EVALUATION OF HEPATO PROTECTIVE ROLE OF *HIBISCUS CANNABINUS* SEEDS EXTRACT AGAINST ALCOHOL RENDERED HEPATOTOXICITY IN RATS

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Summary

The purpose of this study was to determine hepatoprotective and *in-vivo* antioxidant activities of ethanolic extract of the *Hibiscus cannabinus* seeds at different doses against alcohol induced hepatotoxicity in rats. The 70% ethanolic extract of *Hibiscus cannabinus* seeds (EEHCS) was subjected to acute toxicity study as per OECD guideline no. 420. The test extracts was screened for its influence on the GSH levels and lipid peroxidation. The various relevant biochemical markers like SGOT, SGPT, ALP, ACP, total and direct bilirubin were estimated to assess the hepatoprotective potential of the extract. It was further confirmed by histopathological observation. The 70% EEHCS at the dose of (100 mg/kg, 250 mg/kg, 500 mg/kg) produced a dose dependant significant reduction in biochemical levels as well as in morphological parameters in case of alcohol induced hepatotoxicity models. The histopathological study further supported the hepatoprotective activity of the test extract. Maximum protection was seen at 500 mg/kg. The results of the present investigation indicate that EEHCS has significant hepatoprotective and *in-vivo* antioxidant properties. This support the folklore use of the title plant in liver disorders.

Keywords: *Hibiscus cannabinus* seeds, Alcohol, Hepatoprotective, GSH level, Lipid peroxidation, marker enzymes.

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Introduction

Liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply energy provision and reproduction [1]. The liver is expected to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals.

Liver diseases are largest health problem worldwide. Liver disorders are mainly caused by toxic chemicals, excessive consumption of alcohol, infections and autoimmune disorders. Excessive production of reactive oxygen species (ROS) plays an important role in the pathogenesis and progression of various disease involving different organs such as liver [2]. Drug induced hepatic injury is the most common reason cited for the withdrawal of an approved drug from the market [3]. Alcohol is well known to potentiate the hepatotoxicity of various xenobiotics and the information about interaction between alcohol and the hepatotoxins is well documented [4]. Alcoholic liver disease is a common consequence of prolonged and heavy alcohol intake. This disease encompasses a wide spectrum of lesions, the most characteristic being alcoholic steatosis (fatty liver), alcoholic hepatitis, alcoholic fibrosis and cirrhosis [5].

Hibiscus cannabinus L. (Malvaceae) is a woody to herbaceous annual plant producing large cream coloured flowers characterised by a reddish purple or scarlet throat, popular in the western world as “Kenaf” and widely grown as a fibre crop. The plant is a hermaphrodite, mostly unbranched, fast-growing with prickly stems [6]. This plant was traditionally prescribed in traditional folk medicine in Africa and India; reported to contain several active components as tannins, saponins, polyphenolics, alkaloids, essential oils and steroids.

With no effective medication available in the modern medicine the focus is now on “Herbal Drugs” for treating many diseases particularly liver disease. The present study has been Structured to evaluate the hepatoprotective potential of the aqueous extract of *Hibiscus cannabinus* against alcohol induced hepatotoxicity in albino rats.

Materials and Methods

Collection of plant material:

For this study, *Hibiscus cannabinus* seeds were collected from the surrounding gardens of the Harapanahalli after the plant materials authenticated by Professor K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli.

Preparation of ethanolic extract:

Fresh mature seeds were shade dried at room temperature, coarse powdered and extracted with 70% hydro-alcohol by Soxhlet’s extraction method. Thereafter, the extracts were concentrated using rotary flash evaporator to obtain semisolid crude extracts. The percentage yield of seeds extracts was found to be 10.22%. The extracts were stored in airtight container in refrigerator below 10^0C.

Animal models:

Male albino Wistar rats (150 - 200 gm) and female albino Swiss mice (20 - 25 gm) were used in the experiments. Animals were housed in polypropylene cages and maintained under standard environmental conditions such as temperature (26 $\pm$ 2^{0}C), relative humidity (45 - 55%) and 12 hr. dark/light cycle. The animals were fed with rodent pellet diet (Golden Mohur Lipton India Ltd.) and water *ad libitum*.

