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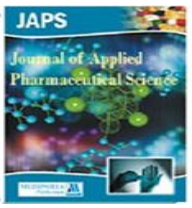

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Hepatoprotective potential of *Andrographis paniculata* aqueous leaf extract on ethanol induced liver toxicity in *albino* rats

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ABSTRACT

To investigate the protective effect of the leaf extract of *Andrographis paniculata* (Ap) against ethanol induced liver toxicity in male albino rats. The liver toxicity was induced by the administration of ethanol to the animals at the optimum dosage of 7.9g/kg body wt., orally for 45 days. After induction of liver toxicity the aqueous plant extract of A.p was administered to the animal 250 mg/kg body wt., for 45 days. The liver toxicity and protective effect of the plant extract was assessed by the estimation of liver marker enzymes, antioxidant enzymes and liver histopathological studies. The ethanol induced animals the liver marker enzymes like ALT, AST, ALP and Bilirubin were significantly elevated ($P<0.001$) when compared to the normal animals. After administration of aqueous extract of A.p the elevated levels of marker enzymes were significantly decreased ($P<0.001$). The antioxidant enzymes were decreased significantly in ethanol induced animals after administration of plant extract the decreased levels were increased significantly ($P<0.001$). The aqueous leaf extract of *A.paniculata* could protect the liver against ethanol induced liver toxicity by possibly reducing the rate of lipid peroxidation and increasing the antioxidant defense mechanism in rats.

Key words: Liver marker enzymes, *Andrographis paniculata,* Antioxidant, lipid per oxidation.

INTRODUCTION

Alcohol related disorders are one of the challenging current health problems with far reaching medical, social and economic consequences. Long term use of alcohol potentially results in serious illness, including alcoholic fatty liver, hyperglyceridaemia, cirrhosis, cardiovascular disease and inflammation of the pancreas (Ponnappa *et al.*, 2000). High alcohol consumption results in critical problems in the body including alcoholic liver disease (ALD) (Pari and Karthikesan, 2001; Sivaraj *et al.*, 2010). The liver is vulnerable to a wide variety of metabolic, toxic, microbial, circulatory and neoplastic insults. In some instances the disease process is primary to some of the most common diseases in humans, such as cardiac decompensation, disseminated cancer, alcoholism and extra hepatic infection (Vinaykumar *et al.*, 1997). Liver injuries may be viral or caused by drugs, chemical and alcohol. One of the factors that play a central role in many pathways of alcohol induced damage is oxidative stress. Oxidative stress in the cells or tissues refers to the enhanced generation of reactive oxygen species (ROS) and/or depletion in the antioxidant defense system, causing an imbalance between pro-oxidants and antioxidants. Antioxidants help organisms deal with oxidative stress, caused by free radical

damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Reactive Oxygen species (ROS) formed *in-vivo*, such as superoxide anion, hydroxyl radical and hydrogen peroxide, are highly reactive and potentially damaging transient chemical species. These are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function. ROS are regulated by endogenous superoxide dismutase, glutathione peroxidase and catalase, as a result of over- production of ROS, due to the exposure to external oxidant substances or a failure of enzyme regulatory mechanisms leading to damage of cell structures, DNA, lipids and proteins (Valko *et al.,* 2006).

Ethanol is known to have a profound effect on the metabolism of lipids and lipoproteins. Accumulation of lipids in the hepatocytes is the most striking initial manifestation of alcohol induced liver injury (Lieber *et el.,* 1994).

India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world (Pal and Shukla, 2003). Plants have been used in traditional medicine for several thousand years. In India, it is reported that traditional healers use 2500 plant species and 100 species of plants serve as regular sources of medicine (Pei, 2001). During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world (Rossato *et al.,* 1999; Hanazaki *et al.,* 2000; Gazzaneo *et al.,* 2005; Lev, 2006). There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various diseases (Azaizeh *et al.,* 2003; Sivaraj *et al.,* 2010).

A number of plants have been shown to possess hepatoprotective property by improving antioxidant status (Vinothkumar *et al.,* 2010). Thus the efficacy of the drug would be preventive and passive for defending against damages. Indian medicinal plants belonging to about 40 families were investigated as liver protective drugs (Handa *et al.,* 1986).

In the present study the folklore medicinal plant *Andrographis paniculata* (Family:*Acanthaceae*) have been selected for hepatoprotective study in ethanol induced liver toxicity in male *albino* rats.

