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Antioxidant Effect of Livomap, A Polyherbal Formulation on Ethanol Induced Hepatotoxicity in Albino Wistar Rats

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ABSTRACT

The present study was designed to evaluate the antioxidant activity of Livomap on ethanol induced hepatotoxicity in albino wistar rats. A total of 30 rats were divided into five groups of six rats in each group. Group 1 served as normal control, Group 2 - normal rat + livomap (50mg/Kg), Group 3 - ethanol control rats, Group 4 - ethanol + livomap (25mg/Kg) and Group 5 - ethanol + livomap (50mg/Kg). The liver toxicity was induced by the administration of ethanol to the animals at a dose of 3 g/kg orally for 35 days. During the period, livomap was co-administered to the rats at doses of 25 and 50 mg/kg for 35 days. Ethanol induced rats exhibited significant increase in the levels of lipid peroxidative products and decrease in the levels of antioxidants. Co-administration of livomap to ethanol-induced rats significantly minimized the alterations in the levels of lipid peroxidation products and antioxidants. Results suggest that livomap might be beneficial in alleviating ethanol-induced oxidative damage and may be attributed to the antioxidant property of polyherbal formulation.

INTRODUCTION

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. Therefore, it has an important role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. It is an important organ for detoxification of xenobiotics, environmental toxicants and liver damage is associated with distortion of several metabolic functions; hence liver diseases are of serious health problem. However, a satisfactory remedy for serious liver diseases is not still available, so search for effective hepatoprotective drugs are continued (Panda et al., 2006). Alcohol abuse and alcoholism are serious current health and socioeconomic problems throughout the world. Ethanol is the psychoactive compound present in alcoholic drinks. Ethanol is a fat-soluble non-electrolyte, which is readily absorbed from the gastrointestinal tract, diffuses rapidly into circulation and is distributed uniformly throughout the body (McDonough, 2003). Alcohol induced hepatotoxicity has been observed to develop mainly through excessive generation of free radicals and reactive oxygen species, as well as impaired anti-oxidant defense mechanism; conditions which result in oxidative stress (Wu and Cederbaum, 2003). Conventional or synthetic drugs used in the treatment of liver disease are inadequate and can have serious adverse effects. So there is a worldwide trend to go back to traditional medicinal plants. A number of plants have been shown to possess hepatoprotective property by improving antioxidant status (Vinothkumar et al., 2010). Herbal drugs are more widely used than allopathic drugs as hepatoprotectives because of them are inexpensive, better cultural acceptability, better compatibility with the human body and minimal side effects.

Livomap is a judicious combination of highly documented hepatoprotective herbs providing perfect protection to the liver in health, infection and inflammation. Livomap is a polyherbal formulation consisting of fourteen medicinal plants (Table 1) derived from the traditional system of medicine in India, the Ayurveda. Since, there is lack of scientific data regarding pharmacological evaluation of Livomap, the present study was aimed to screen Livomap for its hepatoprotective activity.

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Table. 1: Composition of Livomap.

Punarnava (*Boerhavia diffusa*)	60 mg
Nimb (*Melia azadirachta*)	30 mg
Tikta patola (*Trichosanthes cucumerina*)	30 mg
Shunthi (*Zingiber officinale*)	30 mg
Katuki (*Picrorhiza kurroa*)	30 mg
Guduchi (*Tinospora cordifolia*)	30 mg
Devdaru (*Cedrus deodara*)	30 mg
Haritaki (*Terminalia chebula*)	30 mg
Varuna (*Crataeva religiosa*)	30 mg
Shigru (*Moringa oleifera*)	30 mg
Daruharidra (*Berberis aristata*)	30 mg
Afsantin (*Artemisia absinthium*)	30 mg
Sharapunkha (*Tephrosia purpurea*)	30 mg
Bhumiamalaki (*Phyllanthus niruri*)	30 mg

MATERIALS AND METHODS

Experimental animals

Female albino wistar rats weighing 140-150 g, obtained from the Venkateswara Enterprises, Bangalore were used in the present study. They were housed in polypropylene cages (47x34x20 cm) lined with husk, renewed every 24 h under a 12:12 h light/dark cycle at around 22˚C and had free access to water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fibre, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% nitrogen free extract (carbohydrates). The diet provided metabolisable energy of 3,600 kcal. The experiment was carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Drugs and chemicals

Livomap (Maharishi Ayurveda Products) was purchased from Venkateswara Medical Store, Salem. Ethanol was purchased from Changshu Yangyuan Chemical Co., Ltd, China. Butylated hydroxy toluene (BHT), xylenol orange, phenazine methosulphate, nitroblue tetrazolium, dithionitro bis benzoic acid (DTNB), ascorbic acid, 2, 2' dipyridyl, thiobarbituric acid and sodium azide were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. All other chemicals used in this study were of analytical grade.

