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Evaluation Of Hepatoprotective Potential Of *Cocculus hirsutus (L) Diels* On CCl_4 Induced Liver Damage In Albino Wistar Rats

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ABSTRACT

In the present study CCl_4 was used as an hepatotoxicant. Albino Wistar rats of either sex were divided into seven groups. Hepatotoxicity was induced by daily dose of CCl_4 (1ml/kg s.c.on 2nd & 3rd day). Rats were treated for 5 days with methanolic extract along with CCl_4. Blood samples were collected on 6th day of the study. Biochemical parameters were estimated and rats were sacrificed by cervical dislocation and livers from each group were isolated and sent for histopathological studies. The present study showed that after CCl_4 intoxication in animals water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume which was prevented by pretreatment with *Cocculus hirsutus* methanolic extract indicating hepatoprotection. During the hepatic damage cellular enzymes like AST, ALT, LDH, TB, DB present in the liver cells leak into the serum, resulting in increased concentration of these enzymes in body after treatment with *Cocculus hirsutus* it shows a dose dependent hepatoprotection which was further confirmed by studying histopathological results.

Introduction

The liver is the key organ of the metabolic, secretery and excretory functions in the body. For the treatment liver disorders conventional medicine does not provide challenging effect In the recent times folk remedies of both plant and animal origin have long been used for hepato-biliary diseases and recent studies have proved them to be beneficial[I].Thearapy developed for liver diseases along the principles of modern medicine are often limited in efficacy, carry the risk of adverse effects, and are often to costly for the developing world. Therefore treating liver diseases with plant-derived compounds which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive. Furthermore, conventional medicine in the last decades, professionals and the lay public of developed countries pay increasing attention to phytomedicine. Supporters of herbal medicine claim that herbs may both treat and prevent diseases. This adds to a deep belief that these treatments are safe because they are "natural" and fit into the image of a gentle and therefore, harmless alternative to conventional medicine And also there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines which are claimed to possess hepatoprotective activity [II].

Cocculus hirsutus (L) belonging to family Menispermaceae is a perennial climber mainly found in tropical and subtropical climatic condition [III]

The root destroys kapha and vata lessens bile, and burning sensation useful in urethral discharges [IV] Also the *Cocculus hirsutus* is having the hypoglycemic activity [V] Diuretic, laxative activity [VI], Hypolipidemic activity [VII], Spermatogenic activity [VIII] have been reported.

There is an urgent need to develop potent hepatoprotective agents against CCl_4 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.

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 and antioxidant potentials of the Plant against CCl_4 induced hepatotoxicity in Albino Wistar rats.

Materials and methods

Collection and Authentication of plant

The plant was collected from local area near Shirpur region, Dist-Dhule Maharashtra, India. In the month of July. 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 Pet ether (60-80) 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 40-50 ºC 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. [VII]

Phytochemical investigation

It was performed by the method of C.K kokate [IX]

Experimental animals

Three months old Wistar albino rats of either sex weighing 150- 250g were used for the study. The animals were procured from animal house of R. C Patel IPER, Shirpur, Dist- Dhule. 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 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/2007-2008/30.

Toxicity study [X] (OECD 425)

Acute oral toxicity (AOT) was conducted for extract on albino mice. Animal shows toxic effect or mortality in an observation period of 14 days at the dose 2000 mg/kg.

Effective dose (ED_{50}) of extract was selected based on LD_{50} obtained from acute toxicity studies.

Carbon tetrachloride (CCl_4) induced liver damage: [XI]

Albino wistar rats (150-250g) were used. All the animals were divided into the seven groups each group consisting of 6 rats and they received the treatment as follows.

Group I: Normal (Distilled water.p.o.)

Group II: CCl_4 Treated (CCl_4 on 2nd & 3rd day 1ml/kg s.c.)

Group III: Standard drug (Silymarin 50mg/kg p.o).

Group IV: Vehicle treated (1%CMC p.o)

Group V: Methanolic extract of *Cocculus hirsutus* 100 mg/kg p.o

Group VI: Methanolic extract of *Cocculus hirsutus* 200 mg/kg p.o.

Group VII: Methanolic extract of *Cocculus hirsutus* 400mg/kg p.o

The Methanolic extract of Cocculus hirsutus and vehicles (1% CMC in distilled water) were administered orally for 5 days. Hepatotoxicity was induced in IInd, IIIrd, Vth, VIth, VIIth group by an injection of CCl_4 (1 ml/kg, 1:1 with Olive oil s.c.) on 2nd & 3rd day (Shanmugasundarm et.al, 2006). On the 6th day blood sample from all Groups of rats were obtained 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 viz. ALT, AST, ALP, LDH, TB, and DB.

