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Antioxidant activity of *Enicostemma axillare* L., on fructose induced heart disease in swiss albino rats

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ABSTRACT

The antioxidant effect of the ethanolic extract of *Enicostemma axillare*, an indigenous ayurvedic medicinal plant used in India, was studied in rats with fructose-induced cardiac diseases. Rats were divided into four groups: Groups 1 received Normal feed with water. Groups 2 received Fructose treated (The oral administration of 10g of fructose/ 100ml of distilledv water/kg body weight for 21 days), Groups 3 received Fructose and low dose of Enicostemma axillare (150mg/ kg B.wt.)].Groups 4 received Fructose and high dose of Enicostemma axillare treated [oral administration of fructose (10g/ 100ml distilled /kg b. wt) and Enicostemma axillare (250mg/ kg B.wt.)]. For 21 days. The results showed significantly elevated level of serum and tissue thiobarbituric acid reactive substances, and significantly lowered activities/levels of antioxidants such as, catalase, glutathione-S-transferase and reduced glutathione in fructose -treated rats compared with control rats. Administration of ethanolic extract of *Enicostemma axillare* to rats with fructose -induced liver injury significantly decreased the levels of serum and tissue thiobarbituric acid reactive substances and significantly elevated the activities of catalase, glutathione-S-transferase and reduced glutathione in the tissues compared with unsupplemented fructose -treated rats. These findings suggest that ethanolic extract of *Enicostemma axillare* has a modulatory effect on fructose -induced hepatotoxicity in rats.

Keywords: Antioxidant, *Enicostemma axillare*, Lipid peroxidation and Fructose

INTRODUCTION

Heart disease is the leading cause of death, many of the risk factors for heart disease are influenced are by life style. For example smoking, lack of exercise and consumption of a high fat diet all contribute to risk. A healthy diet is important for both prevention and treatment of cardiovascular disease [1, 2].

Atherosclerosis can be defined as the "Focal pathological phenomenon characterized by thickening, hardening, hardening of arteries due to accumulation of lipid, carbohydrates,blood products, fibrous tissue and calcium deposits within the subendothelial space[3]**.** The characteristic feature of atherosclerosis is the buildup of fatty deposits called Deposits called "plague", along the inner artery walls. Subsequently leading to narrowing of the arterial passage way and eventually impaired circulation [4]**.** The atherosclerosis can cause blood clots to form that will ultimately stop blood flow. If this happens in the arteries supplying the heart is stroke where a portion of brain tissue dies.

Hypercholesterolemia is silent. There are no symptoms that are obvious to the naked eye. It is diagnosed by a blood test or after a heart attack or stroke occurs. High plasma cholesterol also impairs the functional intergrity of coronary vasculature and increases ischemia- reperfusion injury to the Heart [5].

Antioxidant compound can protect against lipid peroxidation, but prior presence is required for their protective action. These include vitamin E, ascorbic acid and GSH, which stop the propagation of the lipid peroxidative process [6]. Antioxidants are substances which are capable of recording on preventing the process of fat oxidation are they prevent the oxidation of unsaturated fats. Oxidative cell injury induced by reactive species is protected by the antioxidant defense system, including enzymatic and non-enzyomatic components. Antioxidant strategies, including administration of pharmacological or dietary agent are based on two main mechanisms: The inhibition of reactive species generation and the enhancement of Reactive species elimination. The agent the acting according to the latter mechanism include antioxidant enzyme, which catalyze reactive species degradation, Scavenges neutralizing Reactive species and agents that may enhance intrinsic antioxidant forces [1].

Increased intake of a food may be related to oxidative strees [7] an imbalance between oxidant and antioxidant system in favour of the formes [8]**.** Recent research in our laboratory has shown that increased caloric intake is an important factor decreasing the mitochondrial membrane fluidity and increasing the reactive oxygen species generation [9]. Fructose is readily absorbed from the diet and rapidly metabolized principally in the liver. Fructose can provide carbon atoms for the both the glycerol and the acyl portions of triglyceride. Fructose is thus a highly efficient inducer of denovo lipogenesis. High concentrations of fructose can serve as a relatively unregulated source of acetyl CoA. In contrast toglucose, dietary fructose does not stimulate insulin or leptin (which are both important regulators of energy intake and body adiposity). Stimulated triglyceride synthesis is likely to lead to hepatic ionsulin sensitivity, as well as increased formation of VLDL particles due to hgher substrate availability, increased apoB stability, and MTP, the critical factor in VLDL assembly.

