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Hepatoprotective Effect of *Enicostemma littorale blume* and *Eclipta alba* During Ethanol Induced Oxidative Stress in Albino Rats

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Abstract: The leaves of *Enicostemma littorale blume* (Ens) and *Eclipta alba* (Ecl) have been used for skin infection, antiviral and antibacterial activity in traditional medicine. The present study is aimed at to evaluate the hepato-protective effect of the aqueous leaf extracts of the above two plants during ethanol induced oxidative stress in albino rats. The aqueous leaf extracts of *Enicostemma littorale* and *Eclipta alba* combine (1:1) at dose level of 250 mg kg^{-1} b.wt. were tested for hepato-protective and antioxidant effects during ethanol induced oxidative stress in liver tissue of wistar male albino rats. The degree of hepatoprotection was assessed by measuring the activity levels of the marker enzymes such as serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). Free radicals generated lipid peroxidation was assessed by measuring the tissue levels of thiobarbituric acid reacting substances (TBARS) and the activity levels of the tissue antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD). The ethanol supplemented rats recorded elevated activity levels of serum AST, ALT and ALP revealing ethanol induced hepatotoxicity. The increased levels of TBARS in liver and decreased activity levels of SOD and CAT in ethanol fed animal's revealed oxidative stress. The aqueous leaf extracts supplementation of Ens+Ecl in 1:1 produced significant hepatoprotection and antioxidative effect during ethanol induced hepatotoxicity. The study can be concluded that the therapeutic effect of aqueous leaf extracts of Ens+Ecl in 1:1 is not only hepatoprotective but also possess significant antioxidant property.

Key words: Hepatoprotective, antioxidant, oxidative stress, hepatotoxicity

INTRODUCTION

Alcoholic liver disease is one of the most serious medical consequences of chronic alcohol abuse, which includes alcoholic hepatitis and cirrhosis. Both condition can be fatal and treatment options are limited. 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 has been implicated as important pathological mediation (Bandhopadhy *et al*., 1999) in many clinical disorders such as heart disease, diabetes, cancer and liver disease.

Liver diseases remain as one of the serious health problems. In the absence of reliable liver protective drugs in allopathic medical practices, 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 (Momin, 1987). Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices as well as in traditional systems of medicine in India (Babu *et al*., 2001).

The traditional medicinal plants *Enicostemma littorale Blume* a perennial tropical herb with sessile lanceolate leaves, flowers arranged in clusters, fruit a capsule and *Eclipta alba* an annual herb that is hardy and non-invasive, which grows approximately two feet in height with thin wooden stem, dark green leaves and small white flowers. These plants are locally used for their medicinal properties in Tamilnadu, India. Traditionally *Enicostemma littorale* and *Eclipta alba* have been reported to have antiviral, antibacterial (Phadke and Kulkarni, 1989), anti-inflammatory (Sadique *et al*., 1987; Sureshkumar *et al*., 2005) and antidiabetic (Srinivasan *et al*., 2005; Maroo *et al*., 2003) properties, respectively and used against liver diseases.

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However, systematic studies on these plants against alcohol induced liver damage are scanty. Keeping this in view the present study has been undertaken to investigate hepatoprotective and antioxidant role of the aqueous leaf extracts of the plants during ethanol induced oxidative damage of the liver in rats.

MATERIALS AND METHODS

Plant materials: The traditional medicinal plants viz. Enicostemma littorale Blume (Family: Gentianaceae) and *Eclipta alba* (Family: Asteraceae) were identified, in and around Vellore District, Tamilnadu, India. The plant leaves were collected during the months, September-January in 2004. The leaves were authenticated, cleaned, shade dried and voucher specimens were deposited in the laboratory for future reference.

Preparation of plant extracts: The shade dried leaves were powdered separately in an electrical blender and stored at 5°C until further use. A 100 g of each plant 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 extracts were concentrated under vacuum to get each about 20% solid yield. The leaf extracts were combined in 1:1 tested for hepatoprotective and in vivo antioxidant effects in the male albino rats at the selected optimum dosage of 250 mg kg^{-1} b.wt. and administered orally in aqueous solution.

