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Research Article

Evaluation of hepatoprotective effect of polyherbal formulation - Livomyn

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ABSTRACT

The liver is the most important organ and has great capacity to detoxify and synthesize useful substances. Therefore liver diseases like hepatic fibrosis, liver cirrhosis, hepatitis and hepatocellular carcinoma are of global concern. In absence of reliable hepatoprotective agent in modern medicine the herbal formulations are the most preferred therapy for liver injury. The present study investigates the efficacy of one such polyherbal formulation – Livomyn as a hepatoprotective formula against ethanol, CCl_4 and galactosamine induced hepatotoxicity in Wistar rats. The evaluation of blood serum parameters exhibits significant reduction in the levels of albumin, bilirubin, SGOT, SGPT, LPO, alkaline phosphate and cholesterol whereas significant increase in Total protein and reduced GSH levels in Livomyn treated groups. The hepatoprotective effect is attributed to synergistic effect of potent antioxidant and hepatoprotective property of various medicinal plant extracts in Livomyn formulation.

Keywords: Hepatoprotective; Antioxidant; Polyherbal formulation; Livomyn.

INTRODUCTION

The liver is the most important organ governing essential biochemical activities in the human body. It has great capacity to detoxify and synthesize useful substances, and therefore damage to the liver has grave consequences (Alessandro 2000 & Mageswari 2010). Acute and chronic liver diseases are of global concern (Sung-Hwa Kim 2009). In the absence of reliable liver protective drugs in modern medical practices, herbs play an important role in the management of various liver disorders. A number of plants have shown hepatoprotective property (Scott Luper 1998). Developing therapeutically effective formulation from natural products may reduce the risk of toxicity when the drug is used clinically and can give the benefit of synergetic effect of many medicinal plants. Therefore, considerable efforts to formulate useful polyherbal medicines from documented medicinal plants for a hepatoprotection are successfully made and some herbal formulations are marketed under various brand names. The aim of the present study is to evaluate hepatoprotective activity of one such polyherbal formulation- Livomyn on oral administration in Wistar Rats using Ethanol, Carbon tetrachloride and D-galactosamine as hepatotoxic agents. These compounds induce hepatotoxicity by various mechanisms. Ethanol induces oxidative stress which plays a major role in causing liver injury. A change in the redox state and production of acetaldehyde leads to mitochondrial damage which ultimately results in hepatotoxicy. Carbon tetrachloride (CCl_4) is a well-known hepatotoxin that is widely used to induce toxic liver injuries in laboratory animals. Exposure to CCl_4 results in acute liver injury; continuous exposure to this toxin produces progressive liver injury and fibrosis, eventually causing cirrhosis, portal hypertension, and death (Mageswari 2010). D-galactosamine is known for inducing the features of acute hepatitis in rats resembling to viral hepatitis. The toxic effect of D-galactosamine is related to an insufficiency of UDP-glucose and UDP-galactose and the loss of intracellular calcium homeostasis (Ferenčíková 2003).

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MATERIAL AND METHODS

Animals: Healthy Wistar Rats weighing between 140-225 gm were procured from Haffkine Biopharmaceuticals Corporation Ltd, Parel, Mumbai-12. The animals were maintained in the registered animal house facility (Reg.No: 87/1999/CPCSEA dtd. 2.12.1999) at ICT, Mumbai during the conduct of study. Animals were maintained under standard husbandry conditions (22-28ºC, 60-70% relative humidity, 12 hr L: D cycle) and fed with standard diet and water *ad libitum*. Animals (Rats) were acclimatized for 7 days before beginning the study.

Chemicals: All the chemicals used were of AR grade. The marketed formulation of Livomyn was procured from local Chemist shop.

