EVALUATION OF HEPATOPROTECTIVE EFFECT OF *COCCULUS HIRSUTUS (L)* DIELS ON ETHANOL INDUCED HEPATIC DAMAGE IN ALBINO WISTAR RATS.

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Summary

Herbal drugs are considered and are of particular value in the treatment of chronic diseases requiring prolonged therapy. *Cocculus hirsutus (*Menispermaceae) is a perennial herb growing widely in India. However, there are no reports on the hepatoprotective properties of this plant. Hence, the present study was planned with an objective to evaluate the methanolic extract of this plant for its hepatoprotective properties against ethanol induced hepatotoxicity in rats. Extract was subjected to preliminary phytochemical screening for the presence of different groups of compound. Concentrations of various biochemical markers like AST, ALT, ALP, LDH, Total and Direct Bilirubin and Cholesterol were estimated to determine the extent of hepatic damage. In addition histopathological observation was also made so as to assess the organ protective potential of the extract. The methanolic extract of *Cocculus hirsutus* has shown dose dependant hepatoprotective activity. An oral dose of 100 mg/kg, 200 mg/kg and 400 mg/kg produced significant (P<0.01) protective effect as evidenced by lowering serum levels of AST, ALT, ALP, LDH, Total and Direct Bilirubin and Cholesterol in treated group as compared to positive control. These biochemical observations were supplemented by histopathological examination of liver sections. Protection offered by silymarin (standard reference drug) seemed relatively greater. Based on these results, it was observed that methanolic extract of *Cocculus hirsutus* possess significant hepatoprotective activity against ethanol induced hepatotoxicity in rat and thus helps in evaluation of traditional claim on this plant.

Keywords:- Ethanol, hepatotoxicity, *Cocculus hirsutus,* silymarin.

Introduction

Ethanol is one of the major causes of chronic liver disease, including a variety of liver lesion such as steatosis, steatohepatitis, hepatic fibrosis, cirrhosis and hepatocellular carcinoma. Ethanol-induced liver injury has been extensively reported by studies in vivo. It has been confirmed that ethanol administration initially triggered the up-regulation of lipid biosynthesis genes and down-regulation of fatty acid oxidation gene like peroxisome proliferator-activated receptor (PPAR)-a, resulting in accumulation of lipid including free fatty acid and triglycerides in rat liver [1]. At the second stage, microsomal oxidizing system including cytochrome P450 2E1 (CYP 2E1) and alcohol dehydrogenase were induced and then oxidative stress was produced via ethanol metabolism and cytokine activation [2-3].

Ethanol produces a constellation of dose-related deleterious effects in the liver. It causes many types of injury to body system so there is an ever increasing need of an agent which could protect it from such damage. Recently there is a greater global interest in non synthetic, natural drugs derived from plants/herbal sources due to better tolerance and minimum adverse drug reaction [4]. Herbal drugs play major role in treatment of hepatic disorders. Herbs have attracted a great deal of interest as physiologically functional foods and as a source for the development of drugs. Herbal medicines derived from plant extracts are increasingly being utilized to treat a wide variety of clinical diseases, with relatively little knowledge on their modes of action [5]. There is an urgent need to develop potent hepatoprotective agents against ethanol induced hepatic disorders. We still do not have any specific agent for hepatoprotection. The presently used agents like folic acid, multi-vitamins and few polyherbal preparations provide only a supportive therapy but they do not play effective role in hepatic protection.

Cocculus hirsutus (L) belonging to family Menispermaceae is a perennial climber mainly found in tropical and subtropical climatic condition [6]. The modern literature revealed that the plant is reported to possess hypoglycemic activity [7], Diuretic, laxative activity [8], Hypolipidemic activity [9], Spermatogenic activity [10] etc. The literature survey revealed that there are no scientific studies carried out regarding hepatoprotective activity of the *Cocculus hirsutus*. Hence the present study is focused to evaluate the hepatoprotective activity methanolic extract of *Cocculus hirsutus* against Ethanol induced hepatotoxicity in Albino Wistar rats.

Materials and methods

Plant Material

The plant was collected from local area near Shirpur region, Dist-Dhule (Maharashtra) India, in the month of July. The plant was identified and authenticated from Department of Botany, S.S.V.P.S College of Science, Dhule, Maharashtra, India.

Preparation of Methanolic Extract

The plant was shade dried at room temperature, cut into small pieces and pulverized. The methanolic extract of plant of *Cocculus hirsutus* was prepared in methanol using soxhlet apparatus after de-fatting with Pet ether. The yield of extract was 20 % with reference to dry starting material [9].