Experimental animals: Studies were carried out using Wistar strain male albino rats (160-180 g). The rats 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 guideline of Institutional Animal Ethics Committee.

Experimental design: Distilled ethyl alcohol was obtained from Hayman Ltd., England. The hepatotoxic dose was standardized as 5 mL of 20% ethanol (7.9 g kg^{-1} b.wt.) orally, using an intra-gastric tube daily as described earlier (Rajakrishnan *et al*., 1997) for 45 days.

Thirty rats were randomly divided into 5 groups of 6 each.

- **Group 1:** Normal rats
- **Group 2:** Rats treated with ethanol daily for 45 days using intra gastric tube
- **Group 3:** Rats treated with ethanol for 45 days (1st to 45th day) and later oral administration of combined leaf extract of Ens+Ecl in 1:1 at the dose level of 250 mg kg^{-1} b.wt., for 45 days i.e., 46 to 90th day
- **Group 4:** Rats treated with ethanol for 45 days along with oral administration of combined aqueous leaf extract of Ens+Ecl in 1:1 at the dose level of 2 50 mg kg^{-1} b.wt., for 45 days i.e., 1 to 45th day
- **Group 5:** Rats treated with ethanol simultaneous administration of Sylamarin (a hepatoprotective reference drug) at the dose level of 250 mg kg^{-1} b.wt., for 45 days i.e., 1st to 45th day

Estimation of biochemical parameters: The activity levels of hepato-specific marker enzymes viz. aspartate transaminase (AST), alanine transaminase (ALT) in serum were estimated by the method of Reitman and Frankel (1957) and the activity level of alkaline phosphatase (ALP) in serum was estimated by the method of King and Armstrong (1934). The lipid peroxidation was assessed in tissue by measuring the levels thiobarbituric acid reacting substances (TBARS) by the method of Niehaus and Samuelson (1968). The tissue antioxidant enzymes superoxide-dismutase (SOD) and catalase (CAT) activity levels were estimated by the methods of Kakkar *et al.* (1984) and Sinha (1972), respectively. Serum total protein was estimated by the method of Lowry *et al.* (1951). Analysis of variance (ANOVA) and Student t-test were carried out using SPSS package for statistical analysis.

RESULTS

Administration of ethanol at the dose level of 7.9 g kg^{-1} b.wt., daily for 45 days to albino rats resulted a significant elevation of hepato-specific serum markers AST, ALT, ALP and total protein in comparison with normal animals (Table 1). On administration of aqueous leaf extracts of Ens+Ecl in 1:1 revealed a significant depletion in the activity levels of AST, ALT and ALP.

The ethanol induced oxidative stress in the liver was assessed by measuring the levels of thiobarbituric acid reactive substances (TBARS) and the activity levels of tissue antioxidant defense enzymes, SOD, CAT. Elevated levels of TBARS and a significant reduced activity level of SOD and CAT were recorded in ethanol administered animals (Table 2). The aqueous leaf extracts of Ens, Ecl and Sylamarine (reference drug) administered animals revealed significant depletion in the levels of TBARS and elevation in the activity levels of tissue SOD and CAT (Table 3).

Table 1: Activity levels of AST, ALT, ALP, SOD, CAT and levels of TBARS ethyl alcohol induced hepatotoxicity

Parameter in serum	Normal (group 1)	Changes (%)	ETOH Fed (group 2)
AST (IU/L/min/mg protein)	72.140±1.67	±45.45	104.93±4.42
		p<0.001	
ALT (IU/L/min/mg protein)	25.880±2.52	±139.30	61.93±2.42
		p<0.001	
ALP (KA Units dL^{-1})	10.370±0.96	±196.72	30.77±3.18
		p<0.001	
Parameter in liver			
TBARS (nm100 g^{-1} tissue)	0.675±0.04	+117.77	1.47±0.09
		p<0.001	
SOD ($Unit_1$ mg^{-1} protein)	6.320±0.70	-59.81	2.54±0.24
		p<0.001	
CAT ($Unit_2$ mg^{-1} protein)	170.510±3.58	-46.61	91.03±2.98
		p<0.001	

