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Antioxidant and Hepatoprotective Activity of Livina, A Polyherbal Liquid Formulation

SOUMENDRA DARBAR*, BHASKAR GHOSH, SHYAMAPRASAD CHATTAPADHYAY and MATISH RANJAN CHAKRABORTY
R & D Laboratory, Dey's Medical Stores (Mfg) Ltd., 62, Bondel Road, Kolkata-700 019, India
E-mail: darbar.soumen@gmail.com

Alcohol dependency is a major health and socio-economic problem throughout the world. Hepatic damage by alcohol, alcohol consumption in one from or the other has become almost a routine in the hectic modern world. After heart disease and cancer, alcoholism is the world's biggest health problem and most deaths attributed to alcoholism are caused by cirrhosis of the liver. Damaging effects of reactive oxygen species (ROS) on living systems are well documented. They include oxidative attack on vital cell constituents. Results show that Livina, a polyherbal liquid formulation was effective in blunting ethanol-induced enhanced activities of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), level of serum bilirubin (both total and direct), serum total cholesterol, liver weight loss was also effective in reducing ethanol-induced lipid peroxidation. Livina on the other hand was also effective in blunting ethanol-induced suppressed activities of superoxide dismutase (SOD), catalase (CAT) and decreased level of reduced glutathione (GSH). Results of hepatocellular damage caused by ethanol and its recovery by Livina, suggest that it might be considered as a potential source of natural hepatoprotective agent, which could be related to the free radical scavenging properties.

Key Words: Livina, Ethanol, Hepatotoxicity, ROS enzyme.

INTRODUCTION

Alcohol abuse or alcoholism represents one of the major health, social and economic issues facing the world. It has been observed that almost all ingested alcohol is metabolized in the liver and excessive alcohol use can lead to acute and chronic liver disease[1,2]. It has further been observed that most of the consumed alcohol is eventually broken down by the liver and the products generated and accumulated during alcohol metabolism (*e.g.* acetaldehyde) are more toxic than alcohol itself. In addition, a group of metabolic products called free radicals can damage liver cells and promote inflammation, impairing vital functions such as energy production. The body's natural defenses against free radicals (*e.g.* antioxidants) are inhibited by alcohol consumption, leading to increased liver damage[3-6].

In spite of the hepatic damage by alcohol, alcohol consumption in one form or the other has become almost a routine in the hectic modern world. Hundreds of millions people drink liquor, beer or wine for enjoyment, solace and tranquility. Yet, today, as it has been throughout history, alcohol is troubling mankind. After heart disease and cancer, alcoholism is the world's biggest health problem and most deaths attributed to alcoholism are caused by cirrhosis of the liver[7-9].

Considering the above, it is felt the need to explore the availability of a herbal drug, which could protect against hepatic damage due to ethanol. An indigenous polyhebral drug Livina (Dey's Medical Stores (Mfg.) Ltd., Kolkata, India), has been proved to be very useful in several cases of hepatic involvement, therefore an attempt has been made in this study to find out whether Livina could offer protection against ethanol induced hepatic damage and the assessment has been made by some biochemical and histopathological investigation.

The aim of this study was to investigate the possible protective effects of Livina, a polyherbal formulation on the serum hepatospecific markers and free radical damage of liver caused by ethanol in rats.

EXPERIMENTAL

Male wister albino rats weighing 120 ± 5 g were used in the experiment. They were maintained in a 12 h light and dark cycle at 25 ± 2 °C. They were allowed free access to standard dry pellet diet and water *ad libitum*. All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC).

Experimental design: Group-I: Control rat feed with normal food and water for 28 d; **Group-II:** Experimental rats are feeds with 5 mL (25 %) ethanol/day for 28 d; **Group-III:** Animals were administered Livina (5 mL) along with ethanol for 28 d; **Group-IV:** Animals were feed with 5 mL of Livina only for 28 d.