Experimental animals

Albino Wistar rats (150- 250 g) and Albino mice (22-25 g) of either sex were used for the study. The animals were procured from animal house of R. C Patel IPER, Shirpur, Dist-Dhule, India. All 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, relative humidity of 30-70% and 12-hr light and dark cycle. They were fed on standard balanced diet and provided with water *ad libitum*.

All the experimental procedures and protocols were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of R.C. Patel IPER, Shirpur and were carried out with strict adherence to ethical guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Registration number RCPCOP/IAEC/2007-2008/30.

Phytochemical analysis

The extract was analyzed for the presence of phytochemicals by quantitative analysis [11].

Acute toxicity studies

The Acute toxicity was determined on albino mice as per OECD-423 guidelines [12]. The overnight fasted animals were administered extract orally at the dose level of 5 mg/kg body weight by gastric intubation and were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 hours. Based on these studies the doses were selected for the evaluation of antioxidant and hepatoprotective activity.

Experimental Setup

Albino wistar rats were divided into the six groups, each consisting of six animals and treated as follows.

Group I: Normal (Distilled water p.o.)
Group II: Toxicant (28.50 % Ethanol 3ml/100g/day p.o.)
Group III: Standard (Silymarin 50 mg/kg p.o.)
Group IV: Methanolic extract of *Cocculus hirsutus* 100 mg/kg p.o
Group V: Methanolic extract of *Cocculus hirsutus* 200 mg/kg p.o.
Group VI: Methanolic extract of *Cocculus hirsutus* 400 mg/kg p.o.

The vehicle (distilled water) and Methanolic extract of *Cocculus hirsutus* were administered orally for 30 days. Ethanol (28.50 %) solution in distilled water was administered in a dose of 3ml/100g/day p.o. for 30 days in three divided doses [13].

Twenty-four hours after last dose of Ethanol, blood was withdrawn 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 subjected to biochemical estimations.

Biochemical Estimation

The enzyme activities of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST), alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), as well as Total Bilirubin (TB), Direct Bilirubin (DB), and Cholesterol in blood serum were evaluated spectrophotometrically, using commercially available diagnostic kits [13].

Histopathological studies

After collection of blood, the livers of all animals were removed and washed several times with normal saline and fixed in 10 % v/v formalin solution. Sections (4-5 μm) were prepared and then stained with dye for microscopic observation.

Statistical analysis

The data were expressed as mean ± S.E.M. (n=6). Statistical analysis was performed by one way ANOVA followed by Dunnetts test. $P < 0.05$ was considered to be statistically significant.

Results

Phytochemical analysis

The qualitative phytochemical analysis of extract revealed presence of Alkaloids, Glycosides and Amino acids.

Acute toxicity studies

The LD_{50} of Methanolic extract of *Cocculus hirsutus* was determined. Since no mortality was observed at 2000 mg/kg. Therefore $1/20^{th}$ (100 mg/kg), $1/10^{th}$ (200 mg/kg) and $1/5^{th}$ (400 mg/kg) of LD_{50} were selected for further study.

Biochemical Estimation

The effect of various doses of methanolic extract of *Cocculus hirsutus* on serum biochemical enzymes in ethanol intoxicated rat was studied (Table no. 1and 2). Oral administration of Ethanol at a dose of 3 ml/100g/day caused increased different biochemical parameters like AST, ALT, ALP, LDH, TB, DB and cholesterol (group II), as compared to the normal control group (group I). Elevated levels of these biochemical parameters indicated the damage of hepatic cells. Pre-treatments of animal with 100, 200, 400 mg/kg p.o.of extract of *Cocculus hirsutus* (groups IV, V, VI) as well as treatment with Silymarin 50 mg/kg p.o. (group III) significantly reduced the activities of AST, ALT, ALP, LDH, TB, DB and cholesterol compared to the group of ethanol treated alone (group II) (Table no. 1and 2).

Table No. 1 Effect of *Cocculus hirsutus* extracts on enzyme activities in blood serum.

Treatment	AST	ALT	ALP	LDH
Group I	73±1.2	52±1.7	144±2.5	258±0.72
Group II	139±0.81	100±1.1	248±3.0	353±2.3
Group III	88±0.85**	71±0.56**	174±1.1**	275±0.80**
Group IV	127±0.63**	93±0.67**	232±0.71**	337±0.52**
Group V	120±0.60**	85±0.62**	218±1.0**	330±1.1**
Group VI	105±1.4**	76±0.60**	208±1.1**	309±0.85**

The values are Mean±S.E.M. (n=6), *P< 0.05, ** P< 0.01 compared with the Ethanol treated group (one-way ANOVA followed by Dunnetts test). AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase.

