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Hepatoprotective Effect of *Cleome gynandra* on Carbon Tetrachloride Induced Hepatotoxicity in Wistar Rats

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ABSTRACT

Cleome gynandra has been used widely in Indian indigenous system. In the present study the hepatoprotective effect of methonolic extract of *C. gynandra* (MECG) was investigated against carbon tetrachloride (CCl_4) induced hepatocellular injury in wistar rats. The various parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase (ALP) and total bilirubin (TB) were studied in the present study. The hepatoprotective activity was also supported by histopathological studies of liver tissue. Administration with MECG (200 and 400 mg/kg, P.O.) for 14 days significantly reduced the impact of CCl_4 toxicity on the serum markers of liver damage, serum glutamic oxaloacetic transaminase, serum glutamic-pyruvic transaminase, alkaline phosphatase and total bilirubin. The histopathological studies in the liver of rats also supported that *cleome gynandra* extract markedly reduced the toxicity of CCl_4 and preserved the histoarchitecture of the liver tissue to near normal. Thus, the results suggest that *cleome gynandra* extract acts as a potent hepatoprotective agent against CCl_4 induced hepatotoxicity in rats.

Keywords: Hepatoprotective activity, Carbon tetrachloride, *Cleome gynandra*, Methonolic extract.

INTRODUCTION

Liver plays an important role in regulation of physiological processes, involved in several vital functions such as metabolism, secretion and storage. Liver also detoxifies variety of drugs and xenobiotics and secretes bile that has an important role in digestion[1]. Hepatotoxicity may be defined as the effect of any agent results in a deviation from normal function, morphology and implies chemical/microbial -driven liver damage[2]. Xenobiotics or toxic chemicals directly or indirectly damage the liver's important cellular functions. So it leads to faster accumulation of toxins in the liver which leads to "Liver damage" called as "Hepatotoxicity". Liver damage occurs by either due to direct damage to the liver cells or due to a secondary damage resulting from obstruction of the bile flow. If the natural protective mechanisms of the liver are overpowered during all such exposures, this will lead to hepatic injury. Liver diseases are a problem worldwide, and the conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects[3]. Thus, interest and effort have shifted toward medicinal plants as new sources of hepatoprotective agents[3].

A number of plant-based traditional medicines or formulations containing herbal extracts are sold in the market for liver disorders, especially in countries like India, China, and Malaysia where the management of liver disorders by herbal-based drugs or formulations is still considered to be an intriguing problem[4].

Cleome gynandra (Capparidaceae) is used as a medicinal plant and can be found in all over world .It grows as a weed in paddy fields and also in road sides and in open grass lands. In India it is never cultivated but grows spontaneously everywhere[5]. *C. gynandra* has been reported anti-inflammatory, anti-arthritic activity[6], anti-tumor activity[7], and Antioxidant-activity[8]. It contains Carotenoids, Cardiac glycosides, Cyanogenic Glycosides, Flavonoids, Saponins, Triterpenes, sugars, Tannins[9]. It is claimed to be good for liver toxicity[5].

However, there are no reports focusing on hepato- protective effects of *C. gynandra*. Taken together, the objective of the present study is to evaluate the protective effect of methanolic extract of *C. gynandra* (MECG) against CCl_4 induced liver toxicity.

MATERIALS AND METHODS

Drugs and chemicals

Carbon tetrachloride was procured from S.D. Fine Chemicals Ltd. (India), silymarin was obtained as gift sample from Ranbaxy (Devas, India), standard kit of SGPT, SGOT, ALP and total bilirubin was obtained from ERBA Industries, India. All other reagents used were of analytical grade.

Plant material

Processed leaves of *C. gynandra* were obtained from local area of Kadapa, India. The botanical identity was confirmed at the Department of Botany, SV University, Tirupathi, India by a plant taxonomist. Voucher specimen (no. SV-30475) of the plant is kept in our lab, for reference purposes.

Preparation of the extract

Leaves were shade dried for two weeks and powdered in a Willy Mill to 60-mesh size. Briefly, 1 kg powder was extracted with 6 litres of methanol (95%) at 25°C for 48 h. After extraction the mixture was filtered and the methanol solution was evaporated in a rotary evaporator at 40°C and stored at 4°C for further in vivo investigations.

Experimental animals

Eight-week old Albino rats of either sex, weighing between 180 - 225 g obtained from the Raghavendra enterprises, Bangalore were used for this study. They were fed commercial growers mash and water *ad libitum,* and placed in a controlled environment in the animal house of the PRRM College of pharmacy, Kadapa, India for 2 weeks before the commencement of the experiment (1423/PO/a/11/CPCSEA).

Acute oral toxicity

The acute oral toxicity study was carried out as per the guide lines set by OECD, revised draft Guidelines-423, received from CPCSEA, Ministry of Social Justice and empowerment, Govt. of India.

Experimental design

Five groups of animals containing six each were used for the study

Group 1- Normal*:* The animals received only distilled water for 14 days.

Group 2- Induction of hepatotoxicity by using CCL_4: The animals received 0.1 $ml \cdot kg^{-1}$ /day, I.P. of CCl_4 for 10 days.

Groups 3- Silymarin was administered to animals in the dose of 100 $mg \cdot kg^{-1}$ /day, P.O. for 14 days + 0.1 $ml \cdot kg^{-1}$ /day, I.P. of CCl_4 for 10 days.