EXPERIMENTAL

The experimental protocol was approved by the Institutional Animal Ethics Committee of ICT (approval no. UICT/PH/IAEC/0708/21) and the experimental work

Table 1: Hepatoprotective effect against ethanol induced hepatotoxicity

Parameters	Control	Toxicant	Livomyn- 1 TD	Livomyn- 2 TD
Albumin (g/dl)	4.125 ± 0.184*	3.867 ± 0.158	3.917 ± 0.158*	3.817 ± 0.182*
Alkaline Phosphatase (IU/l)	257.33±13.54**	403.0 ± 11.83	398.0 ±17.60*	374.17 ±18.95*
Bilirubin (mg/dl)	0.350±0.022**	0.517± 0.031	0.40 ±0.037$^{\#}$	0.383 ± 0.031$^{\#}$
Cholesterol (mg/dl)	88.50 ± 2.65**	105.17 ± 2.27	106.5 ± 5.30**	90.0 ± 2.17$^{\#}$
SGOT (IU/l)	273.0 ± 4.54**	336.83 ±7.14	320.83 ± 6.46*	297.17 ± 3.77$^{\#\#}$
Total protein (gm/dl)	7.45 ± 0.34*	6.73 ± 0.22	6.967 ± 0.15*	7.50 ± 0.13*
SGPT (IU/L)	35.0 ±2.11**	41.33 ± 1.76	37.17 ± 0.57*	34.50 ± 2.30$^{\#}$
LPO	0.049 ± 0.019*	0.076± 0.003	0.077± 0.001*	0.067 ±0.003**
GSH	0.286 ± 0.286*	0.231±0.005	0.293 ± 0.015*	0.299± 0.007

N=6, Values are expressed as Mean± S.E.M. Significance at *P<0.05, ** P<0.01 Toxicant Vs Control and $^{\#}P$<0.05, $^{\#\#}P$<0.01 Toxicant Vs Livomyn

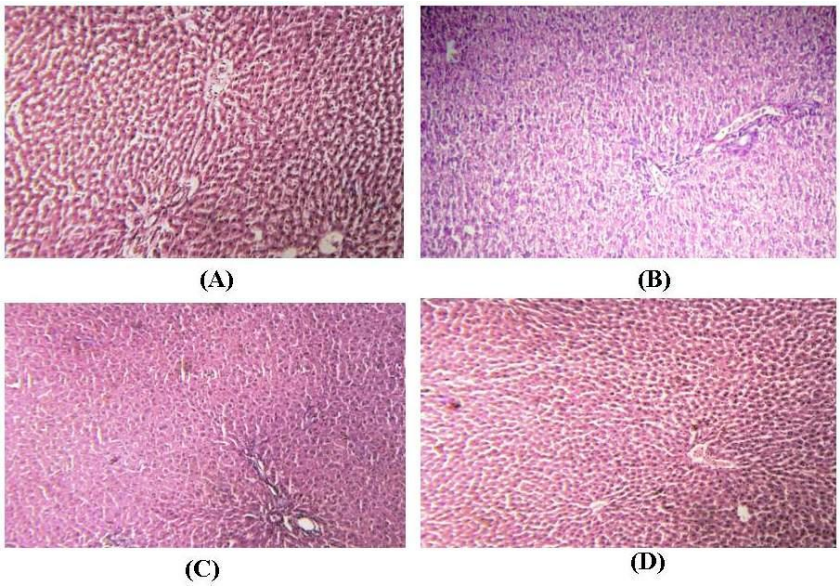

Figure 1: Histopathology of Liver samples of ethanol induced hepatotoxicity

(A) Normal control group: Depicts normal architecture of liver
(B) Toxicant control group: Marked Lobular Disarray, marked glycogen infiltration, Mild diffused lymphocyte infiltration
(C) Livomyn 1 TD: NAD
(D) Livomyn 2 TD: NAD

were carried out as per Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines. The Hepatoprotective effect of Livomyn was evaluated in Wistar Rats using three different experimental models. All the animals were randomly divided into three sets having four different groups.