Experimental design:

Albino rat of wistar strain weighing 150 – 250 gm were selected and divided into six groups of each containing six animals

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Group I – Negative control (received vehicle 1 ml/kg p.o.).

Group II – Rats given 30% alcohol 1 ml/100gm body weight/day for 14 days.

Group III – Normal rats given 30% alcohol 1ml/100g body weight/day+ Standard Drug silymarin at a dose, 100mg/kg body weight/day for 14 days.

Group IV – Normal rats given 30% alcohol 1ml/100g body weight/day +70% EEHCS 100 mg/kg body weight/day for 14 days.

Group V – Normal rats given 30% alcohol 1ml/100g body weight/day + 70% EEHCS 250 mg/kg body weight/day for 14 days.

Group VI – Normal rats given 30% alcohol 1ml/100g body weight/day + 70% EEHCS 500 mg/kg body weight/day for 14 days.

At the end of the experimental period all the animals were sacrificed by cervical decapitation and the following estimations were carried out to assess the liver functions. All the biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) [7], Alkaline phosphatase (ALP) [8], Total bilirubin (TB) [9], Total protein (TP) [10] and Tissue Glycogen [11] were carried out using standard methods. The anti oxidant status of the models were also assayed by studying the Lipid peroxide levels(LPO) [12], Superoxide Dismutase (SOD) [13], Reduced Glutathione (GSH) [14], Glutathione peroxidase (GPx) [15] and Glutathione S Transferase (GST) [16].

Histopathological study on alcohol induced hepatotoxicity.

Histopathological profile of liver from Alcohol (positive control group) intoxicated rats reveals hepatic globular architecture disrupted, hepatic cells have shown various degree of fatty degeneration like ballooning of hepatocytes, fatty cyst, and infiltration of lymphocytes and proliferation of kuffer cells. Protective effect of test extract was confirmed by histopathological examination of liver section. Administration of test extract at the dose of 500 mg/kg that is showed a significant improvement of the hepatic architecture and areas of Kuffer cell proliferation and sinusoid appeared normal on contrary with 100 mg/kg and 250 mg/kg.

Statistical Analysis

The data obtained was expressed as mean ± SEM. The data were subjected to one way ANOVA. The p value < 0.05 was considered statistically significant.

Results

The results of present investigation shows that, the treatment with 70% EEHCS produces dose dependant improvement in the liver architecture as indicated by the histopathological indications in which there was reduction in the kuffer cells proliferation, reduced areas of lymphocytic infiltration and normalization of sinusoids. The biochemical and histopathological observations reveal that the 70% EEHCS possess hepatoprotective activity in alcohol induced hepatotoxicity. Treatment with 500 mg/kg of 70% EEHCS produced the hepatoprotective activity comparable to that of silymarin 100 mg/kg b.w.

Effect of 70% ethanolic extracts of *Hibiscus cannabinus* seeds on biochemical and morphological parameters in alcohol induced hepatotoxicity

Table 1.

Groups	SGPT IU/l	SGOT IU/l	ALP IU/l	ACP IU/l	TB mg/dl	DB mg/dl	Volume/ 100gb.w.	Weight/ 100g b.w
-ve control	100.1 ± 1.59	120.3 ± 3.20	302.7 ± 3.43	31.80 ± 1.14	0.326 ± 0.03	0.279 ± 0.01	2.89 ± 0.20	2.96 ± 0.10
+ve control	244.2 ± 2.62	315.2 ± 3.52	523.9 ± 5.95	66.16 ± 3.65	0.731 ± 0.02	0.534 ± 0.20	4.27 ± 0.17	4.02 ± 0.11
Standard silymarin 100 mg/kg	145.6 ± 2.93***	282.2 ± 3.09***	343.5 ± 3.99***	39.65 ± 2.70***	0.479 ± 0.02***	0.368 ± 0.01***	3.21 ± 0.16**	3.32 ± 0.13***
EEHCS 100 mg/kg	232.5 ± 3.33*	310.4 ± 5.09	504.1 ± 4.16*	52.89 ± 2.78**	0.561 ± 0.02*	0.445 ± 0.025*	3.54 ± 0.17*	3.58 ± 0.12*
EEHCS 250 mg/kg	229.5 ± 2.35**	301.4 ± 2.79*	467.2 ± 2.56***	50.06 ± 2.66**	0.525 ± 0.08**	0.424 ± 0.027**	3.50 ± 0.14*	3.51 ± 0.13**
EEHCS 500 mg/kg	184.1 ± 3.27***	295.1 ± 2.64**	357.2 ± 4.42***	44.67 ± 2.82***	0.445 ± 0.03***	0.419 ± 0.026**	3.30 ± 0.17**	3.47 ± 0.11**

