Research Article

To Study the Effect of Hepasid on Ethanol Induced Hepatotoxicity in Rats

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ABSTRACT

This study investigated hepatoprotective activity of Hepasid syrup in albino rats with liver damage induced by ethanol. Liver is more susceptible to the toxic effect of ethanol. Ethanol induce liver injury is one of the widely used animal model to induce hepatotoxicity in rats. The dose & concentration of ethanol required to induce liver injury varies to great extent. The goal of this study was to compare efficacy of hepasid with standard hepatoprotective drug. The doses of 40% ethanol i.e 2 ml/g of body weight/day orally for 21 days to induce liver injury in either sex Albino rats. The blood was collected at 22nd day & serum parameters of the enzymes like alkaline phosphate (ALP), Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvate transaminase (SGPT), Total Bilirubin (TB), Total Protein (TP), Total Cholesterol (TC) were estimated to determine the level of liver injury.

Keywords: Hepatotoxicity, Silymarin, SGPT, SGOT, ALP, Total bilirubin, Total Protein.

INTRODUCTION

The liver is one of the largest organs in the human body and the major site for intense metabolism and excretion. Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion of reduced glutathione levels. In addition, serum levels of many biochemical markers like transaminases, alkaline phosphatase, bilirubin, triglycerides and cholesterol are elevated in liver disease.[1] Liver diseases pose a serious challenge to international public health. The regulation of homeostasis is done by within the body & involved in almost all the biochemical pathway related to metabolism of fats, carbohydrates, proteins, hormones, synthesis and storage of vitamins. There are over 100 different forms of liver diseases that affect men, women, and children. Alcohol- related liver disease i.e. cirrhosis, and Non-alcoholic such as Hepatitis, Haemochromatosis & primary biliary cirrhosis is one of the types of liver disease. The disturbance of liver function such as illness causes due to liver disease. The liver is liable for several important functions at intervals the body and may it become unhealthy and slashed, the loss of these functions will cause important harm to the body. Liver disease could be a broad term that covers all the potential issues that cause the liver to fail to perform its selected function. Sometime over 75% or 3 quarters of liver tissue has to be affected before a decrease in perform happens.

The study of plant extract for hepatoprotective activity, it is important to induce liver toxicity in experimental animals. Many agents are available, which on administration produce acute and chronic liver toxicity. The drugs like paracetamol or isoniazid are mostly use to induce liver toxicity and the chemicals such as alcohol or carbon tetrachloride also used for induce hepatotoxicity. Due to fungal and parasitic infection, which causes hepatotoxicity e.g. schistosomiasis, aflatoxin. The chemical induce liver toxicity such as ethanol induced toxicity widely used for the hepatoprotective activity of drug. Chronic consumption of alcohol causes liver hepatitis, cirrhosis and fatty liver infiltration. Fatty liver infiltration causes because of alcohol replace fatty acid in the mitochondria. And the liver hepatitis and cirrhosis causes because of chronic consumption of alcohol the lipid peroxidation reaction occur during microsomal metabolism of alcohol.[2] Chronic consumption causes accumulation of reactive oxygen species, it also causes lipid peroxidation of cellular membrane and oxidation of DNA & proteins resulting into liver injury.[3] As shown in figure I, Ethanol produce to constellation of dose related deleterious effect in liver. The major enzyme system i.e. ADH (aldehyde dehydrogenase) is involved in metabolism of alcohol in the liver. ADH converts alcohol to acetaldehyde by removing hydrogen. Acetaldehyde promotes cell death by depleting glutathione levels which impairs major defense mechanism against oxidative damage.