MATERIALS AND METHODS

Plant material

The leaves of *Andrographis paniculata* (Family:*Acanthaceae*) was collected during the month of February-August (2010) from in and around Vellore District, Tamilnadu, India. The plant material was cleaned with distilled water and shade dried at room temperature and authenticated (No:CAHC-04/2009) by Dr.B.Annadurai, Department of Botany, C. Abdul Hakeem College, Melvisharam, Vellore Dt, Tamilnadu., India and voucher specimens were kept at the Department of Botany, C. Abdul Hakeem College, Melvisharam, Vellore Dt., Tamilnadu, India.

Plant extract preparation

The shade dried plant materials were powdered separately in an electrical blender and stored at 5^0C until further use. 100gms of the dried plant powder were taken separately and mixed with 500ml of distilled water and then magnetically stirred in a container overnight at room temperature. The residue was removed by filtration and the aqueous extracts were lyophilized and concentrated under vacuum to get solid yield 10% (leaves).

Animals

Adult male *albino* Wistar rat weighing around 180-220g were purchased from Tamilnadu Veterinary and Animal Sciences University, Chennai, India. The animals were kept in polypropylene cages (three in each) at an ambient temperature of 25±2^{o}C and 55-65% relative humidity 12±1 hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore. India). They had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines.

Experimental design

Group I: Normal rats.

Group II: Control - Rats were treated with ethanol (7.9g/kg body wt by oral administration) daily for 45 days using intragastric tubes.

Group III: Rats were treated with ethanol daily using intragastric tubes for 45 days. Oral administration of aqueous leaf extracts (250mg/kg body wt) of *Andrographis paniculata* was done for 45 days i.e. from 46th to 90th day.

Group IV: Liver toxicity induced animals were treated with the reference drug of Silymarin (25 mg/kg body wt.,) orally for 45 days.

Estimation of Biochemical parameters

The serum biochemical parameters were analyzed. They included, ALT and AST (Reitman and Feankel, 1957), ALP (King and Amstrong, 1934), Serum Bilurubin (Mally and Evelyn, 1937), thiobarbutric acid (TBARS) (Nichans and Samuelson, 1968), Lipid hydroperoxide (Jiang *et al.,* 1992), Super oxide dismutase (Kakkar *et al.,* 1984), Catalase (Sinha, 1972), Glutathione peroxidase (Rotruck and Pope, 1973), and Glutathione -S- tranferase (Habig *et al.,* 1974), Total cholesterol (Zlatkes *et al.,* 1953), TG (Foster and Dunn, 1973), Free fatty acids (Falholt *et al.,* 1973), Phospholipids (Zilversmit *et al.,* 1950).

Preparation of liver tissue homogenate

After 45 days treatment, all the mice were anaesthetized and sacrificed by cervical dislocation. The blood was collected

and serum was separated for estimation of liver biochemical marker enzymes. The liver tissues were excised washed and homogenized in suitable buffer and centrifuged at 12,000×g for 30 minutes at 4°C. The supernatant was collected and used for the experiments. The protein content of the experimental samples was measured by the method of Lowry *et al.*, 1951 using crystalline BSA as standard.

Chemicals

Ethanol was purchased from Hayman Ltd., (Witham, Essex) CM8 3YE, England. Bovine serum albumin (BSA), 1-chloro-2,4-dinitrobenzene (CDNB), 2,4-dinitrophenylhydrazine (DNPH), 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), ethylene diamine tetra acetic acid (EDTA), reduced nicotinamide adenine dinucleotide (NADH), nitro blue tetrazolium (NBT), phenazine methosulfate (PMT), potassium dihydrogen phosphate, reduced glutathione (GSH), sodium azide, sodium pyrophosphate, trichloro acetic acid (TCA), thiobarbituric acid (TBA), 5-thio- 2-nitrobenzoic acid (TNB), hydrogen peroxide (H_2O_2) and ascorbic acid were purchased from Sisco Research Laboratory, Mumbai, India. All chemicals and reagents used were of analytical grade.

Statistical analysis

Data were statistically calculated by utilizing one way ANOVA and expressed as Mean±SEM followed by Fisher's LSD post-hoc test using SPSS 10.0 software (SPSS, Inc, Chicago). The values were considered significant when $P<0.05$.

RESULTS

Biochemical marker enzymes

The serum activities of Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) and bilirubin were used as biochemical markers for the early acute hepatic damage. In Group II the animals were treated with ethanol (7.9g/kg body wt.,) for 45 days. The marker enzymes were significantly elevated, when compared to levels in normal animals. After that, the plant extract *A.paniculata* (250mg/kg body wt.,) was administered to the Group III animals for 45 days (45^{th} day to 90^{th} day). The liver marker enzymes like ALT, AST, ALP and bilirubin were significantly ($P<0.001$) reduced by 42.59%, 30.87%, 42.03% and 28.33% respectively, when compared to the levels in ethanol fed control groups (Table:1).