Hepatoprotective effect against ethanol induced hepatotoxicity (Sadhana 2007 & Pornpen 2007)

The animals were randomly divided in four different groups each containing eight animals as follows:

Group I: Normal Control group (1ml Distilled water)

Group II:Negative Control-Toxicant group (6% Ethanol)

Group III: 6% Ethanol + Livomyn 1TD (Single Therapeutic dose for Rats)

Group IV: 6% Ethanol + Livomyn 2TD (Double Therapeutic dose for Rats)

All animals except from normal control group were orally administered 1ml of 6% v/v Ethanol in distilled water once daily, three hours after the drug administration. Livomyn formulation was diluted with distilled water, to be as extrapolated human dose in rats. The therapeutic dose (TD) was calculated from the human dose of Livomyn and was administered in two doses namely therapeutic (TD) and twice the therapeutic dose (2TD). Both the ethanol and the Livomyn treatment was given for 21 days.

Hepatoprotective effect against CCl_4 induced hepatotoxicity (Mehta 2006a)

The similar procedures were followed as in ethanol induced hepatotoxicity study. The CCl_4 was administered by intra-peritoneal route thrice a week three

Table 2: Hepatoprotective effect against CCl4 induced hepatotoxicity

Parameters	Control	Toxicant	Livomyn-1 TD	Livomyn-2 TD
Albumin (g/dl)	4.125± 0.184*	3.860 ± 0.103	4.00 ± 0.14*	3.867 ±0.178*
Alkaline Phosphatase (IU/l)	257.33±13.54**	388.2 ± 7.43	364.5 ±25.43*	337.67±17.24**
Bilirubin (mg/dl)	0.350±0.022*	0.52± 0.058	0.417 ±0.048*	0.50 ± 0.058*
Cholesterol (mg/dl)	88.50 ± 2.65**	116.2 ± 4.56	98.33 ± 2.55$^{\#\#}$	96.33± 3.073$^{\#\#}$
SGOT (IU/l)	273.0 ± 4.54*	340.67 ±9.58	329.50 ± 10.90*	283.17 ± 7.35$^{\#\#}$
Total protein (gm/dl)	7.45 ± 0.34*	8.00 ± 0.411	7.450± 0.267*	7.33 ± 0.182*
SGPT (IU/L)	35.0 ±2.11**	43.67± 1.48	39.67± 1.453*	36.67 ± 1.47$^{\#}$
LPO	0.049 ± 0.019**	0.078± 0.001	0.073± 0.003*	0.064 ±0.002*
GSH	0.286 ± 0.286**	0.245±0.009	0.266± 0.017*	0.285± 0.016**

N=6, Values are expressed as Mean± S.E.M. Significance at *P<0.05, ** P<0.01 Toxicant Vs Control and $^{\#}P$<0.05, $^{\#\#}P$<0.01 Toxicant Vs Livomyn