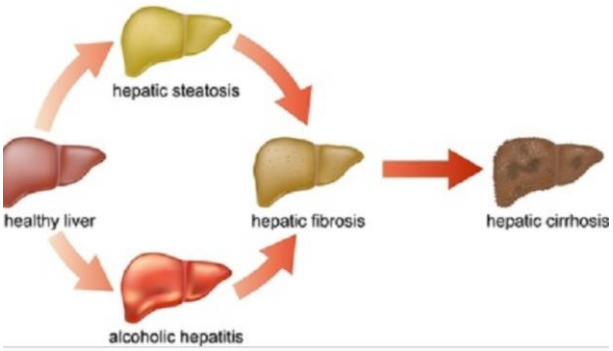

Figure 1: Hepatic Cirrhosis

The hepasid syrup contains a large number of herbs, which are known from ancient time as liver protective. Hepasid contain number of herb such as himsara, kasni, bhringraj, sharpunkha, kutki, punarnava, kakmachi, kasmarda, jhabuka, bhumylaki, guduchi, daruharidra, chitrak, awala, vidang, haritaki, parpata, kumari, parpata, were reported to passes hepatoprotective activity. It relives the problems of digestive system like gases, stomachache, ajirna, weak digestion etc. Other herbs are effective in obstruction of liver and spleen & shows good result in cirrhosis and viral hepatitis. The Hepasid mainly involves membrane stabilization of liver cells as indicated by decrease in levels of SGOT, SGPT and bilirubin, where in it prevents cellular damage and loss of functional integrity of the liver cell membranes caused by various hepatotoxic agents.

MATERIALS AND METHODS

Plant Material collection and authentication:

The Hepasid (Polyherbal formulation) was obtained as a gift sample of testing and analysis, from (Shree Baidynath Ayurved Bhavan Pvt. Ltd. Nagpur).

Procurement of Experimental animals (Albino rats):

The albino rats (wistar strain) of either sex weighing 200-250 gm were obtained from in house animal facility of 'Institute of Pharmaceutical Education and Research'. The animals were housed in polypropylene cage at a temperature of 25 ± 20 ^{0}C with relative humidity of 40-60% and 12 hours light dark cycle. Animals were fed with a balanced diet and water ad libitum during the complete experimental period. The Institutional Animal Ethical Committee was approved all experimental animals, (Registration No.535/02/a/CPCSEA/Jan2002) of Institute of Pharmaceutical Education and Research, Wardha.

Procurement of Diagnostic kits and chemicals

Diagnostic kits for estimation SGPT, SGOT, alkaline phosphate, total protein, bilirubin and cholesterol were obtained from MERCK LTD Nagpur. Alcohol was procured from LOBA Chemie, Pvt. Ltd Nagpur. All the other chemicals used for experimental purpose were of laboratory grade.

Albino rats of either sex were divided into following group with six animals in each group:

Group I	Normal (Distilled water), 5 ml/kg per orally for 21 days.
Group II	Control (40% ethanol), 2 ml/g per orally for 21 days.[4]
Group III	Standard (Silymarin), 25 mg/kg per orally for 21 days.
Group IV	Test Sample (Hepasid), 0.5 ml/kg per orally for 21 days.

RESULTS

Mechanism of Alcohol induce Liver damage

Alcohol related liver damage occur when it is taken longer time and in excessive dose. Alcohol is mainly metabolized by liver through a certain chemical reaction such as oxidation. Most of the alcohol is metabolized in cell systole of the liver by involving enzyme alcohol dehydrogenase which converts the alcohol into the toxic substance[5] aldehyde by removing two hydrogen atoms from ethanol molecule. The acetaldehyde is then further metabolized in mitochondria by the enzyme aldehyde dehydrogenase to acetate[6] by again removing hydrogen and adding oxygen. Another pathway of metabolism of alcohol by microsomal alcohol oxidizing system. This system is activated by long term and heavy consumption of alcohol.[7] This system involve the enzyme Cytochrome P450, 2E1 or CYP2E1 that remove hydrogen away from alcohol to produce Acetaldehyde.[8] The stimulation of enzyme CYP2E1 by alcohol is also contribute to alcohol induce liver disease. As shown in figure II.