Table No. 2 Effect of *Cocculus hirsutus* extracts on Total Bilirubin (TB), Direct Bilirubin (DB), and Cholesterol in blood serum.

Treatment	TB	DB	CHOLESTEROL
Group I	0.17±0.076	0.24±0.14	89 ± 0.71
Group II	1.1±0.00087	0.35±0.011	143 ± 1.0
Group III	0.24±0.018**	0.23±0.055*	100±0.63**
Group IV	0.79±0.014**	0.30 ± 0.006 *	129 ± 0.63 *
Group V	0.67±0.011 **	0.27±0.0056 *	119±0.22 **
Group VI	0.48±0.011 **	0.23±0.0056 *	109±0.86 **

The values are Mean±S.E.M. (n=6), *P< 0.05, ** P< 0.01 compared with the Ethanol treated group (one-way ANOVA followed by Dunnetts test). TB: Total Bilirubin, DB: Direct Bilirubin

Histopathological studies

The histological observations supported the results obtained from serum enzymes assays. Liver sections from control rat showed prominent central vein and normal hepatocytes. The cells have well preserved cytoplasm, prominent nucleus and nucleolus (Fig. 1 A) which were completely lost in rat treated with Ethanol (Fig. 1 B). Vacuolization, fatty change and Cholestasis were severe in Ethanol treated group compared control groups. The liver of the rat treated with silymarin 50 mg/kg (Fig. 1 C) and Methanolic extract of *Cocculus hirsutus* at a dose of 100 mg/kg (Fig. 1 D), 200 mg/kg (Fig. 1 E) and 400 mg/kg (Fig. 1 F) showed significant recovery from Ethanol induced liver damage evidence by normal hepatocyte with nuclei. Dilated central vein, sinusoids, focal fatty changes and decongestion of cells were seen in treatment of extracts and silymarin. The section of rat liver treated with methanolic extract of *Cocculus hirsutus* at 400 mg/kg shows almost normal histology and regeneration of hepatocytes near to normal.