The composition of each 5 mL of Livina syrup is as follows: *Solanum nigrum* (50 mg), *Holarrhena antidysentrica* (25 mg), *Tephrosia purpurea* (100 mg), *Andrographis peniculata* (25 mg), *Phyllanthus niruri* (50 mg), *Tinosphora cordifolia* (25 mg), *Terminalia chebula* (25 mg), *Asteracantha longifolia* (50 mg), *Alstonia scolaris* (50 mg), *Berberis aristata* (100 mg), *Chichorium intybus* (25 mg), *Picrorhiza kurroa* (50 mg).

Biochemical analysis: After the experimental period, animals in different groups were sacrificed. The rats were killed by decapitation after inducing anesthesia (pentobarbitone sodium (60 mg/kg) and blood were collected for serum. Serum levels of SGOT, SGPT, SAP, BUN, cholesterol and bilirubin (total and direct), were determined by standerd biochemical kit. Lipid peroxidation[10-12] were determined by spectrophotometrically.

Histopathological analysis: The liver from all the groups were excised immediately after sacrifice. Tissues were fixed in 10 % formalin in phosphate buffer (pH 7.0) for 24 h at room temperature for histopathology. Tissues were embedded in paraffin wax and sections were cut at 5 mm slices and were stained with haematoxylin eosin and observed under light microscope.

Statistical analysis: The mean ± SD values were calculated for each group and a two-way analysis of variance (ANOVA) was done for each quantitative parameter to determine the significance of inter-group differences.

RESULTS AND DISCUSSION

In this study, rats subjected to ethanol only, developed significant ($p < 0.05$) hepatocellular damage as evident from significant increase in serum activity of SGOT, SGPT, ALP, BUN and bilirubin concentration in compared to normal control group, which has been used as reliable markers of hepatoxicity. Oral administration of Livina (5 mL/day) exhibited significant reduction ($p < 0.05$) (Table-1) in ethanol induced increase in levels of SGOT, SGPT, ALP, BUN and bilirubin concentration (Table-2). Treatment with Livina reversed the hepatotoxicity significantly ($p < 0.05$).

Liver weight: Liver weight of rats treated with ethanol only decreased significantly ($p < 0.05$) which is prevented by Livina supplementation.

TABLE-1
EFFECT OF LIVINA ON DIFFERENT BIOCHEMICAL PARAMETERS OF ETHANOL INDUCED HEPATOTOXICITY IN RATS

Group	SGPT (IU/L)	SGOT (IU/L)	BUN (mg/dl)	SAP (IU/L)	Bilirubin (µmol/L)		Cholesterol (mg/dl)
					Direct	Total	
Control	17.8±1.48	13.4±0.65	19.40±0.74	36.60±2.34	2.60±0.35	4.50±0.65	63.35±2.08
Ethanol	28.7±1.20†	29.9±1.02†	28.22±1.07†	48.90±3.68†	4.98±0.50†	7.80±0.36†	28.87±2.96†
Ethanol + Livina	20.8±1.86‡	15.6±0.56‡	22.30±1.55‡	41.22±2.49‡	3.20±0.63‡	4.70±0.44‡	60.72±3.13‡
Livina	17.9±1.68	14.52±0.89	19.68± 0.54	37.12±2.13	2.66±0.524	4.66±0.69	65.79±3.57

All values are mean ± SEM n = 6 rats in each group.
†$p < 0.05$ as compared with Group-I ; ‡$p < 0.05$ as compared with Group-II.

TABLE-2
EFFECT OF LIVINA ON LIPID PEROXIDATION, ANTIOXIDENT ENZYMES AND GSH IN LIVER OF ETHANOL INDUCED HEPATOTOXICITY IN RATS

Group	SOD (IU/mg protein)	CAT (IU/mg protein)	Lipid peroxides (nmol MDA/mg protein)	GSH (IU/mg protein)
Control	6.01±0.48	76.28±4.2	0.62±0.30	61.59±2.11
Ethanol	3.32±0.38†	52.07±2.7†	1.20±0.34†	34.84±1.44†
Ethanol + Livina	5.62±0.88‡	70.01±5.0‡	0.95±0.42‡	58.73±1.56‡
Livina	6.03±0.67	74.53±2.34	0.59±0.46	60.24±1.36

All values are mean ± SEM n = 6 rats in each group.
†$p < 0.05$ as compared with Group-I; ‡$p < 0.05$ as compared with Group-II.

