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Hypolipidaemic and antioxidant effect of *Enicostemma littorale* Blume

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ABSTRACT

Objective: To investigate the hypolipidaemic and antioxidant effects of aqueous leaf extract of *Enicostemma littorale* (*E. littorale*) Blume (Ens) against ethanol induced hepatic injury in albino rats. **Methods:** Male albino rats of six numbers in each group were undertaken for study. Hypolipidaemic and antioxidant effect of *E. littorale* Blume (Ens) aqueous leaf extract at a dosage of 250 mg/kg bw was evaluated. **Results:** Levels of serum and tissue cholesterol, triglycerides and free fatty acids were elevated and levels of tissue thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxide were increased in ethanol treated rats. The activity levels of liver antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione-s-transferase (GST) were decreased. After administration of extract of *E. littorale* Blume, levels of cholesterol, triglycerides and free fatty acids were decreased in serum and liver tissue, levels of TBARS and lipid hydroperoxide were decreased, and liver antioxidant enzymes were increased in liver tissue. **Conclusions:** It can be concluded that the aqueous leaf extract of *E. littorale* Blume (Ens) has potent restorative effect on hyperlipidaemic and oxidative stress.

1. Introduction

Ethanol is one of the major causes of chronic liver diseases, including a variety of liver lesion such as steatosis, steatohepatitis, hepatic fibrosis, cirrhosis and hepatocellular carcinoma. Ethanol-induced liver injury has been extensively reported by studies *in vivo*. The 'two hit' hypothesis has been well accepted as the first fat accumulation in hepatocytes and the second cell damage^[1]. Long-term heavy consumption of alcohol plays a major role in the development of alcohol-related liver damage^[2]. The close relation between ethanol and liver damage is mainly due to the fact that about 80% of ingested alcohol is metabolized in the liver. Ethanol is metabolized into cytotoxic acetaldehyde by alcohol dehydrogenase enzyme in the liver and acetaldehyde is oxidized to acetate by aldehyde oxidase or xanthine oxidase giving rise to reactive oxygen species (ROS) *via* cytochrome P450 2E1 (CYP 2E1)^[3]. Much of the cell damage that occurs during liver degeneration is believed to be caused by free radicals, highly reactive oxygen species liberated during alcohol metabolism. These radicals react with cell membrane and induce lipid peroxidation, which has been

implicated as important pathological mediation^[4] in many clinical disorders such as heart disease, diabetes, cancer and liver disease. The management of liver diseases is still a challenge to the modern medicine. Several herbal products are available all over the world with an acclaimed hepatoprotective activity. Plant drugs are frequently considered to be less toxic and free from side effects^[5]. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices as well as in traditional systems of medicine in India. There are a number of evidences indicating that natural substances from edible and medicinal plants exhibit strong antioxidative activity, and could work against hepatic toxicity caused by various toxicants^[6,7]. *Enicostemma littorale* (*E. littorale*) Blume (Family: Gentianaceae) is a perennial, tropical traditional medicinal herb with sessile lanceolate leaves, flowers arranged in clusters, fruit in a capsule. The plant is locally used for its medicinal properties in Tamilnadu, India, such as antiinflammatory, antiulcer activity^[8], hypoglycaemic^[9], and antimalarial activities^[10]. The present study aims to investigate the hypolipidaemic and antioxidant effects of *E. littorale* on ethanol induced hepatotoxicity in male albino rats.

2. Materials and methods

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2.1. Plant material

Leaves of *E. littorale* Blume were collected during September to January (2009–2010) in and around Vellore District, Tamilnadu, India. The leaves were cleaned, shade dried, authenticated and voucher specimen was deposited in the Department of Botany, Voorhees College, Vellore, Tamilnadu.

2.2. Preparation of plant extract

The shade dried leaves were powdered separately in an electric blender and stored at 5 °C for further use. A total of 100 g of the leaf powder was taken and mixed with 500 mL of distilled water and stirred magnetically in separate containers overnight at room temperature. The residue was removed by filtration and the aqueous leaf extract was concentrated under vacuum to get 22% solid yield. The plant extract was tested for hypolipidaemic and antioxidant effects in the albino rats at the selected optimum dosage of 250 mg/kg bw and administered orally in aqueous solution.

2.3. Animals

Wistar strain male albino rats (160–180 g) were obtained from Tamilnadu Veterinary and Animal Science University, Chennai, India. The animals were acclimatized to the laboratory conditions, fed with standard pellet diet supplied by Hindustan Lever Ltd., Bangalore, India and had free access to water. The experiments were designed and conducted in accordance with guidelines of Institutional Animal Ethics Committee.

