Pharmacophore 2010, Vol. 1 (2), 82-89. ISSN 2229 – 5402

Pharmacophore

(An International Research Journal)

Available online at http://www.pharmacophorejournal.com/

Original Research Paper

HEPATOPROTECTIVE EFFECT OF *COCCULUS HIRSUTUS* LINN. AGAINST ETHANOL- INDUCED LIVER DAMAGE IN ALBINO WISTAR RATS

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ABSTRACT

The present study was conducted to evaluate the hepatoprotective activity of *Cocculus Hirsutus Linn* against ethanol-induced liver damage in albino wistar rats. Hepatotoxicity was induced by daily dose of 28.5% ethanol (3ml/kg/day p.o.) in three divided doses up to 30 days as manifested by statistically significant increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and bilirubin level. Pretreatment of rats with the extract at doses of 100, 200 and 400 mg/kg prior to 28.50% ethanol dosing at 3ml/100g/day p.o statistically lowered the serum liver enzyme activities. The activity of the extract was comparable to the standard drug, silymarin (50mg/kg, p.o.). Results obtained from histopathological studies also supported hepatoprotective activity against ethanol-induced hepatotoxicity. Thus the study demonstrates that methanolic extract of *Cocculus Hirsutus* possess antihepatotoxic effect against alcohol.

Keywords: *Cocculus Hirsutus*, Ethanol, Hepatoprotective activity and biochemical parameters.

INTRODUCTION

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. Therefore, the maintenance of a healthy liver is vital to overall health and well being.[1] Chronic alcohol consumption increases the capacity of cytochrome P450 2E1 (CYP2E1) to oxidize ethanol up to 10-fold which consequently increases the prooxidative burden. Reactive oxygen species (ROS) generated during ethanol oxidation via CYP2E1 contributes to ethanol induced liver injury. Although the pathogenesis of alcohol-induced liver disease remains the subject of debate, one factor that has been suggested as playing a central role in many pathways of alcohol-induced damage, and which has been the focus of much research is the excessive generation of these free radicals, which can result in a state called oxidative stress. Numerous studies have indicated that excessive ethanol intake induces the mass production of free radicals in the body, which are considered to be associated with alcoholic liver disease.[2]

Therefore, many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal model. Ethanol-induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts.[3, 4]

Cocculus hirsutus (L.) Diels belonging to family menispermaceae is a perennial climber mainly found in tropical and subtropical climatic condition. Traditionally the decoction of the leaves is used for treatment of gonorrhea, spermatorrhoea and diarrhea.[5] Leaves are also used in eczema, dysentery, leucorrhoea, and urinary problems. Leaves and stems are used for treating eye diseases. The literature survey revealed that there is no sufficient detail studies carried out regarding hepatoprotective activity of the *Cocculus hirsutus*. Hence, the present study is focused to evaluate the hepatoprotective of the leaves of *Cocculus hirsutus Linn* against ethanol-induced liver damage in the Wistar rat.

MATERIALS AND METHOD

Collection and Authentication of Plant: The plant was collected from local area of Shirpur, Dhule Maharashtra, India. The plant was authenticated by Dr.D.A. Patil, Department of Botany, S.S.V.P.S College of Science, Dhule, Maharashtra, India.

Extraction methodology: The plant was air dried, cut into small pieces and pulverized into powder. Five hundred gram of dried, powdered plant material was extracted with petroleum ether (60-800C) using soxhlet apparatus to remove lipids. It was then filtered and filtrate was discarded. The residue was then extracted successively with methanol using soxhlet apparatus and methanol was evaporated in a rotary evaporator at 40-50ºC under reduced pressure. The residual extract was suspended

in water for overnight and filtered. The filtrate was dried and stored. The yield of extract was 20% with reference to dry starting material.[6]

Experimental animals:

Three months old Wistar albino rats of either sex weighing 150- 250g were used for the study. The animals were procured from National Toxicology Center, Pune. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2 ^{0}C and relative humidity of 30-70%. A 12/12 h light and dark cycle was followed. All animals were fed on standard balanced diet and provided with water ad libitum**.**

All the experimental procedures and protocols used in the study were reviewed and approved by the (IAEC) Institutional Animal Ethical Committee of R.C.Patel IPER, Shirpur and were in accordance with the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals(CPCSEA). Registration No. RCPCOP/IAEC/2008-2009/30

***Toxicity Study*[7]:** Acute oral toxicity was conducted for extract on albino mice according to OECD 425 and median effective dose (ED50) of extract was selected based on LD50 obtained from acute toxicity studies.