Experimental induction of hepatotoxicity

Ethanol (3 g/kg body weight) was dissolved in water and injected to rats intragastrically, for a period of 35 days (Rajat Sandhir and Gill, 1999).

Experimental design

In the experiment, a total of 30 rats were used in the study. The rats were divided into 5 groups of 6 rats in each group.

Group 1: Normal control rats

Group 2: Normal rats treated with Livomap (50mg/kg)

Group 3: Ethanol control rats

Group 4: Ethanol + Livomap treated (25 mg/kg)

Group 5: Ethanol + Livomap treated (50 mg/kg)

Livomap tablet was powdered with the help of mortar and pestle. Livomap (25 and 50 mg/kg) was dissolved in carboxy methyl cellulose and administered to rats through intragastric intubations for a period of 35 days. After the last treatment, all the rats were sacrificed by cervical decapitation. Blood was collected with anticoagulant and plasma was separated from blood after centrifugation. The liver tissue was excised immediately from the rats, washed off blood with ice-cold physiological saline. A known weight of the liver was homogenized in appropriate buffer solution. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

Biochemical estimations

Plasma thiobarbituric acid reactive substances were estimated by the method of Yagi (1987). The concentration of TBARS in the tissue was estimated by the method of Fraga et al. (1988). The level of HP was estimated by the method of Jiang et al. (1992). The activity of SOD was assayed by the method of Kakkar et al. (1984). The activity of catalase was assayed by the method of Sinha (1972). The activity of GPx was assayed by the method of Rotruck et al. (1973). The level of GSH was estimated by the method of Ellman (1959). The level of vitamin C was estimated by the method of Omaye et al. (1979). The level of vitamin E was estimated by the method of Baker et al. (1980).

Statistical analysis

Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using Statistical Package for the Social Sciences (SPSS) software package version 9.05. P values <0.05 were considered significant.

Table. 2: Effect of livomap on thiobarbituric acid reactive substances (TBARS) in plasma and liver of control and experimental rats.

Groups	Plasma TBARS (nmol/ml)	Liver TBARS (mM/100g tissue)
Normal control	0.34 ± 0.04^{a}	0.82 ± 0.04^{a}
Normal + Livomap (50 mg/kg)	0.32 ± 0.02^{a}	0.80 ± 0.06^{a}
Ethanol control	0.93 ± 0.08^{b}	2.14 ± 0.17^{b}
Ethanol + Livomap (25 mg/kg)	0.54 ± 0.04^{c}	1.42 ± 0.08^{c}
Ethanol + Livomap (50 mg/kg)	0.40 ± 0.02^{d}	0.98 ± 0.05^{d}

Table. 3: Effect of livomap on hydroperoxides (HP) in plasma and liver of control and experimental rats.

Groups	Plasma HP (mmol/ml)	Liver HP (mM/100g tissue)
Normal control	8.41 ± 0.79^{a}	88.72 ± 5.94^{a}
Normal + Livomap (50 mg/kg)	8.36 ± 0.72^{a}	87.05 ± 4.36^{a}
Ethanol control	24.16 ± 1.03^{b}	144.63 ± 8.21^{b}
Ethanol + Livomap (25 mg/kg)	14.73 ± 0.95^{c}	110.18 ± 5.75^{c}
Ethanol + Livomap (50 mg/kg)	10.08 ± 0.72^{d}	98.44 ± 3.73^{d}

RESULTS

The effect of livomap on the levels of thiobarbituric acid reactive substances (TBARS), and hydroperoxides (HP) in plasma and liver of normal and ethanol induced rats is presented in Table 2 and 3. Rats induced with ethanol, exhibited a significant increase

in the levels of TBARS and HP as compared to normal control rats. Administration of livomap to ethanol induced rats significantly decreased the levels of these lipid peroxidative products to near normal. The effect of livomap on the activities of superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) in the liver of normal and ethanol induced rats is depicted in Table 4. A significant decrease in the activities of the antioxidant enzymes SOD, CAT and GPx were observed in the liver of rats induced with ethanol. Livomap co-treatment to ethanol induced rats significantly increased the activities of these antioxidant enzymes to near normal as compared to ethanol induced rats.

