is insulin resistance in rats.[29] Although fructose in the diet alters the activity of several enzymes and regulates hepatic carbohydrate metabolism, leading to hepatic insulin resistance. [30] and hypertriglyceridemia, [31] 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 hyperlipidemia,[32] 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 liver. [33] 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 rats. [34] In our study, administration of fructose for 21 days significantly increased the glucose, insulin and triglyceride levels similar to an earlier study. [15] 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 cholestrol, TGL, VLDL and LDL is observed in tissues. These findings are consistent with the results of Michaelis. [35] The increased conversion of Carbon from fructose in to glycerol-3-phosphate might be responsible for the elevated level of TGL in fructose administered animals. [36]

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-fold. [37] The EA extract might be an inhibitor of HMG CoA reducatse which is responsible responsible for the observed decrease in the level of lipid profile.

ROS can be formed in the heart, and other tissues, by several mechanisms; they can be produced by xanthine oxidase (XO), NAD(P)H oxidases, cytochrome P_{450}, by autooxidation of catecholamines and by uncoupling of NO synthase (NOS) . [38] Apart from lipid profile we

have also estimated the level of TBARS in antioxidants in fructose administered animals. Since oxidative stress is the major factor responsible for the development of age related diseases and other cardiovascular diseases, we have estimated the antioxidants. The antioxidant activity of EA has been reported earler by Jaishree in in-vitro studies. [39]

The ascorbic acid concentration of EA is reported in the Table 1. 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 milieu. [40]

The concentration of flavonoids and polyphenolic compound of EA are mentioned in Table 1. Arai [41] has noted that the intake of flavonoids inversely correlates with the plasma total cholesterol and LDL cholesterol concentrations. Polyphenols reduce the susceptibility of LDL to oxidation in *In-vitro*. [42]

CONCLUSION

In conclusion, EA extract is a rich source of phytoconsituents. 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, TGL and 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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