Ethanol induced liver damage[8]: Albino wistar rats of either sex (150-250g) were used. All the animals were divided into the six groups each group consists of 6 animals and they received the treatment as follows.

Group I: Normal (1%CMC p.o.)

Group II: 28.50 % Ethanol (3ml/100g/day p.o.) for 30 days

Group III: Standard drug (Silymarin 50mg/kg p.o) + Ethanol for 30 days

Group IV: Methanolic extract of *C.Hirsutus* (100 mg/kg p.o.) + Ethanol for 30 days

Group V: Methanolic extract of *C.Hirsutus* (200 mg/kg p.o.) + Ethanol for 30 days

Group VI: Methanolic extract of *C.Hirsutus* (400mg/kg p.o.) + Ethanol for 30 days

All the test drugs were administered orally by suspending in 1% CMC solution. Alcohol (28.50 %) solution in distilled water was administered in a dose of 3ml/100g/day p.o.[8] for 30 days in three divided doses.

Twenty-four hours after last dose of alcohol, blood was obtained from all groups of rats by puncturing retro-orbital plexus. The blood samples were allowed to coagulate for 45 min at room temperature. Serum was separated by centrifugation at 3000 rpm at room temperature for 20 min and used for the biochemical estimation.

Biochemical estimation: The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST)[9], alkaline phosphatase (ALP)[10], lactate dehydrogenase (LDH), total bilirubin (TB) and direct bilirubin (DB) were measured according to the reported methods.

Histopathological examination: The livers of all animals were removed and determine

its liver weight and liver volume. A thin slice of livers preserved in 10% buffered formalin solution for histopathalogical investigations.

Statistical Analysis: All the data expressed as Mean ± S.E.M and analyzed statistically using ANOVA followed by Dunnett test and compare with respective control group. A value was of P<0.05 was considered significant.

RESULTS

Oral administration of Ethanol at a dose of 3 ml/100g/day in three divided doses caused a significant (P<0.01) increased in liver weight, liver volume but no significant change in body weight was observed (Table 1), and showed significant rise (P<0.01) in level of serum marker enzymes such as AST, ALT, ALP, LDH, TB, and DB. Silymarin significantly (P<0.01) reduced these levels near to normal. A significant (P<0.01) decrease was observed in the AST, ALT, ALP, LDH, TB and DB (Table 2) in the animals treated with different doses (100mg/kg, 200mg/kg and 400 mg/kg) of methanolic extract of *C. hirsutus* and showed dose dependant activity. At the dose of 400mg/kg extract showed comparable activity with the standard drug silymarin.

Histopathological studies, showed ethanol to produce extensive vascular degenerative changes and centrilobular necrosis in hepatocytes. Treatment with silymarin and methanolic extract of *C. hirsutus* produced mild degenerative changes and absence of centrilobular necrosis when compared with control (fig. 1). All these results indicate a hepatoprotective potential of *C. hirsutus* showed a dose dependent activity which was confirmed by Histopathological examination.

Table 1: Effect of methanolic extract of *C. hirsutus*, Silymarin and ethanol on liver weight and liver volume.

	Body weight (gm)	Liver weight (gm)	Liver volume (ml)
Normal	245	7	9
Ethanol (3ml/100g/day) Treated	250	15	12
STD drug (Silymarin) + Ethanol	225	9	10
Methanolic extract (100mg/kg) + Ethanol	235	13	11
Methanolic extract (200mg/kg) + Ethanol	243	12	9
Methanolic extract (400mg/kg) + Ethanol	215	9	8

Table 2: Effect of methanolic extract of *C. hirsutus*, Silymarin and ethanol on different biochemical parameters.