Histopathological Examination

The livers of all animals were dissected out and fixed in 10 % formalin and further processed for histopathological investigations.

Statistical analysis

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

Results

The extract revealed presence of various phytochemical constituents Alkaloids, Glycosides and Amino acids. [IX]

The LD_{50} of *Cocculus hirsutus* Methanolic extract was found to be 2000 mg/kg. The effective dose 200 mg/kg was selected based on LD_{50} of plant.

Water is retained in the cytoplasm of hepatocytes leading to enlargement of liver cells, resulting in increased total liver mass and volume which were expressed in [XII] (Table 1).

The activities of various biochemical enzymes in normal, CCl_4 treated, Methanolic extract treated were expressed in (Table 2). In Toxicant control group the enzymes levels are increased above normal and in extract treated showed to be decreased significantly.

Histopathological results showed fatty changes, cholestasis were seen in CCl_4 treated group while methanolic extract 100 mg shows sinusoidal congestion and at higher dose the normal histology and restoration of cells are near to normal are seen shown in (figure 1–6).

Discussion and Conclusion

CCl_4 is metabolized in the liver to the highly reactive trichloromethyl radical. This free radical leads to autooxidation of fatty acids present in the cytoplasmic membrane phospholipids and causes functional and morphological changes in the cell membrane. Furthermore, influx of extracellular Ca into the cell is claimed to be an important step leading to cell death. Free radicals like ($CCl_3^{\bullet}$) and ($Cl_3COO^{\bullet}$).initiate and promote the propagations of lipid peroxidation and leads to cell death. Inhibition of free radicals is important in the protection against CCl_4 induced liver lesion [XIII].

Administration of CCl_4 significantly elevate levels of AST, ALT, ALP, LDH and bilirubin, due to damaged structural integrity of the liver because these are cytoplasm in location and are released into circulation after cellular damage. Treatment with *Coccululs hirsutus* methanolic extracts showed a dose-dependent protection against the injurious effects of CCl_4.

The present study shown that *Cocculus hirsutus* has shown Hepatoprotection which may be due to constituent like B-sitosterol, trilobine, isotrilobine, (+)-syringaresionol and protoquercitol, ginnol, glycosides contributing towards its hepatoprotective activity[VII, VIII]. It is proved that Methanolic extracts of *Cocculus hirsutus* shows

hepatoprotective activity against hepatotoxicant like Carbon tetrachloride and its activity is comparable with the standard drug silymarin. Hepatoprotective activity could be due to various phytochemicals present in extract of test drug.

Acknowledgement

Authors are thankful to Principal Dr. S. J Surana R. C. Patel IPER for providing the necessary facilities for carrying out this research work.

Table 1 Physical Parameters

Groups	Liver weight (gm)	Liver volume (ml)
Normal	8	6
CCl_4 Treated	14	11.5
STD drug (Silymarin) + CCl_4	9	8
Methanolic extract of *Cocculus hirsutus* 100 mg/kg + CCl_4	12	11
Methanolic extract of *Cocculus hirsutus* 200 mg/kg + CCl_4	10.5	8
Methanolic extract of *Cocculus hirsutus* 400 mg/kg + CCl_4	8	7

Table 2 Biochemical Parameters

	AST	ALT	ALP	LDH	TB	DB
Normal	73 ± 1.2	52 ± 1.7	144 ± 2.5	258 ± 0.72	0.17 ± 0.076	0.24 ± 0.14
CCl_4	139 ± 0.81	100 ± 1.1	248 ± 3.0	353 ± 2.3	1.1 ± 0.00087	0.35 ± 0.011
1 % CMC	74 ± 1.4	53 ± 1.2	148 ± 0.5	251 ± 2.1	0.14 ± 0.054	0.25 ± 0.17
Standard (Silymarin)	88 ± 0.85 **	71 ± 0.56 **	174 ± 1.1 **	275 ± 0.80 **	0.24 ± 0.018 **	0.23 ± 0.055 *
Methanolic extract of *Cocculus hirsutus* 100 mg/kg	127 ± 0.63 **	93 ± 0.67 **	232 ± 0.71 **	337 ± 0.52 **	0.079 ± 0.014 **	0.30 ± 0.006 *
Methanolic extract of *Cocculus hirsutus* 200 mg/kg	120 ± 0.60 **	85 ± 0.62 **	218 ± 1.0 **	330 ± 1.1 **	0.67 ± 0.011 **	0.27 ± 0.0056 *
Methanolic extract of *Cocculus hirsutus* 400 mg/kg	105 ±1.4 **	76 ± 0.60 **	208 ± 1.1 **	309 ± 0.85 **	0.48 ± 0.011 **	0.23 ± 0.0056 *