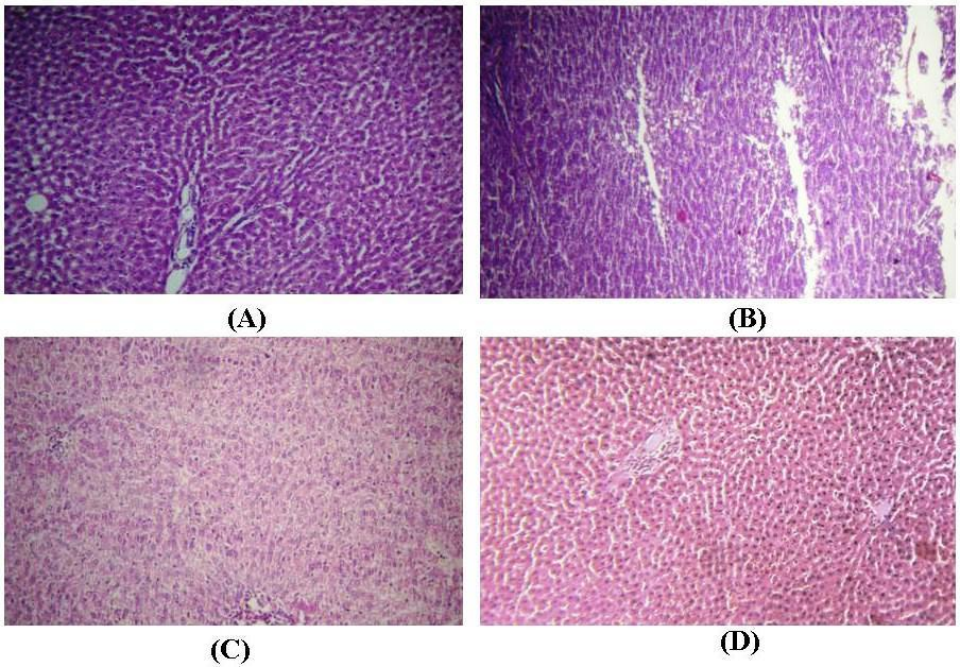

Figure 2: Histopathology of Liver samples of ethanol induced hepatotoxicity

(A)Normal control group: Depicts normal architecture of liver
(B)Toxicant control group: Marked lobular disarry, marked granular degeneration, mild vesicular change
(C)Livomyn 1 TD: Minimal Glycogen infiltration
(D)Livomyn 2 TD: NAD

hours after the drug administration in the dose of 0.8 ml/kg body weight. Livomyn was administered in two doses TD and 2TD for 7 days.

Hepatoprotective effect against D-Galactosamine induced hepatotoxicity (Mehta 2006b)

The animals were divided randomly in four groups as in ethanol induced hepatotoxicity study. D-Galactosamine was administered by intra-peritoneal route once only on the first day of the study in the dose of 700mg/kg of body weight in 1 ml of distilled water. The test drug Livomyn was given for seven days.

All the animals from all the three experimental models were euthanized under ether anesthesia at the end of the study duration. The animals were fasted for 12 hours before sacrifice. The blood samples were collected and serum was separated by centrifugation. Livers were collected in 10% formalin solution and in normal saline. The samples in formalin were sent for histopathology studies (Williamson 1996). The liver homogenate samples in normal saline were analyzed for glutathione and malondialdehyde content. The serum samples were analysed to determine blood serum parameters like Albumin, Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALKP), Total protein, Cholesterol and Bilirubin levels.

Data Analysis

Values are expressed as mean ± SEM. Statistical differences between means were determined by performing one-way ANOVA followed by Dunnett's test. P<0.05 was considered as significant.

Table 3: Hepatoprotective effect against D-galactosamine induced hepatotoxicity

Parameters	Control	Toxicant	Livomyn- 1 TD	Livomyn- 2 TD
Albumin (g/dl)	4.125± 0.184*	3.775 ± 0.170	3.540 ± 0.169*	3.783 ±0.117*
Alkaline Phosphatase (IU/l)	257.33±13.54**	440.66±14.65	370.67±14.90$^{\#}$	284.67 ±17.79$^{\#\#}$
Bilirubin (mg/dl)	0.350±0.022*	0.450± 0.043	0.383±0.031*	0.367 ± 0.033*
Cholesterol (mg/dl)	88.50 ± 2.65**	120.33 ± 2.23	106.00 ± 3.04$^{\#\#}$	93.66± 2.53$^{\#\#}$
SGOT (IU/l)	273.0 ± 4.54*	328.8 ±15.17	306.17 ± 13.23*	277.0 ± 8.83$^{\#}$
Total protein (gm/dl)	7.45 ± 0.34*	6.850± 0.112	6.720± 0.315*	7.125 ±0.063*
SGPT (IU/L)	35.0 ±2.11**	47.67± 2.73	41.67± 1.94*	37.66 ± 1.944$^{\#}$
LPO	0.049 ± 0.019*	0.081± 0.003	0.074± 0.003*	0.070 ±0.003*
GSH	0.286 ± 0.286*	0.215±0.003	0.261± 0.014*	0.282± 0.016**