Group	AST (I.U./L)	ALT (I.U./L)	ALP (I.U./L)	LDH (I.U./L)	T.B. (%mg)	D.B. (%mg)
Normal	60.16 ± 1.1*	59.05 ± 0.4*	175.1 ± 0.4*	124.6 ± 0.8*	0.18 ± 0.005	0.19 ± 0.008**
Ethanol (3ml/100g/day) Treated	183.3 ± 1.9**	145.9 ±0.4	327.1 ± 1.5*	242.4 ± 1.2*	1.12 ± 0.9*	0.49 ± 0.01
Silymarin(50mg/kg) + Ethanol	94.17 ±2.3	69.01 ±0.5	215.7 ± 0.8*	157.7 ± 1.1*	0.42 ± 0.05*	0.22 ± 0.007
Methanolic extract (100mg/kg)+ Ethanol	159.2 ±0.2*	128.2 ±0.7	301.8 ± 0.2*	232.4 ± 1.3*	0.91 ± 0.01**	0.41 ± 0.03*
Methanolic extract (200mg/kg) + Ethanol	131.15 ±0.6	106.8 ±0.3**	268.2 ± 1.1*	211.4 ± 1.7*	0.79 ±0.06	0.32 ± 0.05*
Methanolic extract (400mg/kg)+ Ethanol	115.6 ±1	89.12 ±0.4	222.9 ±1.6*	176.3 ± 0.4*	0.51 ±0.01	0.28 ± 0.08*

Values are expressed as mean ± S.E.M. (n=6)

* P<0.05, when compared with the ethanol treated groups (one-way ANOVA followed by Dunnetts test)

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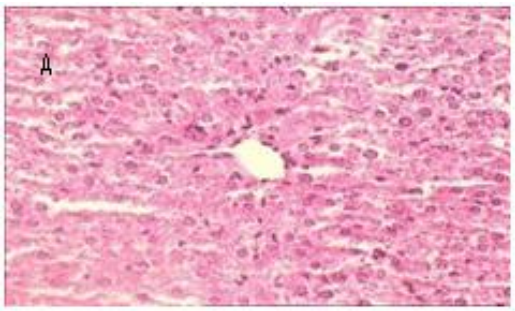
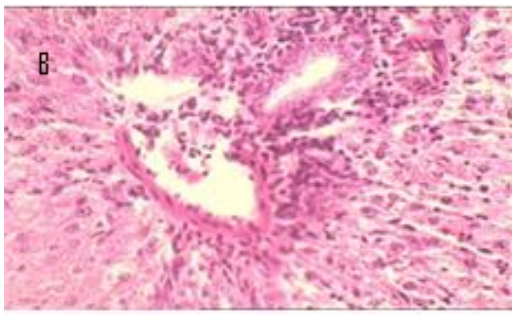
***Histopahological Changes*:**