2.4. Induction of hepatotoxicity

Distilled ethyl alcohol was obtained from Hayman Ltd., England. The hepatotoxic dose was standardized as 5 mL of 20% ethanol and 7.9 g/kg bw of the ethanol was orally given to the rats for 45 days, using an intragastric tube daily as described earlier by Patel *et al*[11].

2.5. Protocol of study

The rats were randomly divided into three groups. Group

I: Normal rats. Group II: Rats were treated with ethanol (7.9 g/kg bw by oral administration) daily for 45 days using intragastric tube. Group III: The ethanol administered rats were fed with leaf extract of *E. littorale* Blume at 250 mg/kg bw for 45 days, *i.e.* from 46th to 90th day.

2.6. Estimation of biochemical parameters

The tissue and serum lipids were extracted according to the method described by Folch *et al*[12]. The lipid parameters including total cholesterol[13], tissue triglycerides[14], non-esterified free fatty acids[15], phospholipids[16], were estimated. The level of lipid peroxidation in liver tissue was estimated by measuring the activity of malondialdehyde and other thiobarbituric acid reactive substances (TBARS) with thiobarbituric acid (TBA)[17]. The lipid hydroperoxides in the tissue were estimated[18]. The tissue levels of enzymatic antioxidants *viz.* superoxidase dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione-S-transferase (GST) were estimated[19–22].

2.7. Statistical analysis

The results were expressed as mean $\pm$ SD. Statistical analysis was carried out by using one way ANOVA with standard statistical software package of social science (SPSS) version 12.

3. Results

3.1. Studies on ethyl alcohol induced hyperlipidaemia and oxidative stress in male albino rats

The serum levels of cholesterol, triglycerides, free fatty acids and phospholipids were significantly elevated by 81.00%, 64.22%, 73.72% and 54.86%, respectively in the ethanol fed animals as compared with normal animals ($P < 0.01$).

Similarly, administration of ethanol caused a significant increment in the levels of cholesterol, triglycerides, free fatty acids by 66.64%, 60.26%, and 72.81%, respectively in the liver tissue ($P < 0.01$) (Table 1). On the other hand, the levels of phospholipids in liver tissue were significantly declined (66.84%).

Table 1

Levels of cholesterol, triglycerides, free fatty acids and phospholipids in serum and liver tissue (Mean $\pm$ SD).

	Parameters	Normal group	Ethanol fed group	Treatment group
Serum	Cholesterol (mg/dL)	76.24 $\pm$ 2.11	138.42 $\pm$ 3.21*	91.16 $\pm$ 1.37*
	Triglycerides (mg/dL)	74.12 $\pm$ 2.16	121.72 $\pm$ 2.87*	87.26 $\pm$ 1.61*
	Free fatty acids (mg/dL)	61.31 $\pm$ 1.90	106.51 $\pm$ 3.12*	70.26 $\pm$ 1.79*
	Phospholipids (mg/dL)	77.62 $\pm$ 2.62	120.21 $\pm$ 2.78*	94.31 $\pm$ 2.10*
Liver tissue	Cholesterol (mg/100 g tissue)	297.21 $\pm$ 5.10	495.29 $\pm$ 5.87*	319.00 $\pm$ 4.12*
	Triglycerides (mg/100 g tissue)	362.65 $\pm$ 4.74	581.21 $\pm$ 6.26*	398.16 $\pm$ 5.37*
	Free fatty acids (mg/100 g tissue)	728.69 $\pm$ 6.79	1 259.29 $\pm$ 7.62*	801.20 $\pm$ 6.21*
	Phospholipids (mg/100 g tissue)	5.61 $\pm$ 1.02	1.86 $\pm$ 0.17*	4.11 $\pm$ 0.18*

* $P < 0.01$.

Table 2Levels of TBARS, hydroperoxide and antioxidant defense enzymes (Mean $\pm$ SD).

Parameters in liver	Normal group	Ethanol fed group	Treatment group
TBARS (nm/100 mg tissue)	0.82 $\pm$ 0.07	1.81 $\pm$ 0.09*	0.88 $\pm$ 0.06*
Hydroperoxide (nm/100 mg tissue)	68.46 $\pm$ 2.81	111.26 $\pm$ 3.14*	73.16 $\pm$ 2.81*
SOD (Unit1/mg protein)	5.89 $\pm$ 0.61	2.11 $\pm$ 0.24*	4.81 $\pm$ 0.91*
CAT (Unit2/mg protein)	164.26 $\pm$ 3.14	88.61 $\pm$ 2.61*	154.51 $\pm$ 2.63*
GPX (Unit3/mg protein)	12.16 $\pm$ 0.87	5.89 $\pm$ 0.49*	9.97 $\pm$ 0.82*
GST (Unit4/mg protein)	5.16 $\pm$ 0.39	2.34 $\pm$ 0.17*	4.87 $\pm$ 0.16*

* $P < 0.01$.

