Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2011; 3(2): 35-37

Research Article ISSN 0975 1556

Hepatoprotective Effect of *Enicostemma littorale* in Alcohol Induced Oxidative Stress in Male Albino Rats

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ABSTRACT
The aqueous extract obtained from the leaves of *Enicostemma littorale* Blume (Gentianaceae) was investigated for antioxidant effect on ethanol induced oxidative stress in male albino rats. The decreased activity level of the antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in the liver tissue of ethanol fed rat recorded elevated activity levels after the administration of leaf extract. The inhibition of lipid peroxide production in the treated animals showed an improved antioxidant status.

Keywords: *Enicostemma littorale*, aqueous extract, oxidative stress, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST).

INTRODUCTION

Free radicals have been implicated in the causation of several diseases such as atherosclerosis, cancer, diabetes, liver cirrhosis etc. and compounds that can scavenge free radicals have great potentials in ameliorating these disease processes. [1] Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species. [2] Chronic alcohol intake produces variety of physiological changes and damages the liver. [3] Excessive alcohol consumption enhances hepatic lipid peroxidation in rats. [4-5] In view to develop a potent hepatoprotective drug against alcohol induced oxidative stress in liver tissue, the efficacy of aqueous leaf extract of perennial herb, *Enicostemma littorale* was tested in rats. *E. littorale* is traditionally used as a stomachic and bitter tonic. Recent preclinical data has proving significant increase in serum insulin levels in alloxan induced diabetic rats. The anti-diabetic efficacy of *E. littorale* was demonstrated earlier by [6-8], it also a potential hypolipidemic plant. [9]

MATERIALS AND METHODS

Animals

Adult male albino rats of Wistar strain (150-200g) were obtained from Tamil Nadu Veterinary and Animal Science University, Chennai, India. They were acclimatized to animal house condition, fed with commercial rat chow (Hindustan Lever Ltd., Bangalore, India) and had free access to water. All the animal experiments were conducted according to the ethical norms approved by Ministry of Social Justice and Empowerment, Government of India and Institutional Animal Ethics Committee guidelines.

Hepatotoxic Agents

Distilled ethyl alcohol was obtained from Hayman Ltd, England. The hepatotoxic dose was standardized as 20% ethanol (7.9g/Kg body wt) orally, using an intragastric tube as described earlier [10] for 45 days.

Plant Materials

The leaves of *E. littorale* were collected from surrounding region (north-east Tamil Nadu). The plant was botanically authenticated and a voucher specimen was deposited in the Herbarium of Department of Botany, Voorhees College, Vellore. The leaves were shade dried at 25°C and the dried leaves were ground with a blender and the powder part was kept in nylon bags in deep freezer until the time of use.

Preparation of Extract

Fifty gram of powdered leaf mixed with 250ml distilled water was stirred magnetically over night at 70°C. The residue was removed by filtration and aqueous extract was concentrated under vacuum to get 20% yield.

Experimental Design

The animals were divided into four groups. Each group consisted of six rats at each dose level. Normal rats (placebo) were kept in group-I. Rats of group-II were given 20% ethyl alcohol (7.9g/Kg body wt) orally, using an intragastric tube for 45 days. Group-III animals received ethyl alcohol alone for 45 days and from 46th day to 90th day the animals were

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received the leaf extract. Group-IV animals received simultaneous oral administration of both ethyl alcohol and aqueous leaf extract of *E. littorale* (250mg/kg body wt.) for 45 days.

Biochemical Estimation of Lipid Peroxidation and Antioxidant Enzymes Activity

Animals were sacrificed by decapitation and the liver was excised, immediately washed with chilled normal saline and homogenates were prepared accordingly for biochemical estimations. The levels of lipid peroxidation in liver tissue were estimated by measuring the activity of malondialdehyde and other thiobarbituric acid reactive substances (TBARS) with thiobarbituric acid (TBA) by the standard method. [11] The lipid hydroperoxide in tissues were estimated by the method. [12] The activity levels of antioxidant defense enzyme superoxide dismutase (SOD) were assayed by the method. [13] The catalase (CAT) activity in liver tissue was estimated by the method. [14] The activity levels of glutathione peroxidase (GPx) in tissue were estimated by the standard method [15] and glutathione–S-transferase (GST) levels was assayed by method. [16]