There are numerous studies in which dietary fructose has been shown to induced hyperlipidemia in rodents [10] Herman et al**.,[11]** reported that's fed a high- fructose diet had sustained elevation in serum triacylglycerol. Increases in VLDL secretion can then leads to chain in other lipoprotein and lipids, such as LDL, TG, Cholesterol. In this context, Kelley et al**.,** [12] hypothesized that pro –oxidant stress response pathways may mediate hepatic increases in VLDL secretion and delayed clearance upon fructose feeding.

The presence invstigation has been undertaken to find out the pharmacological effectiveness of the Hydroalcoholic extract of Enicostemma *axillare* which studies evaluate the potentially of hypocholesterolemic and antioxidant activity.

Swertiamarin, gentiocrucine, enicoflavine, genkwanin, isovitexin, swortistin, sapanarin, 5-oglycosylisoswertisin,gentiocrycine, swertiamarin tetra acetate, 3- acyl- 3,4 dehydrogentiopicroside, ophelic acid, n-hexacosnal, hepatocosane, non acosane, myristic acid, stearic acid, oleic acid, gentianine , betulin, alkaloids.

The plant, E. axillare is bitter tonic, acrid, thermogenic, digestive, carminative, stomachic, luxtive, anthelmintic, anti-inflammatory, liver tonic, Astringent, colic, belminthiasis, abdominal ulcers, hernia constipation, dropsy, swelling, liver disorders, glycosuria, leprosy, skin disese, puritis, intermittent fever and malaise.

This study was designed to test the hypothesis that *Enicostemma axillare* would reduce fructose induced toxicity by modulating lipid peroxidation and antioxidants.

MATERIALS AND METHODS

Plant material

The leaves of *Enicostemma axillare* were collected from S.T.E.T Medical plant garden, Mannargudi, Thiruvarur District and authenticated by Botany Department of A.V.V.M. Sri Pushpam College, Poondi. After anthentification the plant material were washed under running tap water.

Preparation of Plant Extract

Enicostemma axillare leaves were dried (without direct sunlight) and converted to powder form. The powder obtained was successively extracted in methanol and distilled water by using soxhlet apparatus. It was stored at 4°C until used when needed the residual extract was suspended in distilled water and used in the study.

Animals

A healthy swiss albino rats were housed in well ventilated hygienic atomosphere. Animals with 200 – 300g were used our study. Animals were fed with commericial rat feed (Saidurga feeds & foods, Bangalore) and tap water adlibitum. After randamization into various groups, the rats were acclimatized for a period of 2-3 days in the new environment before initiation of experiment.

Chemicals

All of the chemicals were of analytical grades and were obtained from Central Drug House Pvt. Ltd (New Delhi, India).

Groups 1 received Normal feed with water. Groups 2 received Fructose treated (The oral administration of 10g of fructose/ 100ml of distilledv water/kg body weight for 21 days), Groups 3 received Fructose and low dose of *Enicostemma axillare* (150mg/ kg B.wt.)].Groups 4 received Fructose and high dose of *Enicostemma axillare* treated [oral administration of fructose (10g/ 100ml distilled /kg b. wt) and Enicostemma axillare (250mg/ kg B.wt.)].

Experiment design

In the experiment, a total of 16 rats were used. The rats were divided in to following 4 groups of 6 each.

Group I : Normal feed with water

Group II : Fructose treated (The oral administration of 10g of fructose/ 100ml of distilledv water/kg body weight for 21 days),

Group III : Fructose and low dose of *Enicostemma axillare* (150mg/ kg B.wt.).

GroupIV : received Fructose and high dose of *Enicostemma axillare* treated [oral administration of fructose (10g/ 100ml distilled /kg b. wt) and *Enicostemma axillare* (250mg/ kg B.wt.).

Sample Collection

After 21 days of herbal treatment, the blood samples were collected from the anaesthetized rats by puncturing the orbital sinus. After the collection of blood, it was allowed to stand for 10 mts.

Biochemical measurements

Tissue and plasma TBARS [13], CAT [14], GST [15],], GSH [16] were determined.

Statistical analysis

Results are expressed as mean ± SE from six observations

RESULT

Our research is focus on the antioxidant activity of *Enicostemma axillare* which ais evaluated by assessing the Biochemical parameters such as and Antioxidant enzymes namely, catalase, TBARS, GSH, GST, were determined in the plasma and Heart. The results clearly pin points and antioxidant activity of *E. axillare* in hyper cholesterolemic rats.