Values are expressed as mean ± S.E.M. (n=6)
P< 0.05, ** P< 0.01 when compared with the toxicant control groups (One-way ANOVA) followed by Dunnetts test

Histopathological Changes in CCl_4 Model

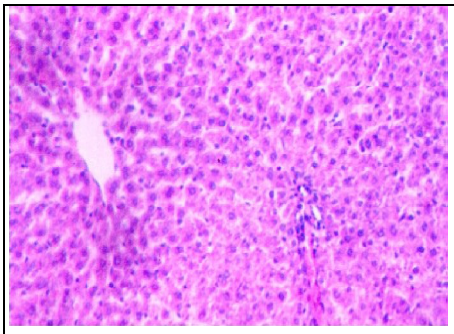

Figure 1 Normal Liver
Sections show Prominent central vein & normal hepatocytes & sinusoids of liver.(H and E X 100)

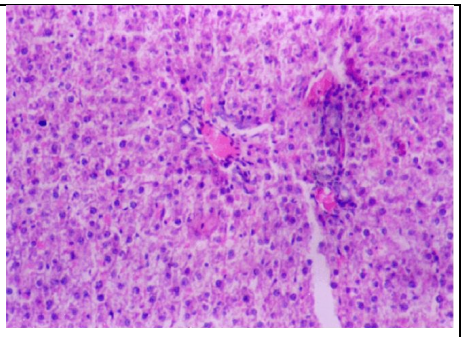

Figure 2 CCl_4 treated Liver
Focal areas of liver cell necrosis and degeneration and fatty changes.
(H and E X 100)

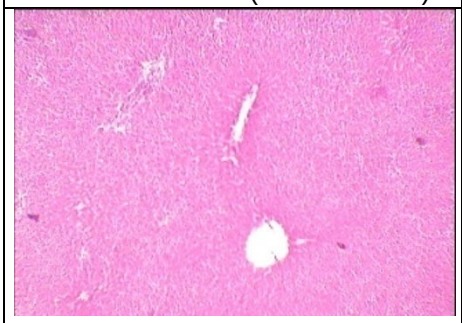

Figure 3 Standard liver
Normal Hepatocytes and shows few areas of necrosis
(H and E X 100)

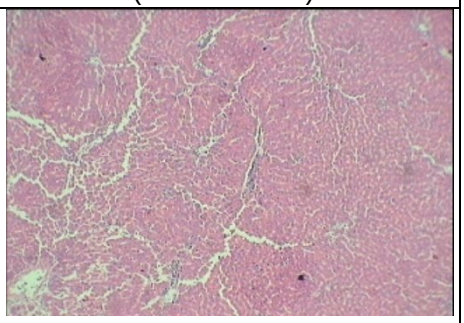

Figure 4 M.E 100 mg/kg
Area show Focal hepatocytes necrosis & inflammation
(H and E X 100)

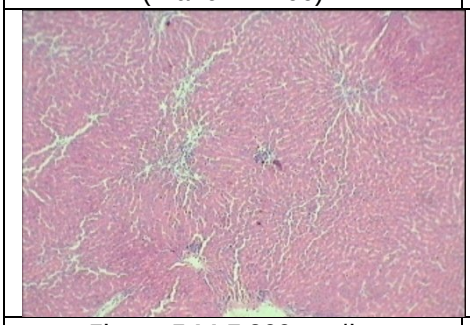

Figure 5 M.E 200 mg/kg
Few areas focal hepatocyte necrosis & degeneration
(H and E X 200)

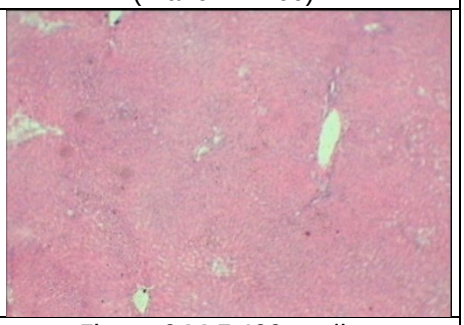

Figure 6 M.E 400 mg/kg
Normal central vein and hepatocytes are seen few area of fatty change.
(H and E X 200)

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