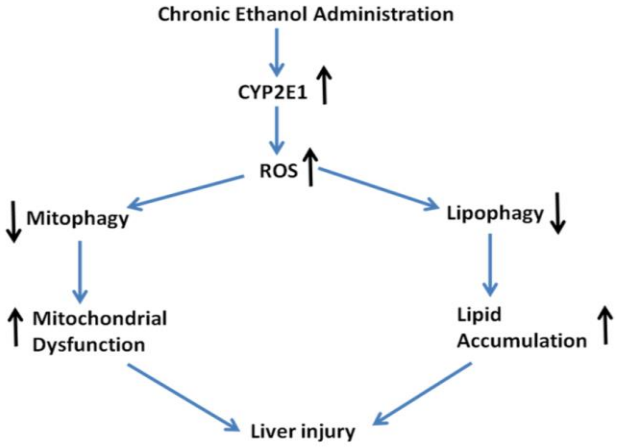

Figure 2: Mechanism of alcohol induced liver damage.

Effect on biochemical parameter

Biochemical serum parameter like SGPT, SGOT, ALP, TB, TP & TC level were found to be elevated in dose dependent manner. Hepatic damage can be assessed by level of cytosolic transaminase including SGPT and SGOT in circulation.[9] A high level of SGOT indicating liver damage that may be due to viral hepatitis as well as cardiac infraction or muscle injury.SGPT catalyze the conversion of alanine to pyruvate and alanine to glutamate and is released similarly. Therefore, SGPT is more reliable as it is more specific to liver as thus the better parameter to detect the liver injury. Alkaline phosphate is excreted normally via bile & liver. Prolong destruction of hepatic cells results in more hepatic releases to exacerbate hepatic dysfunction and also causes elevation in the serum level of ALP and TB.[10] Ethanol induce hepatotoxicity causes rise in serum parameters SGPT, SGOT, ALP, TP, TB and TC. As shown in figure III, IV, V, VI, VII and VIII.