Values are mean ± SEM (n = 6).
* p < 0.05, ** p < 0.01, *** p < 0.001 as compared to positive control.

Effect of 70% ethanolic extract of *Hibiscus canaabinus* seeds on tissue GSH levels in alcohol induced hepatotoxicity

Table 2.

Treatment	Absorbance Mean ± SEM	% Increase
Negative Control (1ml vehicle)	0.095 ± 0.008	--
Positive Control 30% Alcohol (1 ml/100 g b.w. p.o.)	0.032 ± 0.007	--
30% alcohol + Standard (Silymarin) (1 ml/100 g b.w. p.o. + 100 mg/kg p.o.)	0.087 ± 0.009***	91.57%
30% alcohol + 70% EEHCS (1 ml/100 g b.w. p.o. + 100 mg/kg p.o.)	0.065 ± 0.008*	68.42%
30% alcohol + 70% EEHCS (1ml/100 g b.w. p.o. + 250 mg/kg p.o.)	0.077 ± 0.010**	81.05%
30% alcohol + 70% EEHCS (1 ml/100 g b.w. p.o. + 500 mg/kg p.o.)	0.081 ± 0.007***	85.26%

Values are the mean ± S.E.M. of six rats/ treatment.
Significance ***P<0.001, compared to alcohol treatment.

Effect of 70% ethanolic extract of *Hibiscus canaabinus* seeds on *in-vivo* lipid peroxidation in alcohol induced hepatotoxicity

Table 3.

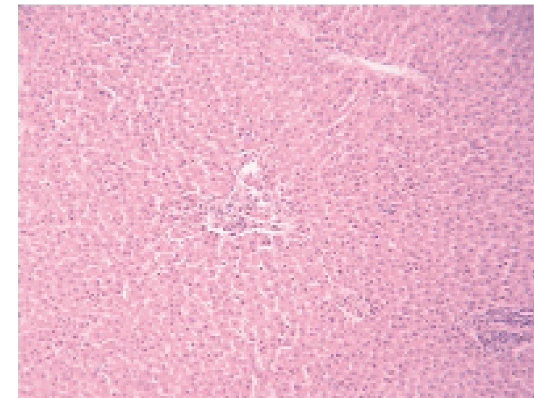
Treatment	Absorbance Mean ± SEM	% Inhibition
Negative Control (1ml vehicle)	0.265 ± 0.016	--
Positive Control 30% Alcohol (1 ml/100 g b.w. p.o.)	0.822 ± 0.018	--
30% alcohol + Standard (Silymarin) (1 ml/100 g b.w. p.o. + 100 mg/kg p.o.)	0.301 ± 0.031***	68.63%
30% alcohol + 70% EEHCS (1 ml/100 g b.w. p.o. + 100 mg/kg p.o.)	0.603 ± 0.041**	36.95%
30% alcohol + 70% EEHCS (1ml/100 g b.w. p.o. + 250 mg/kg p.o.)	0.579 ± 0.040**	44.40%
30% alcohol + 70% EEHCS (1 ml/100 g b.w. p.o. + 500 mg/kg p.o.)	0.421 ± 0.023***	62.42%

Values are the mean ± S.E.M. of six rats/ treatment.
Significance *** P<0.001, **P<0.01 compared to alcohol treatment**.**

Histopathological study on alcohol induced hepatotoxicity:

