

Hepatoprotective Effect of L-35-A Polyherbal Formulation

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Summary

L-35 is a polyherbal formulation, consisting plant ingredients of *Picrorrhiza kurroa* (scrophulariaceae) and *Tephrosia purpurea* L. (Papilionaceae). The L-35 was investigated for *in-vivo* hepatoprotective effects. The ethanol was used as toxicant, and silymarin as standard drug. Different groups of wister albino rats were treated with alcohol (3.76gm/kg p.o., BD), silymarin (100mg/kg p.o., BD) and different dose level of L-35 (5 ml/kg, 10 ml/kg and 15ml/kg p.o., BD) for 25 days. The effects of treated groups on wet liver weight, wet liver volume, serum transaminase (SGOT, SGPT), alkaline phosphatase (ALP), bilirubin (Direct and Total), Total albumin and total protein were measured. L-35 at a dose of 15ml/kg shows significant ($p < 0.01$) hepatoprotective effect compare to that of toxicant group. Histopathological studies also reveal the normal hepatocytes with normal lobular architecture compared to that of toxicant group.

Keywords: L-35, Hepatoprotective effect, Ethanol, Rats.

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Introduction

Nutritional status is a primary factor in the effects of xenobiotics and may be an important consideration in development of safety standards and assessment of risk. One important xenobiotics consumed daily by millions of people worldwide is alcohol. Ethanol is xenobiotics that are known to be associated with beneficial and adverse biological response, depending upon all the factors i.e. age, gender, endocrine status dose, frequency of intake, duration of exposure, and pattern of consumption. Some adverse effects of alcohol, such as alcohol liver disease, have been linked to diet. It is not uncommon for xenobiotics to influence food consumption to such a degree as to result in poor nutritional status (1).

Ayurveda, and indigenous system of medicine in India, has a long tradition of treating liver disorders with plant drugs. L-35 is a polyherbal formulation consisting of *Picrorrhiza kurroa* (scrophulariaceae; roots/ whole plant; one part) and *Tephrosia purpurea* L. (Papilionaceae; whole plant; two parts). Many of the individual ingredients were earlier investigated for their protective activity using different models of experimental hepatotoxicity. Keeping the above information in view, the present study was designed to demonstrate the protective role of L-35, against hepatotoxicity in rats induced by chronic administration of ethanol.

Materials and Methods

Drugs and chemicals

Silymarin was obtained from Micro labs, Bangalore. The kits for all biochemical estimations were purchased from Erba Diagnostics Mannheim GmbH Germany. The other chemicals used were of analytical grade.

Herbal formulation collection

L-35 (Hepaliv) a polyherbal formulation was a kind gift sample from M/S Annapurna Bio Ved Pvt Ltd Hyderabad.

Animals

Albino wistar rats and mice of either sex weighing between 150-200 g and 20-30 g respectively were procured from the Central Animal House of N.E.T Pharmacy College, Raichur, Karnataka. The animals were acclimatized for seven days under laboratory conditions. The animals were fed with commercially available rat pelleted diet (Amrut laboratories Pranava Agro Industries Ltd. Sangli). Water was allowed *ad libitum* under strict hygienic conditions. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC No.576/2002/bc/IAEC/CPCSEA) of N.E.T Pharmacy College, Raichur. Studies were performed in accordance with the CPCSEA guidelines.

Acute toxicity studies

The acute toxicity of a polyherbal formulation L-35 was determined by using albino mice (20-30g). The animals were fasted for 4 hrs prior to the experiment. Animals were administered with twice daily (0.1ml) of formulation and observed for its mortality during 48 hours study period (short term) toxicity. Based on the short term profile of drug, the dose for the next animals were determined as per as OECD guideline 425. All the animals were observed for long term toxicity (0.1ml, twice daily for 25 days). The formulation L-35 did not produce any obvious toxicity or mortality when subjected to chronic toxicity studies for 25 days in mice according to OECD guidelines [2]. Hence, the formulation was ensured to be devoid of any potential toxicity and obvious mortality. Further, the different doses of L-35 used in the study for evaluation of its hepatoprotective activity. Thus the doses 5ml/kg,

10ml/kg, 15ml/kg were used as low, medium and high doses respectively in the entire project.