Antioxidant defense enzymes

The oxidative stress in the liver tissue was assessed by measuring the levels of thiobarbituric acid reactive substances (TBARS), lipid hydro peroxides and antioxidant defense enzymes viz., Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) and Glutathione–S- transferase (GST) in ethanol administrated as well as plant extract (Ap) treated groups. The levels of tissue TBARS and lipid peroxides were significantly elevated in Group-II ethanol treated animals, when compared to levels in normal animals (Table:2). The elevated levels of TBARS and lipid peroxides were significantly reduced in plant extract treated (Group-III) groups by 47.82% and 32.97% respectively (Table-2).

The activity of liver antioxidant defense enzymes viz., SOD, CAT, GPx and GST were significantly decreased by 52.8%, 53.53%, 49.4% and 55.48% respectively in the liver tissue of ethanol fed Group-II animals, when compared to levels in normal animals. When the plant extract (Ap) was administered to the ethanol treated groups, the reduced levels of antioxidant enzymes were significantly increased by 98.98%, 104.73%, 85.04% and 120.57% respectively (Group-III) (Table:2).

Table :1 Effects of plant extract *Andrographis paniculata* (Ap) treatment on ethanol induced hepatotoxicity: Activity levels of ALT, AST, ALP and bilirubin.

Experiments	Parameters in the serum			
	ALT (IU/l/min/mg protein)	**AST** (IU/l/min/mg protein)	**ALP** (IU/l/min/mg protein)	**Bilirubin** (mg/dl)
Group-I Normal	57.16±2.22	147±3.68	232.5±4.96	0 40±.0.02
Group-II Ethanol Control	107±2.04 [b]	217.3±6.02 [b]	426.1±4.44 [b]	0.60±0.02 [b]
Group –III Ethanol +Ap	61.42±1.81 [b]	150.2±3.78 [b]	247±5.86 [b]	0.43±0.01 [b]
Group – IV Ethanol + Silymarin	59.78±1.67 [b]	150±320 [b]	240±4.96 [b]	0.43±0.01 [b]

Values are mean of six individual observations in each group ± S.D
[a]$P<0.05$. [b]$P<0.001$. NS - Non significance.

Table – 2 Effects of plant extract *Andrographis paniculata* (Ap) treatment on ethanol induced hepatotoxicity: Levels of thiobarbituric substances (TBARS), lipid hydroperoxide, Antioxidants defense viz., superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) in the liver tissue.

Experiments	Parameters in the liver					
	TBARS (nm/100g tissue)	Lipid hydro peroxide (nm/100g tissue)	SOD (U_1/mg protein)	CAT (U_2/mg protein)	GPx (U_3/mg protein)	GST (U_4/mg protein)
Group-I Normal	0.66±0.02	69.66±1.63	6.25±0.18	168.3±2.58	11.18±0.21	6.11±0.14
Group-II Ethanol-Control	1.38±0.05 [b]	109.1±2.13 [b]	2.95±0.14 [b]	78.2±1.94 [b]	5.35±0.18 [b]	2±0.11 [b]
Group –III Ethanol +Ap	0.72±0.03 [b]	73.12±1.83 [b]	5.87±0.23 [b]	160.1±2.69 [b]	9.9±0.21 [b]	5.51±0.15 [b]
Group – IV Ethanol + Silymarin	0.70±0.02 [b]	71.72±1.67 [b]	5.91±0.17 [b]	162±1.79 [b]	10.2±0.23	5.87±0.17 [b]

SOD – U_1- One unit of activity was taken as the enzymes reaction which gives 50% inhibition of NBT reduction in one minute. CAT – U_2- µmoles of hydrogen peroxide consumed per minute. GPx – U_3- µg of glutathione consumed per minute. GST – U_4- µmoles of CDNB – GSH conjugate formed per minute

Lipid profile

The serum lipid includes cholesterol, triglycerides, free fatty acids and phospholipids. They were significantly elevated in Group-II ethanol treated control groups by 122.7%, 47.97%,

69.58% and 58.42% respectively when compared to the levels in normal groups (Table:3). Administration of *A.paniculata* leaf extract the elevated levels were significantly reduced by 41.58%, 29.11%, 33.62% and 31.29% respectively (Group-III) (Table:3) when compared to the levels in ethanol induced control groups.

Table – 3 Effects of plant extract *Andrographis paniculata* (Ap) treatment on ethanol induced hepatotoxicity: Levels of cholesterol, triglycerides, free fatty acids and phospholipids in serum.