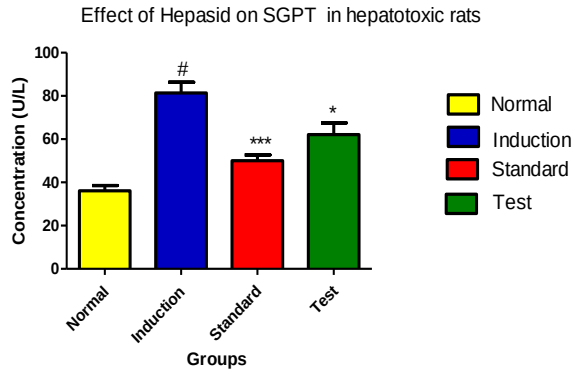

Figure 3: Effect of Hepasid on SGPT

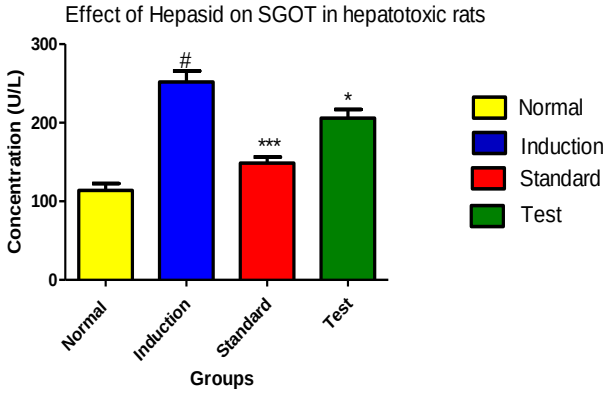

Figure 4: Effect of Hepasid on SGOT

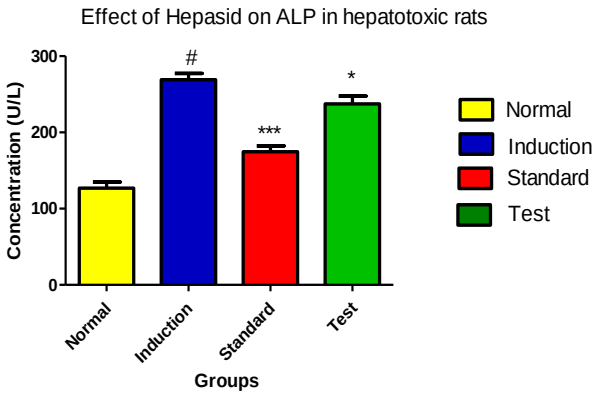

Figure 5: Effect of Hepasid on ALP

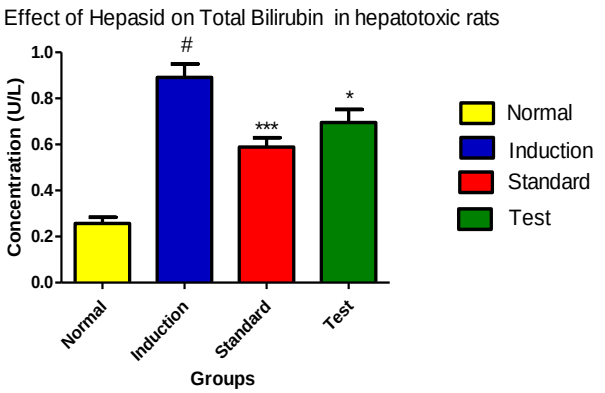

Figure 6: Effect of Hepasid on Total Bilirubin

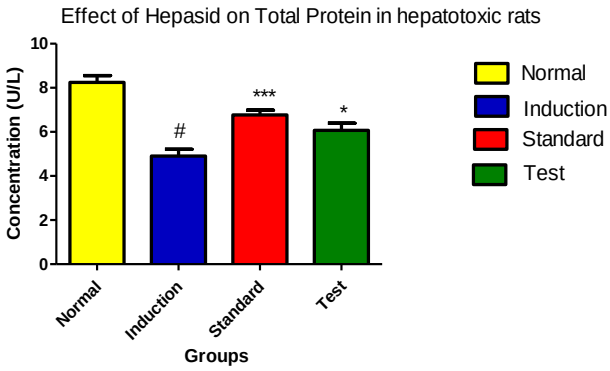

Figure 7: Effect of Hepasid on Total Protein

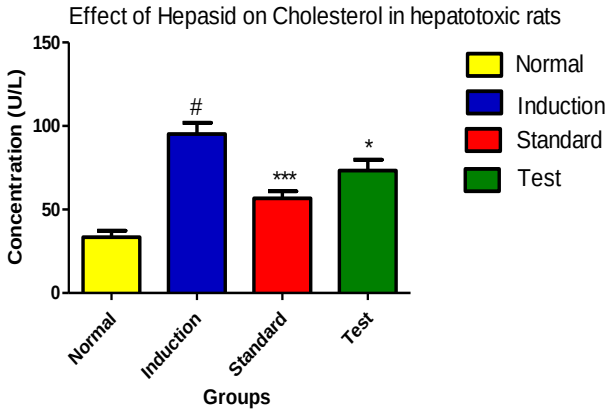

Figure 8: Effect of Hepasid on Total Cholesterol

Table 1: Effect of Hepasid on SGPT, SGOT, ALP, TB, TP and TC

Treatment/ Dose	SGPT [IU/L]	SGOT [IU/L]	ALP [IU/L]	Total Bilirubin [mg/dl]	Total Protein [g/dl]	Total Cholesterol [mg/dl]
Normal (Distilled water)	36.12 ±5.390	114.0 ±19.59	126.9 ± 17.87	0.257 ±0.058	8.248 ± 0.6893	33.38 ±8.669
Inducer (Ethanol)	81.37 ±11.19#	252.0 ±30.92#	269.2 ±18.56#	0.891 ±0.129#	4.900 ±0.6921#	95.16 ±15.16#
Silymarin (Standard)	50.06± 5.895***	148.7 ± 16.92***	174.6 ± 17.31***	0.588 ± 0.090***	6.772 ± 0.4790***	56.72 ±9.382***
Hepasid (Test)	62.12 ± 12.11*	206.0 ± 24.39*	237.5 ±22.78*	0.695 ± 0.127*	6.072 ±0.7305*	73.29 ±14.72*

Statistical Analysis

The mean value ± S.D. are calculated for each parameter. The reduction in biochemical parameters by the test sample against the hepatotoxins was analyzed by considering the differences in biochemical parameters between the control i.e. hepatotoxic and normal group as 100% level of reduction. For determining the significant intergroup differences each parameter was analyzed by one way ANOVA followed by Dunnett's test as shown in table 1.