Alcohol induced-hepatotoxicity

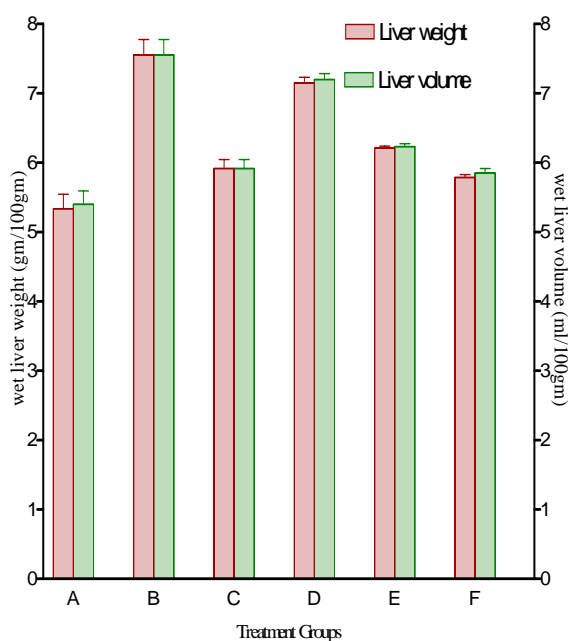
Albino rats of either sex weighing between 150-200 g were selected and divided into six groups of six animals in each. Group A was maintained as normal control, which was given with distilled water only. Group B received ethanol (3.76 gm/kg, twice daily, p.o) and animals in Group C were treated with Silymarin (100 mg/kg, p.o) which served as standard. Animals in Groups D, E and F were treated with three different doses L-35 (5 ml/kg, 10 ml/kg and 15ml/kg p.o., BD) respectively. Group B, C, D, E and, F were intoxicated with ethanol for 25 days. On the 25th day, 3 hrs after last dose of ethanol, the animals were anaesthetized with ether. Blood was withdrawn through retro orbital plexus and biochemical parameters like ALT, AST, ALP, Direct Bilirubin, Total Bilirubin, Total Proteins and Albumin were estimated by standard procedure. The animals were sacrificed by overdose of ether and autopsied. Livers from all animals were removed, washed with saline, weighed and stored in 10% formalin for histopathological studies. Histopathological studies were carried out by a modified method of Luna. The statistical analysis was carried out using one-way ANOVA followed by Dunnett's multiple comparisons for the data which are normally distributed (3, 4, 5, 6).

Results

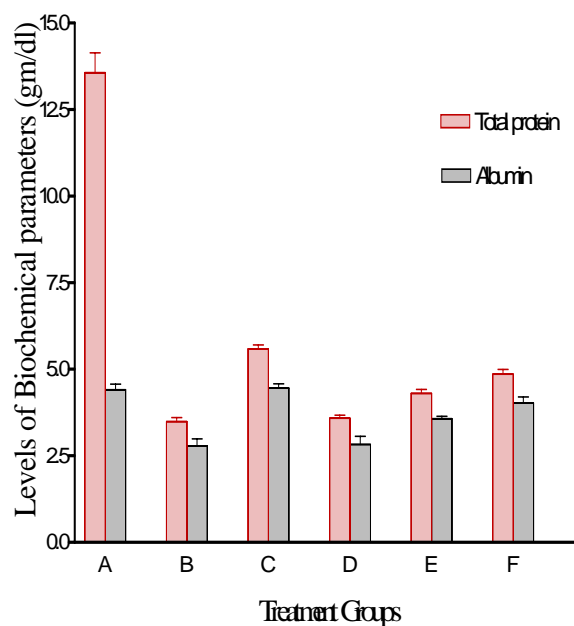
Table 1: Effect of L-35 on wet liver weight, wet liver volume and different biochemical parameters in alcohol-induced Hepatotoxicity