Values are mean of 6 individual observations in each group±SD. p denotes statistical significance, + and – indicate % of changes over the normal group; AST: Aspartate tranaminase, ALP: Alkaline phosphatase, ALT: Alanine transaminase, TBARS: Thiobarbituric reactive substances, SOD: Superoxide dismutase, CAT: Catalase. $Unit_1$: One unit of activity was taken as the enzymes reaction which gives 50% inhibition of NBT reduction in one minute.$Unit_2$: µmoles of hydrogen peroxide consumed per min

Table 2: Hepatoprotective effect of the aqueous leaf extracts of *Eclipta alba* and *Enicostemma littorale* on ethanol induced hepatotoxicity

Groups	AST (IU/L/min/mg protein)	ALT (IU/L/min/mg protein)	ALP (KA units dL^{-1})	TBARS (nm 100 g^{-1} tissue)	SOD ($Unit_1$ mg^{-1} protein)	CAT ($Unit_2$ mg^{-1} protein)
Normal (group 1)	72.14±1.67	25.88±2.52	10.37±0.96	0.675±0.04	6.32±0.70	170.51±3.58
ETOH Fed (1st to 45th day) (group 2)	104.93±4.42	61.93±2.42	30.77±3.18	1.47±0.09	2.54±0.24	91.03±2.98
Ecl+Ens Fed (45 to 90th day) (group 3)	79.64±2.53	32.79±1.59	13.92±0.81	0.725±0.02	5.03±0.14	155.43±4.19
Changes of (%)	-24.10	-47.05	-54.76	-50.68	+98.03	+70.75
(ETOH Fed vs. Ecl+Ens Fed)	*p<0.001	*p<0.001	*p<0.001	*p<0.001	*p<0.001	*p<0.001
**P(ANOVA)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Values are mean of 6 individual observations in each group±SD. - indicate % of changes over the ETOH fed rats. *The group compared with ETOH fed rats by using Student t-test; **p denotes statistical significance of ANOVA to the difference between the ETOH fed and plant extract, $Unit_1$: One unit of activity was taken as the enzymes reaction which gives 50% inhibition of NBT reduction in one min. $Unit_2$: µmoles of hydrogen peroxide consumed per min

Table 3: Synergistic hepatoprotection of *Enicostemma littorale* and *Eclipta alba* during ethanol induced hepatotoxicity

Groups	AST (IU/L/min/mg protein)	ALT (IU/L/min/mg protein)	ALP (KA units dL^{-1})	TBARS (nm 100 g^{-1} tissue)	SOD ($Unit_1$ mg^{-1} protein)	CAT ($Unit_2$ mg^{-1} protein)
Normal (group 1)	72.14±1.67	25.88±2.52	10.37±0.96	0.675±0.04	6.32±0.70	170.51±3.58
ETOH Fed (1st to 45th day) (group 2)	104.93±4.42	61.93±2.42	30.77±3.18	1.47±0.09	2.54±0.24	91.03±2.98
ETOH +(Ecl+Ens) (1st to 45th day) (Group 4)	75.56±1.9	28.82±1.6	11.03±0.82	0.701±0.04	5.27±0.59	161.42±4.12
	*p<0.001	*p<0.001	*p<0.001	*p<0.001	*p<0.001	*p<0.001
Changes of (%) (ETOH Fed vs. ETOH+ (Ecl+Ens)	-27.99	-53.46	-64.15	-52.31	+107.48	+77.32
	**p<0.001	**p<0.001	**p<0.001	**p<0.001	**p<0.001	**p<0.001
ETOH+ (silymarin) (1st to45th day) (group 5)	85.89±2.12	36.24±1.12	20.18±1.1	0.934±0.03	4.28±0.36	123.98±3.46
changes of (%) (ETOH vs. ETOH+(silymarin)	-18.15	-41.48	-34.42	-36.46	±68.50	±36.20
	**p<0.02	**p<0.001	**p<0.001	**p<0.001	**p<0.02	**p<0.001
**P(ANOVA)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Values are mean of 6 individual observations in each group±SD. - indicate % of changes over the ETOH fed rats; *The combined (Ecl+Ens) compared with silymarin fed model by using student 't'test; **The group compared with ETOH fed rats by using Student t test; $Unit_1$: One unit of activity was taken as the enzymes reaction which gives 50% inhibition of NBT reduction in one min.$Unit_2$: µmoles of hydrogen peroxide consumed per min