The levels of vitamin C, vitamin E and reduced glutathione (GSH) in plasma and liver of normal and ethanol induced rats are shown in Figure 1, 2 and 3 respectively. Ethanol administration significantly decreased the levels of the non-enzymatic antioxidants vitamin C, vitamin E and reduced glutathione in plasma and liver. Rats co-treated with livomap significantly increased the levels of the non-enzymatic antioxidants to near normal as compared to ethanol induced rats.

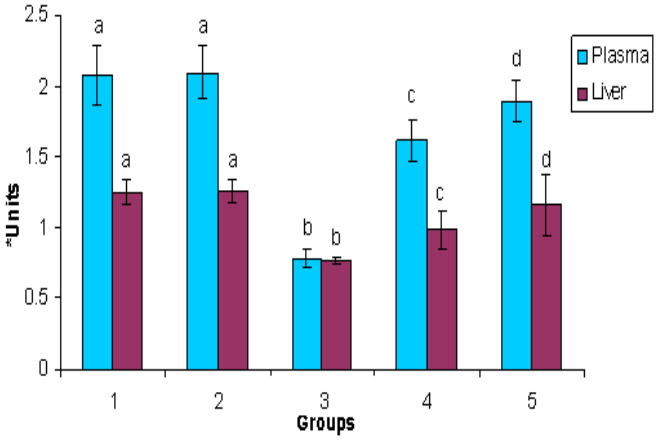

Fig. 1: Effect of livomap on vitamin C in plasma and liver of control and experimental rats.
*Plasma – mg/dl, *Liver – mg/100g protein, Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

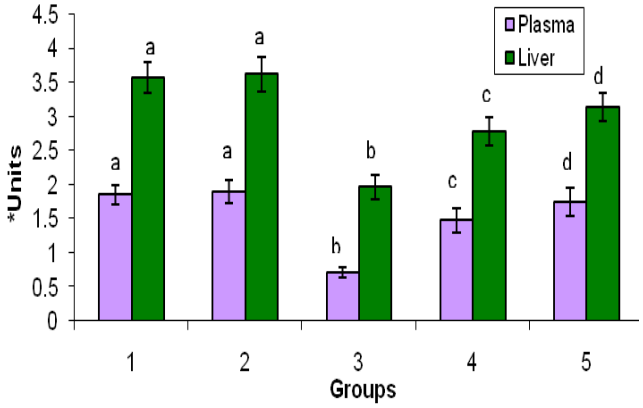

Fig. 2: Effect of livomap on vitamin E in plasma and liver of control and experimental rats
*Plasma – mg/dl, *Liver – mg/100g protein
Each value is mean ± S.D. for six rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

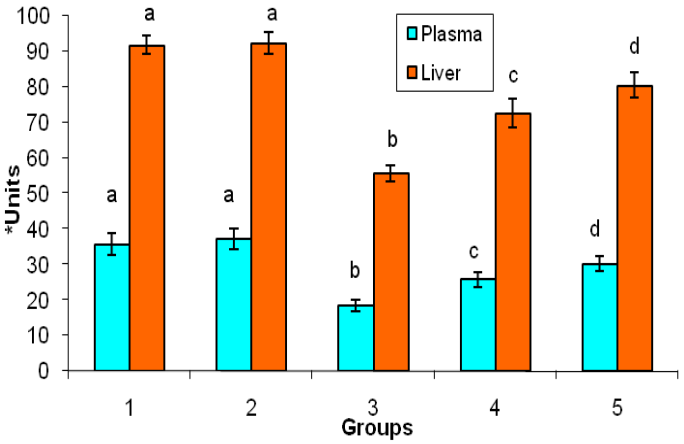

Fig. 3: Effect of livomap on reduced glutathione in plasma and liver of control and experimental rats
*Plasma – mg/dl, *Liver – mg/100g protein
Each value is mean ± S.D. for six rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table. 4: Effect of livomap on superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in liver of control and experimental rats.