Group	Wet liver weight (gm/100gm)	Wet liver volume (ml/100gm)	Serum Biochemical Parameters						
			Alanine transaminase (u/l)	Aspartate transaminase (u/l)	Alkaline phosphatase (u/l)	Direct bilirubin (mg/dl)	Total bilirubin (mg/dl)	Total protein (mg/dl)	Albumin (mg/dl)
Normal control (10ml/kg)	5.33 ± 0.20	5.40 ± 0.19	40.28± 4.11	107.23 ± 15.35	107.22 ± 7.39	0.20 ± 0.02	0.29± 0.02	13.56± 0.57	4.40± 0.16
Toxicant (Ethanol 3.76 gm/kg, p.o)	7.55 ± 0.22	7.55 ± 0.22	84.91±2.78	216.83±0.76	207.53±1.82	1.75±0.07	1.74±0.04	3.48±0.11	2.79±0.19
Silymarin (100mg/kg)+ Ethanol	5.91 ± 0.12**	5.91 ± 0.12**	42.80±0.43**	109.22±5.09**	105.68±1.51**	0.34±0.01**	0.35±0.07**	5.58±0.11**	4.46±0.11**
Low dose (5ml/kg) BID+Ethanol	7.15 ± 0.08	7.20 ± 0.08	78.95±2.86*	210±1.57*	201.7±1.21*	1.58±0.024*	1.6±0.024*	3.59±0.08	2.83±0.23
Medium dose (10ml/kg) BID+ Ethanol	6.20± 0.03**	6.23 ± 0.04**	50.82±1.70**	186.53±1.00**	146.16±1.06**	1.13±0.034**	1.20±0.03**	4.30±0.11**	3.57±0.07*
High dose (15ml/kg) BID+ Ethanol	5.78 ± 0.04**	5.85 ± 0.06**	40.31±0.89**	136.65±0.86**	126.58±0.69**	0.77±0.04**	0.87±0.01**	4.86±0.13**	4.03±0.17**

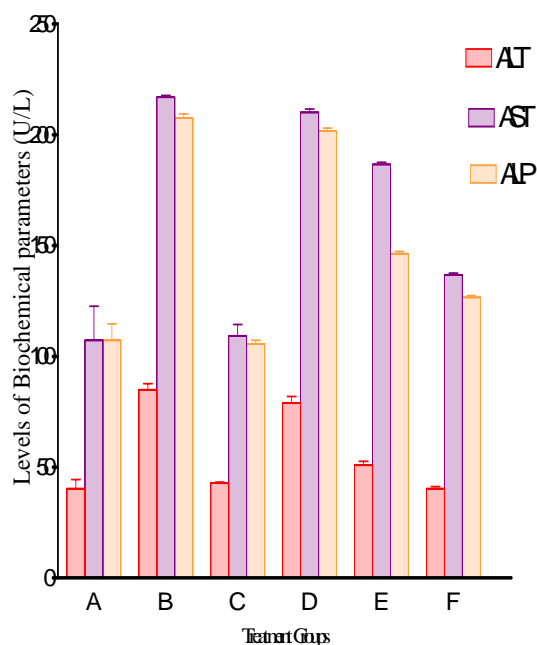
Values are mean ± SEM (n=6) one way ANOVA followed by Dunnett's 't' test. Where, * represents significant at p<0.05, ** represents highly significant at p< 0.01, *** represents very significant at p<0.001.



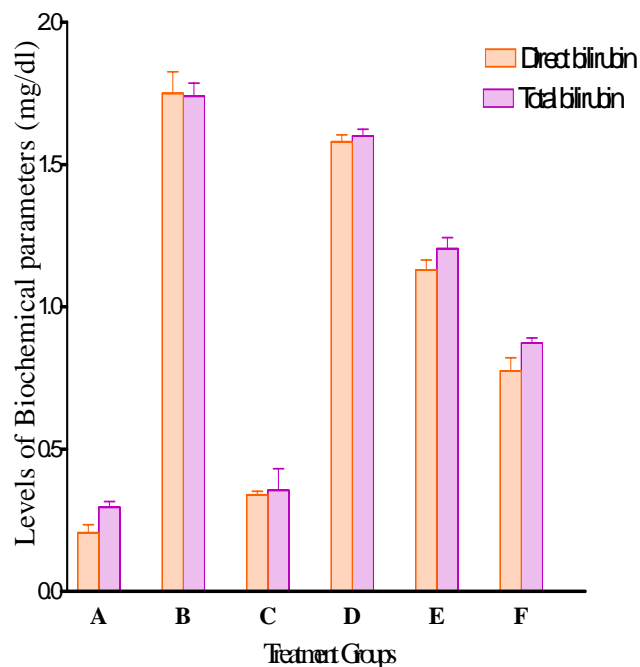
Graph 1. Effect of various treatments on ethanol-induced wet liver weight (gm/100gm) and liver volume (ml/100gm) in rats after 25 days