DISCUSSION

Measurement of the enzymes activity in the body fluids is a useful monitor of disease state (Porchezhian, 2005). An obvious sign of hepatic injury is leakage of cellular enzymes into serum (Savides and Oehme, 1983). In the present study the elevated levels of serum AST, ALT and ALP in ethanol administered animals might be due to the damage of the liver tissue and/or changes in cell permeability that all AST, ALT and ALP to leak into serum (Paul and Giboney, 2005). Similar observations during ethanol induced hepatotoxicity were elsewhere recorded (Daniell *et al*., 2002).

Intake of alcohol results in excessive generation of free radicals (Isayama *et al*., 2003). Free radicals are the Reactive Oxygen Species (ROS) are known to cause

oxidative damage to number of molecules in cell, including membrane-lipids, proteins and nucleic acids. In the present study the hepatic cellular injury in ethanol administered animals might be due to increased oxidative stress leading to lipid per oxidation, cell membrane damage and thereby leakage of the cellular enzymes. The level of lipid per oxidation was assessed by measuring the levels of thiobarbituric acid reactive substances (TBARS) in the liver tissue (Rukkmani *et al*., 2004). The significant increase of the tissue TBARS levels in the ethanol administered animals revealed lipid peroxidation in the liver. The depleted activity levels of cellular antioxidant enzymes viz. The SOD and CAT recorded in the present study revealed reduced cellular antioxidant defense mechanism and thereby leading to increased lipid peroxidation. However, the administration of aqueous leaf extracts of the Ens and Ecl revealed hepatoprotective effect during ethanol induced hepatotoxicity. The significant decrement in the levels of AST, ALT and ALP in plant extract fed animals might be due to decreased leakage from liver cells. This suggests that the plant extracts could be able to repair the probable hepatic injury and/or restore the altered cellular permeability. Thus, reducing the toxic effect of ethanol in the liver tissue. The significant depletion in tissue levels of TBARS in the plant extracts fed animals might be due to reduced lipid peroxidation and/or elevation of tissue antioxidant defense enzymes activity levels indicating that the plant extracts could reduce the free radical generation and increased free radicals scavenging mechanism. The significant increment in the activity levels of SOD and CAT in the plant extract fed animals are in corroboration with the increased free radicals scavenging mechanism. Similar studies reported that significant decrease in the activity of liver SOD, CAT in ethyl alcohol intoxicated rat was observed and the therapeutic treatment with *A. indica* herbal drug promoted the hepatoprotection by elevating free radical scavenging activity (Maruthappan and Sakthi, 2009).

In the present study, a significant higher hepatoprotective and antioxidant effect were revealed in the plant extract simultaneously fed along with ethanol supplemented group animal (1st to 45th day) when compared to that of the plant extract fed group after 45th day onwards (45th to 90th day).

The results of the study envisaged that alcohol induced oxidative stress leading to hepatic tissue damage. The aqueous leaf extract of Ens and Ecl supplementation recorded significant hepatoprotective effect against ethanol induced oxidative stress, when compared with sylimerin, the reference hepato protective drug. The above results suggest further scope of studies on isolation of the potent hepatoprotective ingredients from leaf of Ens+Ecl to combat the ethanol induced hepatotoxicity.

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