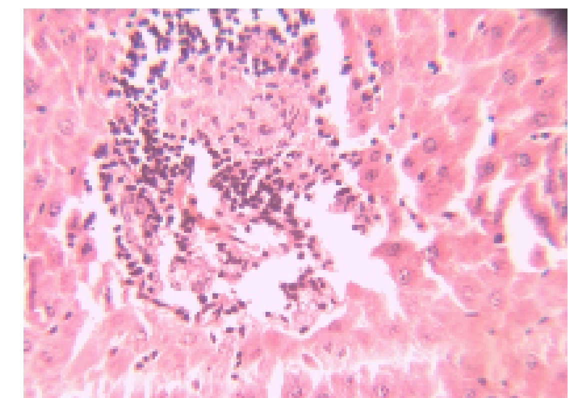
Histopathological profile of liver from Alcohol (positive control group) intoxicated rats reveals hepatic globular architecture disrupted, hepatic cells have shown various degree of fatty degeneration like ballooning of hepatocytes, fatty cyst, infiltration of lymphocytes and proliferation of kuffer cells. Congestion of liver sinusoids. Protective effect of test extract was confirmed by histopathological examination of liver section. Administration of test extract at the dose of 500 mg/kg that is showed a significant improvement of the hepatic architecture and areas of Kuffer cell proliferation and sinusoid appeared normal on contrary with 100 mg/kg and 250 mg/kg.