Table I represents the level of TBARS in plasma, in our experimental study. The TBARS level in plasma was markedly increased in the disease group rats, when compared to normal group. Then the level was decreased in the hydroalcoholic extract of high dose (250mg/kg) treated group and low dose (150mg/kg) treated group. The level of TBAR showed reverse effect of above parameters. The level is increased after the administration of low dose treated groups. The high dose (250 mg/kg) treated groups showed significant results when compared to low dose treated groups.

Table II represents the level of Antioxidant activity in plasma, in our experimental study. The catalase, GSH, anf GST level of antioxidant Activity in plasma was markedly decreased in the disease group rats, when compared to normal

group. Then the level was increased in the hydro alcoholic extract of high dose (250mg/kg) treated group and low dose (150mg/kg) treated group. The level of TBAR showed reverse effect of above parameters. The level is decreased after the administration of low dose treated groups. The high dose (250 mg/kg) treated groups showed significant results when compared to low dose treated groups.

Table III represent the level of antioxidant activity in Heart, in our experimental study. The catalase, GSH, and GST level of antioxidant activity in Heart was markedly decreased in the disease group's rats, when compared to normal group. Then the level was increased in the hydro alcoholic extract of high dose (250mg/kg) treated group and low dose (150mg/kg) treated group. The level of TBARS showed reverse effect of above parameters. The level is decreased after the administration of low dose treated groups. The high dose (250mg/kg) treated groups showed significant results when compared to low dose treated groups.

TABLE I

Table showing level of TBARS in plasma and heart.

S.No	Groups	TBARS	
		Plasma (mM/100ml)	Tissue (mM/100g tissue)
1.	GP-I	0.02+0.006	0.34+0.15
2.	GP-II	0.05+0.01	1.16+0.01
3.	GP-III	0.02+0.01	0.49+0.03
4.	GP-IV	0.01+0.008	0.25+0.061

(Values are mean ± S.E from 6 rats in each group).

TABLE II

Table showing level of catalase, GSH and glutathione-s- transfarase in plasma

S.No	Groups	Catalase (U^B/mg protein)	GSH (mg/100g tissue)	GST (U^C/mg protein)
1.	GP-I	37.2+3.05	2.2+0.05	1.3+0.01
2.	GP-II	30.5+3.4	1.4+0.01	0.58+0.02
3.	GP-III	34.36+04	1.75+0.02	0.89=0.02
4.	GP-IV	39.92+4.38	2.0+0.01	1.12+0.01

(Values are mean ± S.E from 6 rats in each group).
U^B µ moles of H2O2 liberated/min/mg protein
U^C µ moles of CDNB conjugate formed/min/protein

TABLE III

Table showing level of catalase, GSH and glutathione-s- transfarase in heart.

S.No	Groups	Catalase (U^B/mg protein)	GSH (mg/100g tissue)	GST (U^C/mg protein)
1.	GP-I	33.39+3.35	2.3+0.02	1.2+0.01
2.	GP-II	1.34+0.01	1.5+0.01	0.6+0.03
3.	GP-III	12.56+9.65	1.8+0.02	0.9+0.01
4.	GP-IV	42.90+4.05	2.1+0.03	1.1+0.02

(Values are mean ± S.E from 6 rats in each group).

U^B µ moles of H2O2 liberated/min/mg protein
U^C µ moles of CDNB conjugate formed/min/protein

DISCISSION

The body has an effective mechanism, to prevelant and neutralizes the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as catalase, GSH etc. When the balance between reactive oxygen species production and antioxidant defenses lost, "oxidant stress" results which through a series of events deregulated the cellular functions leading to pathological conditions.

Specific enzymes such as superoxide dismutase, catalase and glutathione peroxide can protect the organism against the reactive oxygen species effects [17].Increased consumption of fruits and vegetables may protect of antioxidants [18].

The most abundant oxidative free radical generated in living cells are superoxide anions and derivatives particularly the highly reactive and damaging hydroxyl radicals which induces peroxidation of cell membrane lipids. The end products of lipid peroxidation are known to induce cellular damage and have been known to be responsible for oxidative free radical induced human disease [19].