N=6, Values are expressed as Mean± S.E.M. Significance at *P<0.05, ** P<0.01 Toxicant Vs Control and $^{\#}P$<0.05, $^{\#\#}P$<0.01 Toxicant Vs Livomyn

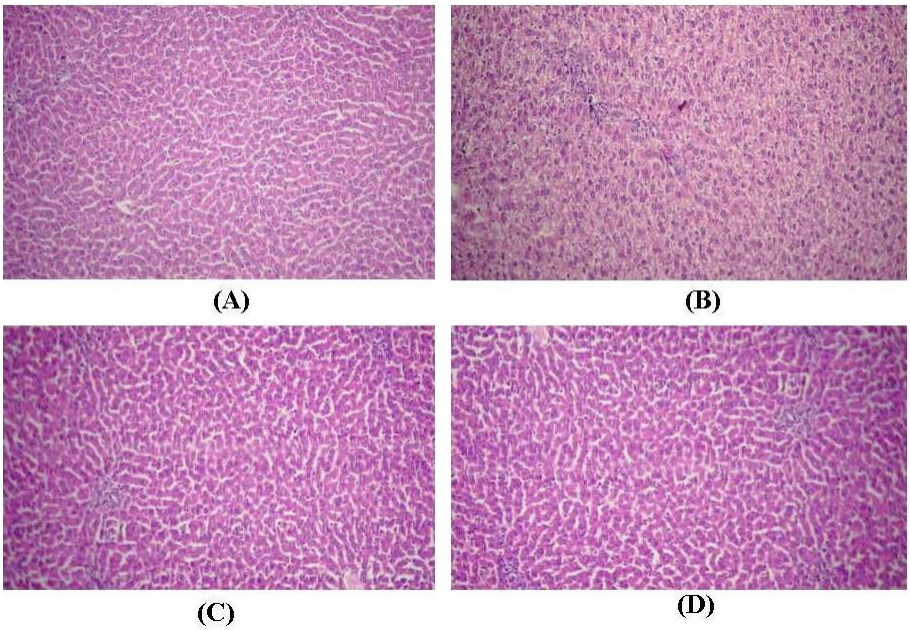

Figure 3: Histopathology of Liver samples of D-galactosamine induced hepatotoxicity

(A)Normal control group: Depicts normal architecture of liver

(B)Toxicant control group: Depicts marked glycogen infiltration, moderate multifocal periportal lymphocytic infiltration

(C)Livomyn 1 TD: Depicts minimal granular degeneration, minimally multifocal minimal periportal lymphocytic infiltration, mild biliary hyperplasia

(D)Livomyn 2 TD: Minimal multifocal minimal periportal lymphocytic

RESULTS

In the ethanol induced hepatoprotective model administration of ethanol produced significant rise in ALKP, bilirubin, Cholesterol, SGOT, and LPO indicating significant liver injury. Whereas in Livomyn treated group the level of SGOT, SGPT, ALKP and bilirubin were considerably decreased than that of toxicant group as shown in Table-1. There was significant reduction in the levels of cholesterol and albumin in Livomyn group and increase in the levels of total protein as well as GSH in dose dependant manner. Thus Livomyn arrested alcohol induced liver injury significantly, and hence can be used to protect the liver damage due to alcohol.

Evaluation of hepatoprotective effect using CCl_4 induced hepatotoxicity in Wistar rats indicated prominent hepatoprotective effect of Livomyn (Table-2). The serum biochemical parameters like ALKP, cholesterol, SGOT, SGPT, and LPO were significantly increased on administration of carbon tetrachloride. This was due to free radical generation by CCl_4. Livomyn significantly reversed the rise in level of Cholesterol, SGOT, SGPT, and LPO indicating protection to the liver. Livomyn in both the doses increased the reduced GSH levels as well as total protein content. In fact at higher dose, Livomyn almost brought the levels of reduced GSH back to normal, in spite of concomitant administration of CCl_4.