Graph 3. Effect of various treatments on ethanol-induced Total protein and Albumin (gm/dl) in rats after 25 days

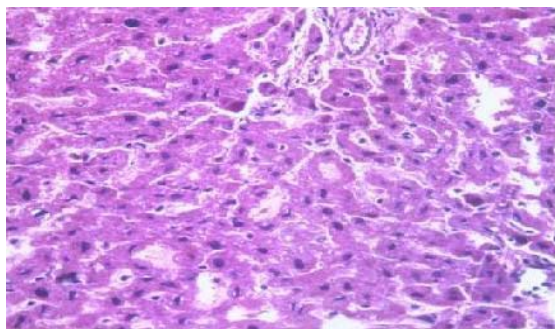


Graph 2. Effect of various treatments on ethanol-induced ALT, AST, ALP (U/L) in rats after 25 days

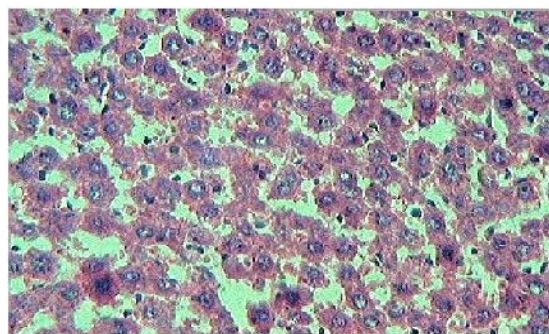


Graph 4. Effect of various treatments on ethanol-induced direct bilirubin and Total bilirubin (mg/dl) in rats after 25 days

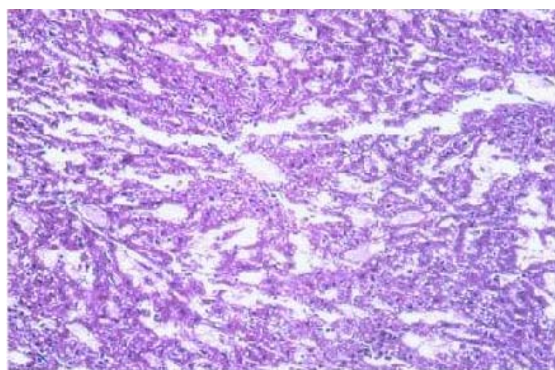
Histopathology of Rats liver



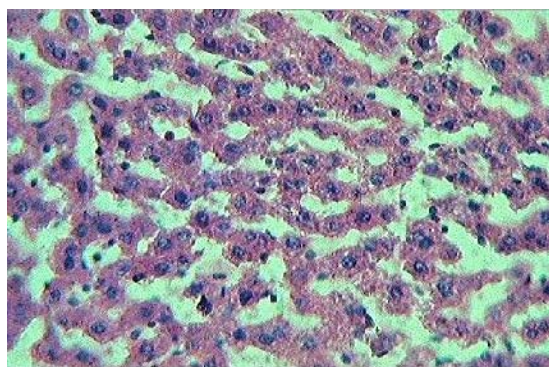
Graph 1. Liver sections showed the normal lobular architecture with hepatocyte arranged in single cords. There is a centrally placed nucleus, the sinusoidal cells against which kupffer cell nucleus are abutting.