Experiments	Parameters in the liver			
	TC (mg/dl)	TG (mg/dl)	FFA (mg/dl)	Phospholipids (mg/dl)
Group-I Normal	38.66±2.16	116.3±2.42	60.5±3.14	79.66±2.42
Group-II Ethanol Control	86.13±2.92 [b]	172.1±4.19 [b]	102.6±3.06 [b]	126.2±2.94 [b]
Group –III Ethanol +Ap	50.31±3.12 [b]	122±1.47 [b]	68.1±4.03 [b]	86.71±1.87 [b]
Group – IV Ethanol + Silymarin	46.78±2.89 [b]	120.1±1.39 [b]	65.31±3.97 [b]	83.62±1.6 [b]

Values are mean of six individual observations in each group ± S.D
[a]P<0.05. [b]P<0.001. NS - Non significance.

DISCUSSION

The increased levels of ALT, AST, ALP and bilirubin in serum of the ethanol induced animals might be due to the leakage of the biochemical marker enzymes and bilirubin in to the serum. Generally, measurement of ALT, AST and ALP are commonly used as marker enzymes of hepatotoxicty (Yanpallewar *et al.,* 2002; Asha *et al.,* 2004; Yen *et al.,* 2007). The significant decrease in the levels of biochemical marker enzymes like ALT, AST, ALP and bilirubin in plant extract administered animals might be due to decreased leakage of the enzymes in liver cells. This suggests that the *A.paniculata* plant extract could repair the hepatic injury and/or restore the cellular permeability, thus reducing the toxic effect of ethanol induced liver toxicity and preventing enzymes leakage into the blood circulation. Other investigators have reported similar observations (Molina *et al.,* 2003; Ozaras *et al.,* 2003; Uzun *et al.,* 2003).

The significant depletion of levels of TBARS and lipid peroxides in the liver tissue of the plant extract administered animal group might be due to reduced lipid peroxidation and/or elevation of tissue antioxidant defense enzymes activity levels, indicating that the plant extract could reduce the generation of free radicals and increase free radicals scavenging mechanism.

The decreased levels of antioxidant viz., SOD, CAT, GPx and GST activities may be due, in part, to an overwhelming oxidative modification of the enzymatic proteins by excessive ROS generation. More so, reduction in the activities of these enzymes may stem from decrease in their rate of synthesis. In this study, SOD was found to play an important role in the elimination of ROS derived from the peroxidative process of xenobiotics in liver tissue. The observed increase of SOD activity suggests that the *A.paniculata* extract has an efficient protective activity in response to ROS. These findings also indicate that *A.paniculata* may be associated with decreased oxidative stress and free radical mediated tissue damage.

CAT is a key component of the antioxidant defense system. Inhibition of this protective mechanism results in enhanced sensitivity to free radical induced cellular damage. Excessive production of free radicals may result in alteration of the biological activity of cellular macromolecules. Therefore the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. Administration of *A.paniculata* increases the activities of catalase in ethanol induced liver damaged rat to prevent the accumulation of excessive free radicals and protects the liver from ethanol induced toxication.

The significant increment in the activity levels of GPx and GST, increase the free radicals scavenging mechanism. Similar reduction in lipid peroxidation increased antioxidant enzyme activity levels during plant extract supplementation were recorded (Mari and Cederbaum, 2001; Zhanxiand Zhou *et al.,* 2002; Adamska *et al.,* 2003; Izabela Dobrzynska *et al.,* 2004) .

The ethanol acts as a surfactant and suppresses the action of enzyme LCAT to block the uptake of lipoprotein from circulation by extra hepatic tissue, resulting in an increase in blood lipid levels. The hyper-lipidemic condition revealed in the serum of ethanol administered animals was restored to normal after the supplementation of the plant extract. The decreased serum cholesterol in the plant extract administrated rat might be due to increased activity of enzyme LCAT involved in esterification of cholesterol in the plasma. The significant decrease in the triglycerides (TG) in serum in the *A.paniculata* plant extract administrated animals might be due to decreased accumulation of lipoprotein. This might be due to increased activity of lipoprotein lipase, which is involved in the uptake of TG rich lipoprotein by extra hepatic tissue. The significant decrease in the free fatty acid accumulation in serum of plant extract administrated animals, might be due to decreased lipid breakdown, which corroborates with results obtained where in a decreased lipid peroxidation and increased activity levels of antioxidant defense enzymes were recorded. The significant decrease of the phospholipids in the serum of plant extract administrated animals might be due to decreased peroxidation in the bio-membrane of hepatocytes.

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