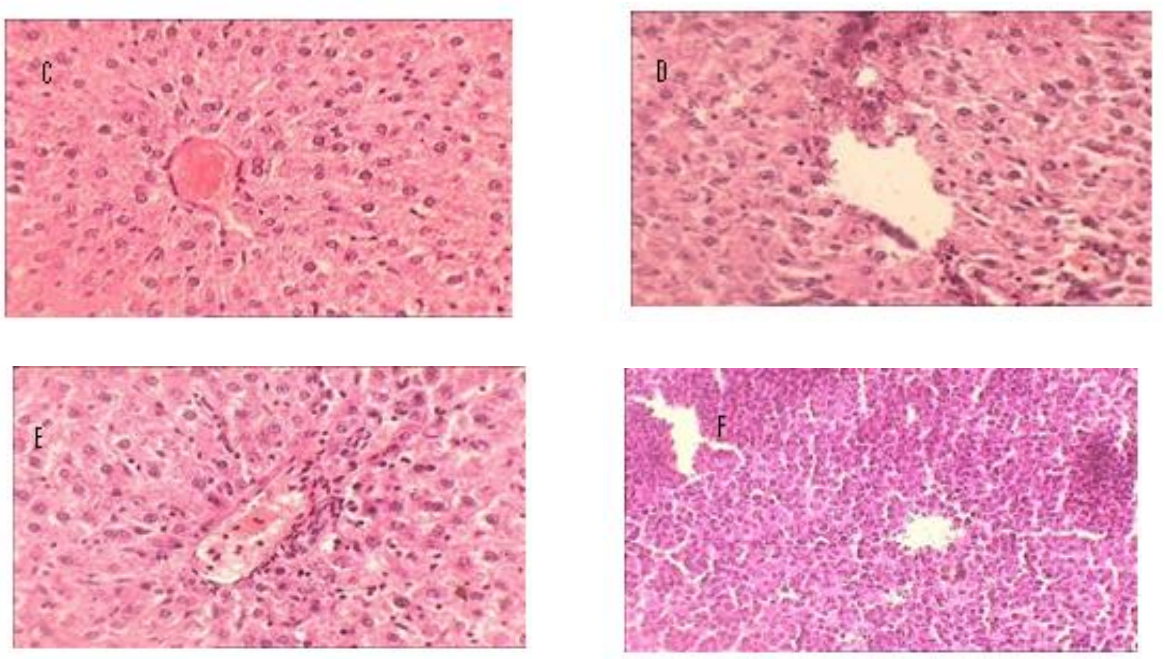


Figure 1: Effect of *C. hirsutus* on ethanol induced liver damage in rats (X 100)

A) Normal liver Section showing prominent central vein & normal hepatocytes & sinusoids of liver; B) Ethanol treated liver showing focal areas of liver cell necrosis and degeneration and fatty changes ; C) Silymarin treated liver showing normal hepatocytes and few areas of necrosis ; D) Methanolic extract (100mg/kg) treated liver showing focal hepatocytes necrosis & inflammation ; E) Methanolic extract (200mg/kg) treated liver showing few areas of liver cell necrosis & degeneration; F) Methanolic extract (400mg/kg) treated liver showing normal central vein and hepatocytes are seen few area of fatty change .

DISCUSSION

Chronic alcohol intake leads to many cellular and tissue abnormalities.[11] Chronic alcohol abuse proved successive hepatic changes, consisting of alcoholic Steatosis (Fatty liver), alcoholic hepatitis and cirrhosis.

The hepatocytes contain three main pathways for ethanol metabolisms to give acetaldehyde; each located in the different subcellular compartment namely alcohol dehydrogenase pathway of cytosol, the microsomal ethanol oxidizing system located in the endoplasmic reticulum, and catalase located in the peroxisomes. Chronic ethanol feeding results in the appearance of a form of Cytochrome P-450 also differing by its catalytic activity from other Cytochrome P-450 species, which leads to glutathione depletion and due to CYP-450 directly or through its active metabolites like acetaldehyde, increases the free radical concentration in body.[12] These free radicals trigger the oxidative damage and further stimulate lipid peroxidation. Ethanol

induced hypoxia has also been evoked as a possible cause of hepatotoxicity.[13]

Liver weight was found to be increased in the hepatotoxicant model as compare to normal, standard and methanolic extract of *C. hirsutus* treated groups. Hepatomegaly occurs due to accumulation of lipids and proteins in hepatocytes, with an impaired protein secretion by hepatocytes. Water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume. Steatosis and Cholestasis increases the liver weight.[14] Pretreatment with *C. hirsutus* decrease the liver weight of rats. *C. hirsutus* showed the inhibitory effect on Steatosis and Cholestasis.

In histopathological assessment, it was found that treatment with *C. hirsutus* showed as depression of hepatotoxicity. Thus, histological architecture of *C. hirsutus* to prevent hepatotoxicity.

The present study revealed a significant in activities of AST, ALT, ALP, LDH and serum bilirubin level on exposure to Ethanol, indicating considerable Hepatocellular injury. Administration of methanolic extract of *C. hirsutus* at 100mg/kg ,200mg/kg and 400mg/kg dose level attenuated the increased level of the serum enzymes, produced by Ethanol and caused a subsequent recovery towards normalization almost like that of Silymarin treatment.

CONCLUSION

In conclusion, methanolic extract of *C. hirsutus* has hepatoprotective activity against hepatotoxicant like ethanol and its activity is comparable with the standard drug silymarin. Drug has shown the ability to maintain the normal functional statues of the liver. From the above preliminary study, we conclude that *C. hirsutus* proved to be one of the herbal medication for liver ailment.

ACKNOWLEDGEMENT

I am thankful to. Prof. P. H. Patil HOD, Dept. of Pharmacology**,** Dr. C. R. Patil Dept. of pharmacology, R. C. Patel Institute of Pharmaceutical Education & Research, Shirpur**.** Mr. Pardeshi Histolopathologist, Jalgaon.

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