***In vivo* antioxidant activity:** *In vivo* lipid peroxidation study revealed that ethanol treated group showed significant increase ($p < 0.05$) in malondialdehyde (MDA) level when compared with normal control group. Administration of Livina was able to prevent significantly ($p < 0.05$) the rise in malondialdehyde level. There

was a marked decreased in the level of GSH and the activities of SOD and CAT in ethanol treated group when compared with normal control group. The GSH level and the activity of SOD and CAT were significantly increased ($p < 0.05$) in Livina treated groups.

Histopathological changes in liver: Liver of control animals showed hepatocytes arranged around the central vein resembling the spokes of a wheel. The liver sections of animals administered 25 % ethanol revealed fatty degeneration predominantly of the macro vesicular type. The ruptured cells on intoxicated liver were reformed and the cytoarchitecture was restored to the same as normal liver in the case of Livina administration (Table-3, Figs. 1 and 2).

TABLE-3
HISTOPATHOLOGICAL CHANGES IN LIVER OF WISTAR RATS

Groups	Microscopic observation
Control	Liver samples show normal architecture without any degeneration, necrosis or inflammation seen.
Ethanol	Prominent cetrilobular necrosis with prominent and enlarged central vein. There is significant periportal inflammation. Fatty deposition also seen reflecting liver damage.
Ethanol + Livina	Liver sample showed a significant reduction in portal inflammation and in the sinusoidal dilatation. The central vein was clearly visible. Liver samples also showed good recovery with absence of necrosis and fatty depositions.
Livina	Liver histology was almost normal with only very little sinusoidal dilatation seen in some hepatic lobules. Central vein appeared clearly with the disappearance of fatty deposition and necrosis thus indicating a potent anti-hepatoprotective activity.

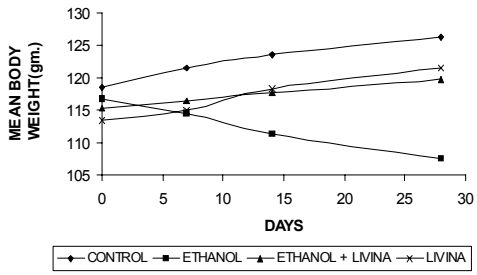

Fig. 1. Effect of livina on mean body weight in ethanol induced hepatotoxiccity in rats

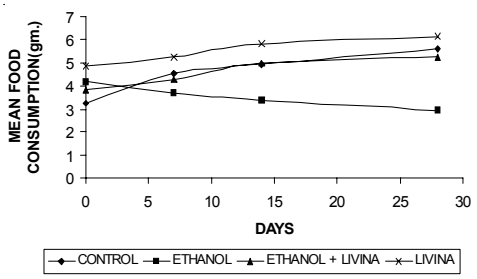

Fig. 2. Effect of livina on food consumption in ethanol induced hepatotoxicity in rats

Liver is a versatile organ in the body concerned with regulation of internal chemical environment. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences.

Ethanol induced liver necrosis was inhibited significantly by administration of Livina, which confirms the protective action of the Livina, a polyherbal liquid formulation against experimentally induced liver damage in rats. SGOT, SGPT, ALP, BUN and bilirubin are the most sensitive tests employed in the diagnosis of hepatic disease. The elevated levels of these parameters were significantly reduced by the treatment of Livina. It can be concluded from this investigation that Livina possess hepatoprotective activity. Ethanol treatment decreased SOD, CAT, GSH and increased lipid peroxidation. Pretreatment with polyherbal drug Livina (5 mL/day/rat) improved the SOD, CAT, GSH and reduced lipid peroxidation. The histological study showed recovery of the damaged liver cells in the ethanol treated group caused by Livina.

Conclusion

The results of the present study thus demonstrate that Livina, a polyherbal liquid formulation protects liver from ethanol-induced damage by preventing the peroxidation of membrane lipids. Further studies are, however, needed to isolate the specific components responsible for the antioxidant action of this multiherbal drug and to establish its mechanism of action.

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