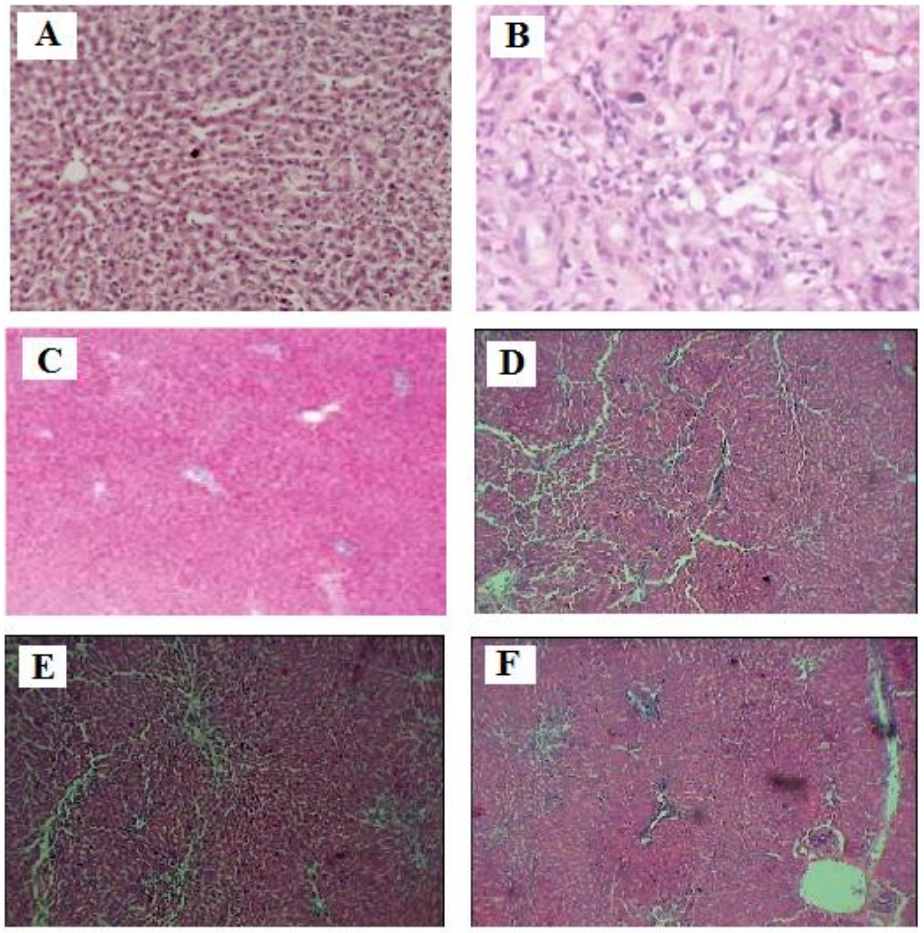

Fig.1. Effect of *Cocculus hirsutus* extracts on hepatic histopathological change in Ethanol induced hepatic injury in rats. Histological examination was performed under a light microscope (magnification 100X) with haematoxylin and eosin staining on liver tissues.
(A) Normal liver, (B) Ethanol (3ml/g/day) treated, (C) Silymarin (50 mg/kg) + Ethanol treated, (D) Methanolic extract of *Cocculus hirsutus* (100 mg/kg) + Ethanol treated, (E) Methanolic extract of *Cocculus hirsutus* (200 mg/kg) + Ethanol treated, (F) Methanolic extract of *Cocculus hirsutus* (400 mg/kg) + Ethanol treated.

Discussion and conclusion

Approximately 80% of ingested alcohol is metabolized in the liver, so excessive alcohol consumption can lead to acute and chronic liver disease [14]. Ethanol produces a constellation of dose-related deleterious effects in the liver. 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 [15]. In this during the metabolism of ethanol to acetaldehyde in the body, a state of oxidative stress is created by excessive ROS generation, which plays a vital role in the development of alcoholic liver disease [14]. The body has an effective defence mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as SOD, Catalase and GSH etc. These enzymes constitute a mutually supportive team of defence against reactive oxygen species. Hepatotoxicants produces imbalance between ROS production and these antioxidant defence which results into oxidative stress through a series of events deregulates the cellular functions leading to hepatic damage [16].
High levels of oxidative stress caused by ethanol-treatment in cells contribute to hepatotoxicity, as evidenced by a significant elevation in cellular ROS, suggesting a role of oxidative stress in ethanol-induced hepatic damage. Restoration of the intra-cellular ROS level by pre-treatment with methanolic extract indicates that its protective effect against ethanol- induced liver damage was mainly due to the suppression of an increase in intra-cellular ROS [17].

Ethanol-induced hepatic damage is characterized by hepatic marker enzymes such as ALT, AST, ALP, and LDH. The elevation of these enzymes in serum suggests hepatocytic damage [18]. In this study, there were significant decreases in serum enzymes by the co-administration of *Coccuus hirsutus* extract with ethanol, confirming that the methanolic extract of *Coccuus hirsutus* effectively protected the rat livers against severe damage caused by ethanol ingestion. The reduction in the levels of ALT, AST, ALP, and LDH towards the normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by ethanol.

Bilirubin increased in the blood because of regurgitation of bile due to obstruction within the liver by the damage or inflammation [19]. The present observation that the levels of bilirubin were increases than normal levels in the Ethanol treated rats, while significant decrease in bilirubin were indicates that the liver is restored to its normal activity by the hepatoprotective action of methanolic extract.
Intoxication with ethanol also resulted in inhibition of synthesis of the bile acids from cholesterol which is synthesized in liver or derived from plasma lipids, leading to increase in cholesterol levels. Suppression of cholesterol levels suggests the inhibition of the synthesis of bile acids from cholesterol is reversed by the methanolic extract.

The histological observations basically supported the results obtained from serum enzyme assays. The liver of ethanol intoxicated rats showed massive fatty changes gross necrosis, and fibrosis. The histopathological observations of the liver of rats pre-treated with *Cocculus hirsutus* and subsequently given ethanol showed a more or less normal architecture of the liver having reversed to a large extent, the hepatic lesions produced by the toxins, almost comparable to the normal control and silymarin groups.

In conclusion, the result of this study demonstrated that Methanolic extract of *Cocculus hirsutus* shows significant hepatoprotective activity against Ethanol induced liver damage rats. It may also be hypothesized that B-sitosterol, trilobine, isotrilobine, (+)-syringaresionol and protoquercitol, ginnol, glycosides, alkaloids which are present in the methanolic extract, could be considered responsible for the hepatoprotective activity. Further research is necessary to determine the possible phytoconstituents responsible for hepatoprotective activity and their pharmacological mechanism of action.

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