Fig. No. 1



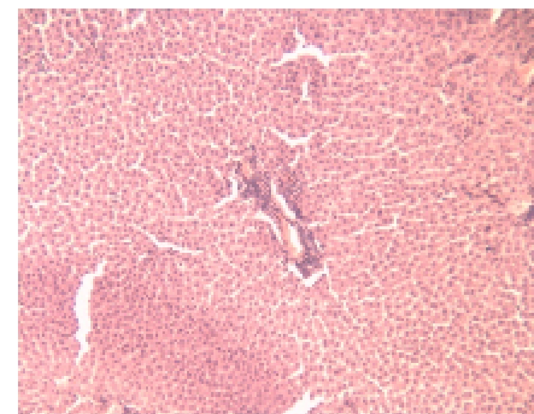
NEGATIVE CONTROL GROUP

Fig. No. 2



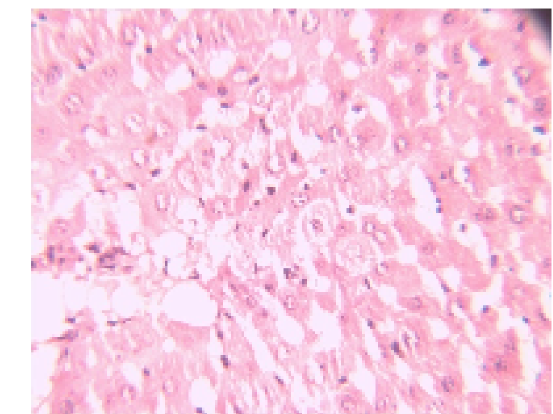
POSITIVE CONTROL GROUP

Fig. No. 3



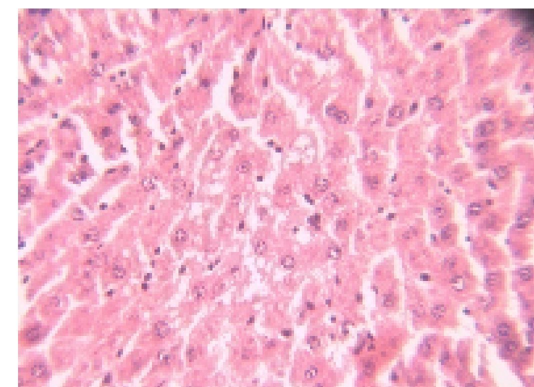
100 mg/kg SILYMARIN

Fig. No. 4



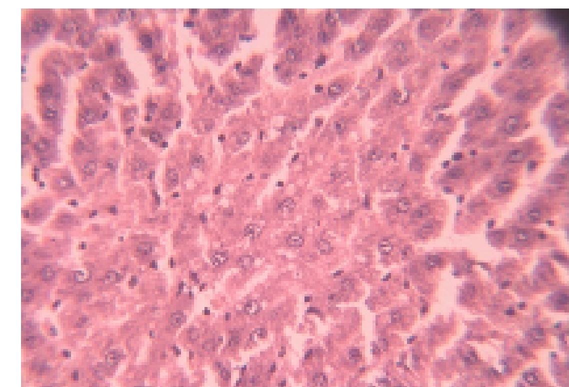
100 mg/ kg 70% EEHCS

Fig. No. 5



250 mg/kg 70% EEHCS

Fig. No. 6



500 mg/kg 70% EEHCS

Discussion

Spectroscopic determination of total phenolic and flavonoid contents and found to contain 65.47 mg/G and 57.0 mg/G of the respective constituents from 70% EEHCS. This 70% EEHCS contains higher concentration of total phenolic and flavonoid and hence selected for further study like *in-vivo* antioxidant and hepatoprotective activity.

GSH is considered as inbuilt antioxidant substance which prevents lipid peroxidation, estimation of tissue GSH and extent of lipid peroxidation were considered as parameters of screening in vivo antioxidant properties. Treatment with 70% of EEHCS (100 mg/kg, 250 mg/kg and 500 mg/kg b.w.) prevented depletion of GSH to the extent of 68.42%, 81.05% and 85.26% in case of alcohol induced GSH depletion, whereas the same extract showed 20.68%, 35.86% and 63.79% prevention of GSH depletion incase of paracetamol challenged route.

Treatment with 70% of EEHCS (100 mg/kg, 250 mg/kg and 500 mg/kg b.w.) reduced the lipid peroxidation to the extent of 62.42% at 500 mg/kg dose in alcohol elevated lipid peroxidation. Similar type of protection was offered against paracetamol induced lipid peroxidation. Therefore this extract was selected for screening hepatoprotective activity against alcohol and paracetamol induced hepatotoxicity in rats.

The hepatoprotective activity was assessed by measuring the biochemical markers like SGPT, SGOT, ALP, and ACP, bilirubin (total and direct) in the hepatotoxic models. Further the wet liver weight and liver volume; histopathological observations were made to assess hepatotoxicity/ hepatoprotectivity.

After administration of 30% alcohol for 14 days elevated the SGPT U/L (244.2), SGOT U/L (315.2), ALP, ACP, total bilirubin mg/dl (0.731), direct bilirubin mg/dl (0.534). Pretreatment with 70% ethanolic extract (100 mg/kg, 250 mg/kg and 500 mg/kg p.o.) for 14 days significantly reduced the elevated biochemical markers in a dose dependent manner. Treatment with 500 mg/kg of 70% EEHCS produced the hepatoprotective activity comparable to that of silymarin 100 mg/kg b.w. Similarly alcohol altered the liver architecture as indicated by the histopathological observations that hepatic cells shown extensive fatty change, more around central vein and microvasculisation fatty change, congestion of liver sinusoids. Treatment with 70% EEHCS showed dose dependant improvement in the liver architecture as indicated by the histopathological indications that there was reduction in the kuffer cells proliferation, reduced areas of lymphocytic infiltration and normalization of sinusoids. The biochemical and histopathological observations reveal that the 70% EEHCS possess hepatoprotective activity in alcohol induced hepatotoxicity. Toxicity of alcohol was found to be related to its metabolism by alcohol dehydrogenase (ADHs) and also to the metabolism by CYP2E1. The major route of ethanol oxidation in the liver is via alcohol dehydrogenase to acetaldehyde, which is associated with the reduction of NAD to NADH. NADH in turn increases xanthine oxidase activity, which elevates the production of superoxides. Metabolism of ethanol by ADH influences redox status of the liver in other ways. Thereby tissue GSH levels are depleted and lipid peroxidation is increase.

Conclusion

The result of this study demonstrated that ethanolic extract of *Hibiscus cannabinus* shows significant hepatoprotective activity against alcohol induced liver damage rats. Hence the present study justified the traditional use of *Hibiscus cannabinus* in the treatment of liver diseases.

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