DISCUSSION

The Liver damage or injured due to many factors such as chemicals and drugs also. In the present study ethanol was used to induce hepatotoxicity, since it is relevant. Ethanol in larger doses shows deleterious effect in the liver.[11] The majority of ethanol is metabolized in the liver and individual who abuse alcohol by routinely (about 4-5 drinks) per day. It shows the risk for developing the alcoholic liver disease.[12] In addition, both acute and chronic administration causes liver injury. The development of steatosis (fatty liver) another sign of excessive ethanol consumption is liver enlargement and protein accumulation, both are common in heavy drinkers.[13,14]

Elevated levels of serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT) are indication of hepatocellular injury, but in the present study the polyherbal formulation of hepasid (0.5 ml p.o.). Cause a significant inhibition of levels of SGPT and SGOT toward the normal range. Also suppress the level towards the normal range of ALP, Total bilirubin, Total protein and Total cholesterol.

The polyherbal formulation of Hepasid contains himsara, kasni, bhringraj, sharpunkha, kutki, punarnava, kakmachi, kasmarda, jhabuka, bhumylaki, guduchi, daruharidra, chitrak, awala, vidang, haritaki, parpata, kumari, parpata, were reported to passes hepatoprotective activity. Therefore, it has been suggested that the hepatoprotective activity shown by Hepasid can be because of these active constitute present in this syrup, which is being also confirmed by biochemical parameters.

CONCLUSION

In conclusion, administration of hepasid to hepatotoxic rats had shown hepatoprotective activity, which was evaluated and confirmed by experimental studies. Hepasid is significant effective against 40% ethanol induced hepatic damage in rats by reversal of increased serum level of SGOT, SGPT, ALP, Cholesterol, Bilirubin, and decreased level of Total Protein, which occurs during hepatotoxicity, has been reduced by the administration of Hepasid. Hence, we can conclude that the Hepasid is potential hepatoprotective against hepatotoxic effect of 40% ethanol. The elevated level of these marker enzyme in alcohol induce hepatic injury in rats in the present study corresponded to the extensive liver damage induce by toxin. Hepasid treated in ethanol induce rats showed significant ($P < 0.05$) decrease in level of SGOT, which is indication of hepatoprotective activity. Serum level of SGPT can increase due to damage of the tissue producting acute hepatic necrosis, such as viral hepatitis & cholestatis. Treatment with hepasid in alcohol induce hepatotoxicity in rats showed significant ($P < 0.05$) decrease in SGPT level.

Alkaline phosphatase is a membrane bound enzyme and its elevation in plasma indicates membrane disruption in the organ. Although ALP is not a liver specific enzyme, the liver is the main source of this enzyme. The level of this enzyme increases in the hepatic injury. ALP levels showed significant ($P < 0.05$) reduction in their high concentration induce by alcohol after treated with Hepasid in rats. Protein are synthesized in liver, if liver injured by any hepatotoxin its cell are unable to perform their work and thus serum or plasma protein concentration decrease in liver. Decrease in elevated level of the above enzymes would indicate reversal of the induced toxicity of the liver. Hepasid treated in ethanol induce hepatotoxic rats showed significant increase in enzyme level of Total protein ($P < 0.05$). The poly herbal formulation i.e. Hepasid also had shown significant decrease in Bilirubin ($P < 0.05$) and Serum Cholesterol ($P < 0.05$) level. Thus, hepatoprotective action of these Hepasid formulations is likely to be due to its ability to induce microsomal enzymes.

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