Statistical Analysis

All the data were statistically analyzed using student's t-test. The results were expressed as mean ± SD method described earlier. [17]

Table 1: Activity Levels of TBARS, Lipid Hydroperoxide, SOD, CAT, GP_X and GST during Alcohol Induced (7.9g/kg p.o. x 45 days) Oxidative Stress in Male Albino Rats*

Parameters	Group-I (Placebo)	% of Changes	Group-II
TBARS (nm/100g tissue)	0.673 ± 0.040	+ 96.13 P< 0.001	1.230 ± 0.20
Lipid Hydroperoxide (mm/100g tissue)	76.10 ± 5.69	+ 27.29 P< 0.001	96.87 ± 6.09
SOD (unit[1]/mg protein)	6.060 ± 0.75	- 50.49 P< 0.001	3.00 ± 0.50
CAT (unit[2]/mg protein)	151.08 ± 9.12	- 32.38 P< 0.001	102.16 ± 8.71
GPx (unit[3]/mg protein)	11.83 ± 1.42	-50.54 P< 0.001	5.85 ± 0.94
GST (unit[4]/mg protein)	6.13 ± 0.74	- 42.90 P< 0.001	3.50 ± 0.21

Table 2: Activity Levels of TBARS, Lipid Hydroperoxide, SOD, CAT, GP_X and GST during Alcohol Induced (7.9g/kg p.o. x 45 days) Oxidative Stress in Male Albino Rats*

Parameters	Group-I (Placebo)	Group-II	% of Changes	Group-III
TBARS (nm/100g tissue)	0.673 ± 0.040	1.230 ± 0.20	-32.42 P< 0.001	0.892 ± 0.091
Lipid Hydroperoxide (mm/100g tissue)	76.10 ± 5.69	96.87 ± 6.09	-11.89 P< 0.001	85.35 ± 3.82
SOD (unit[1]/mg protein)	6.060 ± 0.75	3.00 ± 0.50	+67.93 P< 0.001	5.038 ± 0.096
CAT (unit[2]/mg protein)	151.08 ± 9.12	102.16 ± 8.71	+35.76 P< 0.001	138.70 ± 6.86
GPx (unit[3]/mg protein)	11.83 ± 1.42	5.85 ± 0.94	+47.06 P< 0.01	8.603 ± 1.20
GST (unit[4]/mg protein)	6.13 ± 0.74	3.50 ± 0.21	+36.91 P< 0.001	4.792 ± 0.54

RESULTS AND DISCUSSION

The activity levels of malondialdehyde and other TBARS with TBA and the lipid hydroperoxide in the liver tissue were increased 96.13% and 27.39% respectively in alcohol fed rats (group-II) when compare to that of the placebo (group-I). The activity levels of the antioxidant enzyme viz. SOD, CAT, GPx and GST were significantly decreased (50.49%, 30.38%, 50.54%, 42.9%) respectively in group-II animals when compared to that of group-I. The restorative studies conducted using the leaf extract of *E. littorale* on the ethanol induced oxidative stress in group-II rats revealed a reduced lipid peroxidation and an enhanced activity levels of the antioxidant enzymes in the liver tissues.

Table 3: Activity Levels of TBARS, Lipid Hydroperoxide, SOD, CAT, GP_X and GST during Alcohol Induced (7.9g/kg p.o. x 45 days) Oxidative Stress in Male Albino Rats*

Parameters	Group-I (Placebo)	Group-II	% of Changes	Group-IV
TBARS (nm/100g tissue)	0.673 ± 0.040	1.230 ± 0.20	-34.92 P< 0.001	0.859 ±0.051
Lipid Hydroperoxide (mm/100g tissue)	76.10 ± 5.69	96.87 ± 6.09	-16.42 P< 0.001	80.96 ± 4.07
SOD (unit[1]/mg protein)	6.060 ± 0.75	3.00 ± 0.50	+81.33 P< 0.001	5.44 ± 0.76
CAT (unit[2]/mg protein)	151.08 ± 9.12	102.16 ± 8.71	+38.67 P< 0.001	141.67 ± 8.86
GPx (unit[3]/mg protein)	11.83 ± 1.42	5.85 ± 0.94	+61.02 P< 0.01	9.42 ± 1.1
GST (unit[4]/mg protein)	6.13 ± 0.74	3.50 ± 0.21	+44.0 P< 0.001	5.04 ± 0.30