Similar results were observed in the D-galactosamine induced hepatotoxicity where the levels of ALKP, Cholesterol, SGPT and LPO were significantly increased on galactosamine administration, indicating injury to the

liver. The levels of reduced GSH were significantly decreased due to administration of D-galactosamine, indicating reduced potential of liver to scavenge free radicals. This is a hallmark of injury to the liver. Livomyn treatment decreased the increased level of SGPT, bilirubin, ALKP, LPO and SGPT as indicated by serum parameters in Table 3. Also the level of Cholesterol in Livomyn treated group was reduced to normal level and the levels of total protein and GSH were increased which was comparable to the normal control group exhibiting the remarkable hepatoprotective effect of Livomyn.

These results were supported by the histopathograms where liver histopathology signifies hepatoprotective effect of Livomyn (Figure-1, 2& 3 Group C and D)

DISCUSSION

Phytopharmaceuticals are coming up as an effective source of disease treatment. Ayurvedic system of medicine has always used this potential of plant products (Manisha 2008). The hepatoprotective effect of one such poly herbal formulation Livomyn may be attributed to its herbal ingredients which possess very potent antioxidants and hepatoprotective phytoconstituents. Livomyn contains extracts of the plants like *Andrographis paniculata, Phyllanthus niruri, Cichorium intybus, Boerhaavia diffusa, Tinospora cordifolia* and *Picrorrhiza kurroa*.

Andrographis paniculata is very well known plant for its liver protective effect. Andrographolide is the major active constituent isolated from the plant and it has been reported that it protected against paracetamol-induced toxicity on isolated rat hepatocytes (Visen 1993). *Phyllanthus niruri*, a widely distributed herb is in use in herbal medicinal systems and is effective against toxicity caused by several hepatotoxicants. The hepatoprotective nature of *Phyllanthus niruri* can be attributed to the presence of several bioactive compounds such as lignans, alkaloids, terpenoids and tannins especially phyllanthin and hypophyllanthin, quercetin, astragalin, gallic acid, ellagic acid and corilagin. It also possesses glycosides, flavonoids, flavonols, polyphenols, phenylpropanoids, which are known antioxidants (Mageswari 2010 & Masturah 2007). The presence of all these phenolic compounds may contribute to the high free radical scavenging activity of *Phyllanthus niruri*(Rajeshkumar 2002). Moreover *Phyllanthus niruri* extract has been found to inhibit many drugs metabolizing P450 enzymes (Kuzhuvelil 2006).

Cichorium intybus is a popular ayurvedic remedy for the treatment of liver diseases. It is commonly known as “kasani” and it is a part of polyherbal formulations used in the treatment of liver diseases (Jindal 1975). In recent years many researchers have examined the effects of *Cichorium intybus* roots, leaves and seeds on hepatotoxic damages (Sadeghi 2008). *Boerhaavia diffusa* is a plant known for its medicinal properties, employed in folkloric medicine in Nigeria, and Ayurvedic medicine system of India. The plant is consumed as vegetable as it is believed to be a rich source of vitamins, minerals, protein and carbohydrate (Cho 2004). It has been shown to contain a large number of compounds such as flavonoid, saponins, steroids and alkaloids. Traditionally, *Boerhaavia diffusa* has been evaluated for its hepatoprotective, anti-diabetic, diuretic, anti-inflammatory, antibacterial, antiviral and cancer chemo preventive properties (Rawat 2008 & Adenubi 2010).

Extracts of all these plants in Livomyn may be active individually or in combination to give the hepatoprotective benefits. The phytoconstituents of all these plants may act synergistically producing greater hepatoprotective effect. Inhibition of CYP450 metabolising enzymes which could illicit protective effect by reducing free radical formation due to oxidative metabolism of toxicants like ethanol.

CONCLUSION

It is evident from the study that Livomyn is a potent polyherbal formulation against ethanol, CCl_4 and D-galactosamine induced hepatotoxicity in experimental animals. It can be used to arrest the alcohol, drug or viral induced hepatotoxicity as well as to treat any other type of liver injury and reversing the hepatocyte damage.

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