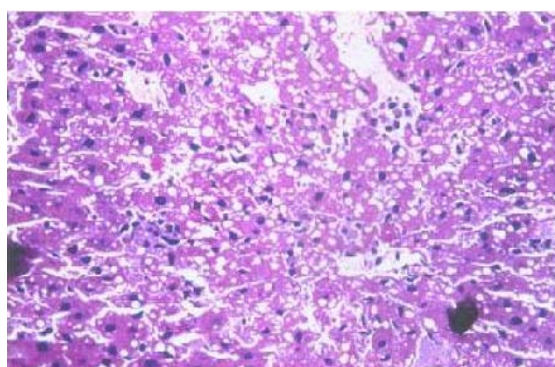
Graph 4. The sections from the liver show Inflammation and focal areas of granular degeneration of hepatocytes.



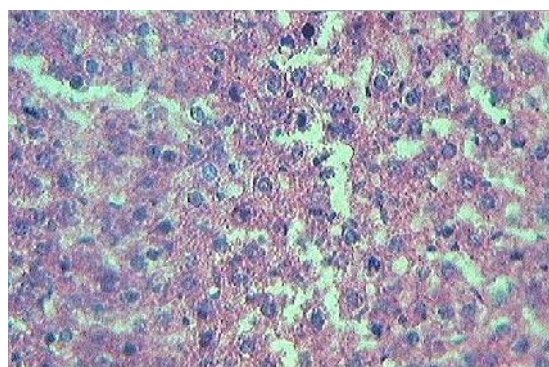
Graph 2: Hepatocytes showed microvesicular fatty changes surrounded by large number of hepatocytes showing hydropic degeneration with loss of nuclear architecture.



Graph 5. The liver shows mild Inflammation and liver cells appear normal.



Graph 3. No centrilobular necrosis or severe hydropic degeneration was not observed that indicates protective effect of drug against hepatic damage induced by ethanol.



Graph 6. The section show normal hepatocytes with normal lobular architecture.

Discussion

Alcohol is well established xenobiotics that affects on phase I and phase II metabolic systems. Alcohol increased formation of lipoperoxides, conjugated dienes and malondialdehyde (MDA) and reduced levels of antioxidants like vitamin E and glutathione in the tissues have been demonstrated in experimental animals administered with ethanol as well as alcoholic human subjects and also increases level of AST, ALT, ALP and bilirubin, is conventional indicator of liver injury (7). Oxidative stress is one major factor in etiology of ethanol injury, mainly by Kupffer cell derived reactive oxygen species (ROS) and ethanol activates Kupffer cells primarily through the action of a substance called endotoxin which activates Kupffer cell to generates ROS and pro inflammatory cytokines (TNF alpha, IL-1), both of them can lead to liver damage (8).

Water is retained in the cytoplasm of hepatocyte leading to enlargement of liver cells, resulting in increased total liver weight and volume (9) This alcohol-induced increase in wet liver weight and volume was prevented by treatment of L-35, thus indicating hepatoprotective activity. During hepatic damage, cellular enzymes like alanine transaminase, aspartate transaminase, and alkaline phosphatase present in liver cells leak into serum, resulting in increased concentrations(10). Alcohol administration for 25 days increased all serum enzymes, whereas silymarin and L-35 treated groups showed reduced alanine transaminase, aspartate transaminase, and alkaline phosphatase levels and increased total protein and serum albumin levels indicating hepatoprotective effect against alcohol-induced liver damage.

The protective effect of L-35 in ethanol induced hepatotoxicity is probably due to the increase of the activities of the antioxidant enzymes, or to a counteraction of the free radicals by the presence of the electrophilic constituents picoside I, picoside II and kutkoside (11) or to an activated conjugation of antitubercular drugs with reduced glutathione in liver or due to membrane stabilizing action (12) or due to antilipid peroxidation activity (14).

The histopathological studies shows that L-35 treated group inhibit microvesicular fatty changes surrounded by large number of hepatocytes showing hydropic degeneration with loss of nuclear architecture induced by alcohol toxicant.

Conclusion

In the present investigation, the poly herbal formulation L-35 has shown a potent hepatoprotective action at a high dose of 15ml/kg, p.o. in ethanol intoxicated animal models.

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