The potential toxicity of oxygen has been attributed to formation of H_2O_2; recently, however, the case with O_2 can be reduced in tissue to the superoxide anion free radical $(O2^{\cdot})^-$ and the occurrence of super oxide dismutase in aerobic organism have suggested that the toxicity of O_2 is due to its conversion to superoxide is formed when reduced are deoxidized by molecular oxygen. Superoxide dismutase is a family of metallo enzyme which are known to catalyze dismutation of superoxide radical to H_2O_2 and molecular oxygen.

Elevated level of TBARS in the plasma and Heart of group II (Disease) rats is a clear manifestation of excessice formation of free radicals and activation of lipid peroxidation.

Since thiobarbituric acid is not highly specific for malondialdehyde, the results of this measurement are generally expressed as the measurement of thiobarbituric acid reactive substances TBARS. Erythrocyte TBARS production was significantly higher in patients with coronary atherosclerosis than in the controls. In conclusion, our results indicate that erythrocytes from patients with coronary atherosclerosis are more susceptible to oxidation than those of controls and that these patients have lowered antioxidant capacity as revealed by decreased plasma levels of vitamins C and E [20]

Catalase is one of the important enzymes in the supportive team of defense against ROS. Catalase is hemoprotein containing four heme groups. This enzyme for catalyses the decompositions of H_2O_2 to H_2O and oxygen and thus protecting the cell from oxidative by H_2O_2 and OH [21]. The generation of H_2O_2 may also lead to inactivation of this enzyme. Catalase, which acts as preventive antioxidant plays on important role in protection against the deleterious effect of lipid peroxidation [22]. The findings in our study reveals that the activity of CAT was significantly lowered in heart and plasma of fructose treated rats may increase their susceptibility to oxidative injury. After treatment with *Ecinostemma axillare*, evaluate the activity of catalase was significantly increased.

Glutathione-S-Transfuse another scavenging enzyme, binds to many different lipophilic compounds. So it would be expected to bind to lipophilic CCl_4 and act as an enzyme for GSH in conjugation reaction. GST is a member of a complex supergene-encoded family of detoxification may be due to the down regulation of glutathione-S-transferase subunits. The depression of hepatic GST activity might will be an adaptive response to the increased production of oxidized glutathione in the tissue of fructose hepatotoxic animals. Since the efflux of oxidized glutathione and glutathione-S-transferase used the same transport system. The decrease in hepatic GST activity may favour the excretion of oxidized glutathione, there by maintaining the thiol redox status in tissues. The moderate decline in GST activity in liver seen in this study might have been due to the decreased availability of GSH this is consistent with a reported study [23]. Which showed a retection of GST activity in liver? These findings led to the conclusion that GSH and GSH dependent enzyme system may be directly related the pathogenic mechanism of hepatitis. In our study use observed a significant reduction in GST levels

in plasma and heart homogenates in fructose induced rats.

GSH is a tripe tide consisting of glutamate, cysteine and glycine. It acts as an antioxidant both intracellullary and extracellularly in conjuction with various enzymatic process that reduced hydrogen peroxide and hydro peroxides as GSH is oxidized to GSSG and other mixed disulfides. GSH is produced in the liver and maintained higher concentration is most tissue**.** There is substantial evidence that oxidative injury plays a major role in the atherosclerotic process. Thus, antioxidants may protect against development of atherosclerosis. Glutahione, an intracellular tripeptide with antioxidant properties, may beprotective. This study that low GSH in adolescent boys is a significant independent predictor of parental CHD, in addition to elevated LDL cholesterol, low HDL cholesterol, and elevated total serum homocysteine concentrations [24]. In our study use observed a significant reduction in GSH levels in plasma and heart homogenates in fructose treated rats.

GSH is a tripeptide consisting of glutamate, cysteine and glycine. It acts as an antioxidant both intracellularly and extracellulary in conjunction with various enzymatic process that reduced hydrogen peroxide and hydroperoxides as GSH is oxidized to GSSG and other mixed disulfides. GSH is produced in the liver and maintained at a higher concentration is most tissue [25]. GSH is a critical determinant of tissue susceptibility to oxidative damage. The depletion of GSH levels has been shown to be associated with enhanced fructose toxicity [26].

CONCLUSION

In the present bring out the antioxidant activity on *Enicostemma axillare* against CCl_4 induced hepatotoxicity in rats. The use of *Enicostemma axillare* as chronic cough syrup seems to be effective. To rationalise use of the plant however, more work needs to be carried out at molecular level.

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