Table 4: Activity Levels of TBARS, Lipid Hydroperoxide, SOD, CAT, GP_X and GST during Alcohol Induced (7.9g/kg p.o. x 45 days) Oxidative Stress in Male Albino Rats*

Parameters	Group-I (Placebo)	Group-III	% of Changes	Group-IV
TBARS (nm/100g tissue)	0.673 ± 0.040	0.892 ± 0.091	-3.69 NS	0.859 ±0.051
Lipid Hydroperoxide (mm/100g tissue)	76.10 ± 5.69	85.35 ± 3.82	-5.76 P< 0.1	80.96 ± 4.07
SOD (unit[1]/mg protein)	6.060 ± 0.75	5.038 ± 0.096	+7.97 NS	5.44 ± 0.76
CAT (unit[2]/mg protein)	151.08 ± 9.12	138.70 ± 6.86	+2.14 NS	141.67 ± 8.86
GPx (unit[3]/mg protein)	11.83 ± 1.42	8.603 ± 1.20	+ 9.49 NS	9.42 ± 1.1
GST (unit[4]/mg protein)	6.13 ± 0.74	4.792 ± 0.54	+ 5.17 NS	5.04 ± 0.30

* Values are mean ± SD, n=6, Groups are compared with other group, using student's t-test

NS = non significant

TBARS - content in tissue, expressed as 'n'moles /100g tissue

Lipid Hydroperoxide- content in tissue, expressed as mm/100g tissue

Unit[1] SOD = Superoxide dismutase activity, expressed as units/mg protein/min (one unit of SOD activity is defined as the enzyme reaction, which give 50% inhibition of NBT reduction in one minute under the assay condition)

Unit[2] CAT = Catalase activity, expressed as µmoles of H_2O_2 consumed/min/mg protein

Unit[3] GST = Glutathione-S-transferase activity, expressed as 'n'moles of CDNB(1-chloro-2,4-dinitrobenzene)-GSH conjugate formed/min/mg protein.

Unit[4] GPX = Glutathione peroxidase activity, expressed as mg of GSH consumed/min/mg protein.

Chronic alcohol abuse provokes successive hepatic changes, consisting of alcoholic fibrosis, alcoholic hepatitis and cirrhoris [18-19] an increased oxidative damage. [20-22] Chronic alcohol intake leads to many cellular and tissue abnormalities in the liver, probably due to the changes in the permeability of plasma membrane. This might be due to peroxidation of lipid by the generation of oxygen as free radical. [23] In the present investigation the levels of TBARS and lipid hydroperoxide in the liver tissue of alcohol fed animals (group-II) were elevated indicating lipid peroxidation due to alcohol induced oxidative stress. Thus chronic alcohol intake

increase lipid peroxidation in liver tissue. [20-22] The activity level of antioxidant enzymes such as SOD, CAT, GPx and GST were reduced in the liver tissue of group–II animals (Table 1) indicating the probable increased levels of lipid peroxidation. Therefore, the increased lipid peroxidation and decreased activity level of antioxidant enzymes in the tissue reveals the oxidative damage. [22, 24-28] The supplementation of the leaf extract of *E. littorale* to the alcohol fed animals (group-II) revealed, decreased levels of TBARS and lipid hydroperoxide indicating the depleted lipid peroxidation along with the elevated activity levels of antioxidant enzymes (group-III, Table-2). Further the study on simultaneous supplementation of leaf extract along with alcohol to the animals from day-1 to day-45 (group-IV, Table-3) revealed non significant change in lipid peroxidation and activity level of antioxidant enzymes in the liver tissue when compare to that of group–III animals (Table-4).

Thus, the present study envisages the hepatoprotective effect of *Enicostemma littorale* leaf extract in ethanol induced oxidative stress in rats.

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