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(Research Article)

Attenuation of Fructose Induced Hyperlipidemia of *Enicostemma axillare*

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ABSTRACT

Hypercholesterolemia has become the leading cause for the development of various diseases. It has drawn the attention of pharmaceutical companies to turn towards the herbal products, having fewer side effects. Preliminary phytochemical analyses were carried out in different extracts of Enicostemma axillare (EA) like hexane, chloroform, ethyl acetate and 85 % methanol. The hypocholesterolaemic effect of 85 % methanolic extract of EA were 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 activity and HDL significantly ($p<0.05$).The results reveal that EA is a rich source for phytoconstituents like tannin, Vitamin C and Vitamin E and can be used as a potent hypocholesterolemic and antioxidant agent in pharmaceutical industry. In the present research, the hypocholesterolaemic activity of EA along with their phytochemical evaluation has been done.

Key Words: Antioxidants, Hypocholesterolaemia, *Enicostemma axillare*, Fructose, Lipid profile.

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. However, a healthy diet is felt important for both prevention and treatment of cardiovascular disease.[1] Hypercholesterolaemia is a well-recognized risk factor for coronary artery disease.[2] "Atherosclerosis" is the primary factor leading to coronary heart disease. It is the commonest cause of death in industrialized world in addition to stroke and peripheral vascular disease, which are treated as a major cause of morbidity and mortality.[3]

Increased intake of food may be related to oxidative stress[4,5] has observed that increased caloric intake is an important factor in 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 hyperlipidaemic rabbits.[6] In addition, flavonoids and phenolic compounds 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.[8] 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 anti-tumor 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 attempted to quantify the phytoconstituents in different extracts of EA along with the evaluation of hypocholesterolaemic and antioxidant activity of phyto-constituents rich extract.

MATERIALS AND METHODS

Plant Materials

The root of EA was collected from Madurai district, Tamil Nadu, 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, Tamil Nadu, 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 desiccator until used for experiments. The yield of extract was calculated as 1.86 % in hexane, 2.52 % in chloroform, 1.56 % in ethyl acetate and 3.34 % in 85 % methanol extract.

Preliminary Phytochemical Analysis

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 and alkaloids by following the method of Trease and Evans (1996).[18]

Evaluation of EA on Hypocholesterolaemic Activity

Experimental animals

Albino Wistar Rats of 150-200 g were obtained from Central GLP complainant animal house, Centre for Advanced Research in Indian System of Medicine, SASTRA University, Tamil Nadu, 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 pellets of laboratory animals 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 consisted of six animals. 10 % fructose was used as inducing agent for hyperlipidaemia. 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 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 *et al.,* (1957)[19]. Plasma was separated by centrifuging at 2000 rpm for 10 minutes. Various antioxidants like Lipid peroxidation (TBARS)[20] Glutathione peroxidase (GPx)[21] Reduced glutathione (GSH)[22] and 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 and Dunn (1973).[27] Very Low Density Lipoprotein (VLDL) is TGL/5. Low Density Lipoprotein - Cholesterol (LDL-C) concentrations were then determined using the Friedewald equation.[28]

Statistical analysis

Values are of Mean ± Standard Error (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 found to differ significantly at $p<0.05$.

RESULTS

The EA was observed to be a rich source of phytoconstituents like phenol, tannin, carbohydrate and, vitamins like Vitamin C and Vitamin E. The percentage yield of all the phytoconstituents in 85 % methanolic extract was found to be lower than that of completely raw plant (Table-1).

Qualitative analysis of EA extract showed the presence of various phytoconstituents. alkaloids and phytosterol were eluted in hexane and chloroform extracts. Flavonoids, polyphenols and phytosterol were eluted in ethyl acetate extract.

85 % methanolic extract has proved positive for flavonoids, polyphenols, phytosterol and carbohydrate (Table-2).

Fructose administration was 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 III). On treating animals with EA extract at the dose of 150 and 250 mg/kg b.wt., all the above mentioned lipid profiles were found to be decreased significantly ($p<0.05$). Likewise, the HDL level was seen to be decreased in diseased animals and increased in treatment ($p<0.05$, Table-3).

TBARS, the reflection of oxidative stress was 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 IV and V). The antioxidants like GSH, GPX and catalase were noticed to be decreased in plasma and heart homogenate of diseased animals ($p<0.05$, Table- 4 and 4). Pretreating the animals with extract was noted to increase the level of antioxidants.

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 phytoconstituents (Table I). Among the different extracts of EA, 85 % methanolic extract contains most of the phytoconstituents like flavonoids, tannin, etc. Based on the presence of higher concentration of phytoconstituents, 85% methanolic extract of EA alone is taken for further pharmacological evaluation.

Hyperlipidaemia 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 hypolipidaemic agents. In this present research work, we have attempted to evaluate the hypolipidaemic and antioxidant activity of EA in fructose-induced hyperlipidaemia. Though the exact mechanism for the hypoglyceamic effect of EA has been reported earlier[29], the hypoglyceamic 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 hyperglyceamia and hyperlipidaemia are involved.

Research findings on the molecular mechanism for the fructose-induced hyperlidaemia are underway. Some mechanisms of high fructose diet induced hypertriglyceridaemia are 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 hypertriglyceridaemia[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 hyperlipidaemia[32] (Park et. al., 1992), insulin resistance, hyperinsulinaemia 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 the most suitable one for the production of insulin resistance in rats.[34] In our study, administration of fructose for 21 days has significantly increased the glucose, insulin and triglyceride levels similar to an earlier study.[35] Administering EA (150 mg/kg) has prevented the development of hyperlipidaemia.

Lipid changes observed in fructose-treated rats are noted to have elevated levels of cholesterol, TGL, LDL, VLDL and HDL-C. Accumulation of cholestrol, TGL, VLDL and LDL is observed in tissues. These findings are consistent with the results of Michaelis et. al., 1975[36]. 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.[37] 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.[38] The EA extract might be an inhibitor of HMG CoA reducatse, which is 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 P450, by auto-oxidation of catecholamine and by uncoupling of NO synthase (NOS).[39] Apart from lipid profile, we have also estimated the level of TBARS the marker of 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 earlier in *in-vitro* studies.[40]

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 milieu.[41]

The concentration of flavonoids and polyphenolic compound of EA are mentioned in Table I. Arai et. al., (2000)[42] 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*.[43]

CONCLUSION

EA extract is a rich source of phytoconstituents. Among the different extracts of EA, 85% methanolic extract is rich in phytoconstituents. The pharmacological evaluation of EA has proved that, 85% methanolic extract of EA exhibits hypolipidaemic activity by decreasing the level of total cholesterol, LDL, VLDL, TGL and increasing 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 hypolipidaemic and antioxidant activity. In future, the mechanism of action and isolation of active compound responsible for the hypocholesterolaemic effect of EA should be carried out.

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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

Note: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

Note: 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

Note: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

Note: 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 $p<0.05$.

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