TBARS: Content in tissue, expressed as μ moles of malondialdehyde (MDA)/mg protein; SOD–U1: One unit of activity was taken as the enzymes reaction which gives 50% inhibition of NBT reduction in one minute; CAT–U2: μ moles of hydrogen peroxide consumed per minute; GPX–U3: μ g of glutathione consumed per minute; GST–U4: μ moles of CDNB–GSH conjugate formed per minute.

The significant increments in the levels of tissue TBARS and lipid hydroperoxides by 122.08% and 62.51%, respectively were recorded in ethanol fed rats ($P < 0.01$). The activity levels of liver antioxidant defense enzymes *viz.* SOD, CAT, GPX, GST were significantly decreased by 64.17%, 46.05%, 51.56% and 54.65%, respectively in the liver tissue of ethanol fed animals (Table 2).

3.2. Effect of the leaf extracts of *E. littorale* Blume on ethanol induced hyperlipidaemia and oxidative stress

After administration of leaf extract of *E. littorale*, the levels of cholesterol, triglycerides and free fatty acids in serum and liver tissue were decreased. Phospholipids were significantly elevated in the liver tissue of the plant extract treated groups but declined in serum ($P < 0.01$) (Table 1).

The increased levels of TBARS and hydroperoxides in the liver tissue of ethanol fed rats were significantly reduced in the plant extract supplemented animals ($P < 0.01$). On the other hand, there was significant elevation of tissue antioxidant enzymes defense *viz.*, SOD, CAT, GPX and GST in extract treated ($P < 0.01$) (Table 2).

4. Discussion

Hyperlipidaemia is an important complication of alcohol induced liver injury[23]. Administration of ethanol can produce accumulation of cholesterol and triglycerides in the liver as well as in the blood of rats[24]. The imbalance in the lipid metabolism plays a role in aggravating the lipid peroxidation.

The hyperlipidemia and elevated lipid peroxidation are interrelated[25]. The possible mechanism is that the hyperlipidemic condition would stimulate the catabolic pathway *via* oxidative breakdown. Ethanol is rich in calories and devoid of nutrients, thus contributing to fat accumulation in the liver, and causing fatty liver complex. The main pathway for hepatic oxidation of ethanol to acetaldehyde proceeds through acetaldehyde dehydrogenase[26], which is associated with the reduction of nicotinamide adenine dinucleotide (NAD) and striking redox change associated

with metabolic disorders[27]. Reducing equivalents inhibit tricarboxylic acid cycle activity and fatty acid oxidation. Ethanol also inhibits lipoprotein export and increases fatty acids uptake[28]. Furthermore, interaction of ethanol with biological membranes including lipid and protein components can cause significant changes in the function of membranes[29]. HMG CoA reductase catalyses rate limiting in cholesterol biosynthesis and its activity directly correlates with the extent of tissue cholesterol synthesis which is significantly activated during ethanol ingestion[30]. Plasma lecithin cholesterol acyl transferase (LCAT) is the enzyme involved in the esterification of cholesterol in the plasma[31]. The enzymes lipoprotein lipase are involved in the uptake of triglycerides rich lipoprotein by extra hepatic tissue. Phospholipids are vital components of biomembranes, with peroxidation as primary target.

The results show significant increase of cholesterol in both liver as well as serum of the ethanol fed animals. This might be due to the increased activity of HMG CoA reductase which catalyzes the cholesterol biosynthesis in the liver tissue[32]. The increased serum cholesterol in the ethanol fed rats might be due to the decreased activity of the enzymes LCAT involved in the esterification cholesterol in plasma. The significant increase of the triglycerides in serum and liver tissue of ethanol treated animal signifies a simultaneous accumulation of lipoproteins. This might be due to decreased activity of lipoprotein lipase, which is involved in the uptake of triglycerides rich lipoprotein by extra hepatic tissue. However, it is supposed that increased synthesis or decreased lipid deposition or both result in simultaneous accumulation of triglycerides in the serum and liver[33].