Groups	SOD (Units[a] / mg protein)	Catalase (Units[b] / mg protein)	GPx (Units[c] /min/mg protein)
Normal control	7.55 ± 0.62[a]	78.17 ± 5.41[a]	9.54 ± 0.97[a]
Normal + Livomap (50 mg/kg)	7.88 ± 0.74[a]	80.47 ± 4.94[a]	9.75 ± 0.81[a]
Ethanol control	3.75 ± 0.37[b]	48.92 ± 3.78[b]	5.07 ± 0.43[b]
Ethanol + Livomap (25 mg/kg)	5.72 ± 0.64[c]	62.56 ± 6.13[c]	7.14 ± 0.72[c]
Ethanol + Livomap (50 mg/kg)	6.84 ± 0.58[d]	70.52 ± 5.24[d]	8.23 ± 0.74[d]

U[a] – Enzyme concentration required to inhibit the chromogen produced by 50% in one minute.
U[b] – µmol of hydrogen peroxide consumed per minute.
U[c] – µg glutathione consumed.
Each value is mean ± S.D. for six rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

DISCUSSION

The extent of lipid peroxidation in liver tissue was determined by measuring the levels of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP). In the present study, significantly elevated levels of lipid peroxidation products such as TBARS and HP were observed in ethanol administered rats, which might be due to excessive formation of free radicals and activation of peroxidation system resulting in hepatic and other cellular damage by ethanol (McCord et al., 2000). Alcohol consumption is known to cause fatty infiltration and cirrhosis. It enhanced lipid peroxidation produced during the microsomal metabolism of ethanol. The significant decrease in the levels of TBARS and HP in plasma and liver of livomap administered rats might be due to reduced lipid peroxidation and or elevation of the levels of tissue antioxidant defense enzymes. It's well known that,

Ayurvedic medicines could reduce the generation of free radicals and increase free radicals scavenging mechanism.

Antioxidant enzymes are important components of the cellular defense system against ROS and reactive nitrogen species (RNS). SOD and catalase are defense against oxidative damage by supplying NADPH, which is needed for the regeneration of GSH. Moreover oxidative stress is known to decrease GST activity and also lower GSH content (Bast and Haenen, 1988). Our results showed decreased activity of SOD, CAT, and GPx in ethanol administered rats. It has been reported that ethanol impairs the antioxidant system of the tissues in proportion to the amount of ethanol ingestion (Scott et al., 2000).

Superoxide dismutase catalyzes the reaction of superoxide anion radicals (O_2•) dismutation to hydrogen peroxide (H_2O_2), whereas catalase degrades H_2O_2 into a molecular oxygen and water. Superoxide ion (O_2•) and hydroxyl radicals are known to cause marked injuries to the surrounding tissues and organs. Therefore, removing superoxide ion and hydroxyl radicals is probably one of the most effective defense mechanisms against a variety of diseases. Decreased activities of SOD and catalase will result in the accumulation of these highly reactive free radicals leading to deleterious effects such as loss of cell membrane integrity and membrane function (Krishnakantha and Lokesh, 1993).

Oxidant scavenging at the intracellular level within the cytosol appears to rely on GP_X for elimination of low molecular levels of hydroperoxides and lipid peroxidation. Decreased level of GP_X in the liver of rats that ingested with alcohol could be due to either free radical dependent inactivation of enzyme or depletion of its co-substrates, that is GSH and NADPH. Catalase activity was decreased in alcohol induced rats, which could possibly be due to loss of NADPH, or generation of superoxide, or increased activity of lipid peroxidation or combination of all (Das and Vasudevan, 2005). GSH acts as an antioxidant both intracellularly and extracellularly in conjunction with various enzymatic processes. GSH is a major non-protein thiol in living organisms and plays a central role in coordinating the body's antioxidant defense process (Videla and Valenzuda, 1982).

The decrease in the levels of serum vitamin C and E in alcohol treated group could be as a result of increased utilization of this antioxidant in scavenging the free radicals during alcohol induction (Shen et al., 1997). Treatment with livomap significantly increased the activities of antioxidant enzymes and the level of non enzymatic antioxidants. This could be due to the protective effect of livomap on the hepatocytes, which minimized the destruction of hepatocytes and the permeability of liver cells. The phytoingredients present in the medicinal plants of livomap might be responsible for its hepatoprotective effect.

CONCLUSION

In conclusion, ethanol-induced oxidative stress led to liver damage, which was attenuated by treatment with Livomap. The formulation at a dose of 50 mg/kg body weight was found to be superior to 25 mg/kg. The active phytoconstituents present in Livomap may repair the hepatic tissue damage induced by the alcohol by stimulating the hepatic enzymatic and nonenzymatic antioxidant activities and attenuating the lipid peroxidation level in the target organ. Thus, our finding confirmed the hepatoprotective effect of the polyherbal formulation, Livomap against ethanol-induced hepatotoxicity.

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