The free fatty acid accumulation in both serum and liver of ethanol supplemented animals might be due to increased lipid breakdown. In our study an increased lipid peroxidation and decreased activity levels of antiperoxidation enzymes were recorded. The phospholipids were significantly reduced in liver tissue decreased in the serum. This may be due to increased peroxidation of the biomembrane of the hepatocyte, while the increased levels of phospholipids in the serum might be due to seepage into the serum during lipid peroxidation.

Intake of alcohol results in excessive generation of free radicals. Free radicals are the reactive oxygen species (ROS) which are known to cause oxidative damage to number of molecule in cell, including membrane–lipids, proteins, and nucleic acids^[34]. In the present study the hepatic cellular injury might be due to increased oxidative stress and thereby leading to lipid peroxidation. The level of lipid peroxidation in the ethanol fed rats was assessed by measuring the levels of TBARS and hydroperoxide in the liver tissue^[35].

The increased TBARS and hydroperoxide levels in the liver of ethanol fed animals indicate enhanced lipid peroxidation leading to tissue injury. The cellular antioxidant defense mechanism, which includes scavenging activities of enzymes *viz.* SOD, CAT, GPX and GST plays an important role in scavenging toxic intermediates of reactive oxygen species. During hepatotoxicity these enzymes might be functionally impaired due to excess generation of free radicals creating oxidative imbalance.

SOD is metalloproteins catalyzing the dismutation of superoxide anion to hydrogen and oxygen. Numerous studies have shown the importance of SOD in protecting cells against oxidative stress^[36]. The SOD activity could be decreased in tissue during alcohol ingestion. This decrease could be due to the feedback inhibition or oxidative inactivation of enzyme protein due to excess ROS generation^[37].

CAT, heme protein, catalyzes the reduction of hydrogen peroxides^[38], acts as preventive antioxidant and plays an important role in protection against the deleterious effects of lipid peroxidation^[39]. The activity levels of catalase in tissue decreased in ethanol fed animals might be due to the inhibition of CAT activity, which is suggestive of enhanced synthesis of O_2^- during the ingestion of alcohol since O_2^- is a powerful inhibitor of catalase^[40].

GPX is an enzyme with selenium in the form of selenocysteine and can catalyze the reduction of hydrogen peroxide and hydroperoxides to non toxic products. GPX has a well–established role in protecting cells against oxidative injury. GPX is non–specific for H_2O_2 and lipid peroxide generated during alcohol ingestion which are efficiently scavenged by GPX activity. The depression of this enzyme activity reflects perturbations in normal oxidative mechanism during alcohol ingestion.

GST is an important enzyme which can utilize glutathione as the substrate. GST is capable of binding with xenobiotics through covalent bond on the enzymes surface which may lead to the inactivation of the enzyme but prevent xenobiotic action. This enzyme, which is most abundant in the liver, is thought to play an important role in the biotransformation of many xenobiotics.

The cellular antioxidant defense enzymes *viz.* SOD, CAT, GPX and GST were significantly reduced in the ethanol fed rats. This might lead to decreased antioxidant defense and increased oxidative stress and thereby the tissue injury occur. Similar studies also indicate the failure of cellular antioxidant defense system during hepatotoxicity were recorded^[41,42].

The hyperlipidaemic condition in the serum and liver

tissue of ethanol fed animals restored to normal levels after the administration of the plant extract. The decreased serum cholesterol in the extract fed rats might be due to increased activity of enzyme LCAT involved in esterification of cholesterol in the plasma. The significant decrease in the triglycerides in serum and liver tissue of the plant extract fed animals might be due to decreased accumulation of lipoprotein. This might be due to increased activity of lipoprotein lipase which involved in the uptake of triglycerides rich lipoprotein by extra hepatic tissue. The significant decrease in the free fatty acid accumulation in both serum and liver tissue of plant extract fed animals might be due to decreased lipid breakdown and/or decreased lipid peroxidation and increased activity levels of antioxidant defense enzymes. The levels of phospholipids were increased in the liver tissue but decreased in the serum of plant extract fed animals. It might be due to decreased peroxidation of biomembranes of hepatocytes. Similar studies on the hypolipidemic effect of *E. littorale* Blume were reported in cholesterol fed rats^[30].

The above study revealed that ethanol induced hyperlipidaemic and oxidative stress were observed on the 46th day after a continuous administration of ethanol, but it was significantly reduced after administration of the aqueous leaf extract of *E. littorale* Blume.

In conclusion, *E. littorale* leaf extract has restorative effect on the ethanol induced hyperlipidaemia and oxidative stress. Further study is needed to evaluate the hepatoprotective effect of aqueous leaf extract of the folklore medicinal plant *E. littorale* Blume.

Conflict of interest statement

We declare that we have no conflict of interest.

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