Groups 4- The animals received MECG 200 $mg \cdot kg^{-1}$ b.wt/day for 14 days (P.O.). + 0.1 $ml \cdot kg^{-1}$ /day, I.P. of CCl_4 for 10 days.

Groups 5- The animals received MECG 400 $mg \cdot kg^{-1}$ b.wt/day for 14 days (P.O.). +0.1 $ml \cdot kg^{-1}$ /day, I.P. of CCl_4 for 10 days.

The CCl_4, Silymarin and the extracts were administered concomitantly to the respective group of animals. On 14th day the blood samples were withdrawn from the retro orbital venous plexus of rats without any coagulant for the separation of serum. After collecting the blood in eppendorf tubes kept aside for 1 hr at room temperature and then serum was isolated by centrifugation at 2000 rpm for 15 min and stored until analyzed for various biochemical parameters.

Assessment of serum marker enzymes

Serum analysis of various liver marker enzymes such SGOT, SGPT, ALP and TB were estimated by using standard diagnostic kits.

Histopathological studies

One representative animal from the each group was utilized for this purpose. The liver specimens obtained from the control and treated groups of animals were fixed in 10% buffered formalin for 24 h. The formalin fixed liver samples were stained with haematoxylin-eosin for photo microscopic observations of the liver histopathological architecture.

Statistical analysis

In the present study, all the data was expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Tukey test using computer based fitting program (Prism graph pad 5.0). Statistical significance was set accordingly.

RESULTS

Acute toxicity

Methonolic extract of *Cleome gynandra* was found to be safe since no animal died at the maximum single dose of 4000 $mg \cdot kg^{-1}$ when administered orally and the animals did not show any gross behavioral changes. Hence $1/10^{th}$ of this dose i.e. 400 $mg \cdot kg^{-1}$ were used as high dose and 200 $mg \cdot kg^{-1}$ were used as low dose in the subsequent study respectively.

Effect of MECG on serum marker enzymes

Rats treated with carbon tetrachloride showed drastic and significant hepatic damage as observed from elevated

serum level of hepatic enzymes (Table 1). SGPT, SGOT, ALP, and total bilirubin in serum were increased in carbon tetrachloride intoxicated control animals (Table 1). Treatment with the methanolic extract of *C. gynandra* caused significant protection against CCl_4 induced increase in serum enzyme level in a dose dependant manner and were comparable to the silymarin (Table 1). The degree of protection observed was maximum with higher dose of the extract (400 $mg \cdot kg^{-1}$).

Effect of MECG on histopathology of liver

The Hepatoprotective effect of methonolic extract of *C. gynandra* was confirmed by histopathological examination of the liver tissue of control and treated animals. The CCL_4 treated liver sections showed severe central vein congestion, sinus congestion, entrilobular degeneration and centrilobular necrosis along with moderate portal triaditis and inflammation. Fatty changes and ballooning of hepatocytes were also showed (Fig. 1- B). The liver sections of the rats treated with MECG and silymarin followed by CCl_4 intoxication showed a sign of protection as it was evident the absence of necrosis and vacuoles (Fig. 1- C, D, E).

DISCUSSION

Carbon tetrachloride is a routinely used hepatotoxin for experimental study of liver diseases[10]. In the present investigation administration of CCl_4 to rats causes acute liver damage that mimics natural causes. It mediates changes in liver function that ultimately leads to destruction of hepatocellular membrane. CYP 450 activates CCl_4 to form various free radicals (trichloromethyl, Cl_3C-CCl_3 (hexachloro-ethane), $COCl_2$ (phosgene), etc.) which are involved in the pathogenesis of liver damage in chain reactions resulting in peroxidation of lipids, covalent binding of macromolecules, disruption of metabolic mechanisms in mitochondria, decreasing levels of phospholipids, increasing triglyceride levels, inhibition of calcium pumps of microsomes thus leading to liver necrosis[11]. Therefore, inhibition of the cytochrome P450 dependent oxygenase activity could cause a reduction in the level of toxic reactive metabolites and a decrease in tissue injury. On the other hand, an elevation of plasma SGOT, SGPT, ALP and bilirubin activities could be regarded as a sign of damage to the liver cell membrane. Many compounds known to beneficial against carbon tetrachloride mediated liver injury exert their protective action by toxin mediated lipid peroxidation either via a decreased production of CCl_4 derived free radicals or through antioxidant activity of the protective agent themselves[12-14].

So in the present study, CCl_4 was employed as toxic agent and the protective effect of *Cleome gynandra* against the CCl_4 induced hepatotoxicity was studied. The extent of toxicity was estimated by biochemical enzyme markers like SGOT, SGPT, ALP and bilirubin levels and histopathological studies. All these parameters were found to be elevated in control group when compared normal group. Treatment with MECG showed significant decrease of these parameters, and values were found parallel with normal group. Interestingly, effect of high dose was found more significant than low dose. Report shows flavonoids and steroids are may be responsible for Hepatoprotective effect[15]. Perhaps flavonoids present in the *Cleome gynandra* may be responsible for the marked Hepatoprotective effects, observed in the present study.

CONCLUSION

It can be concluded that *C. gynandra* possess a protective effect against CCl_4 induced hepatotoxicity in rats as evidenced by the biochemical, histological parameters.

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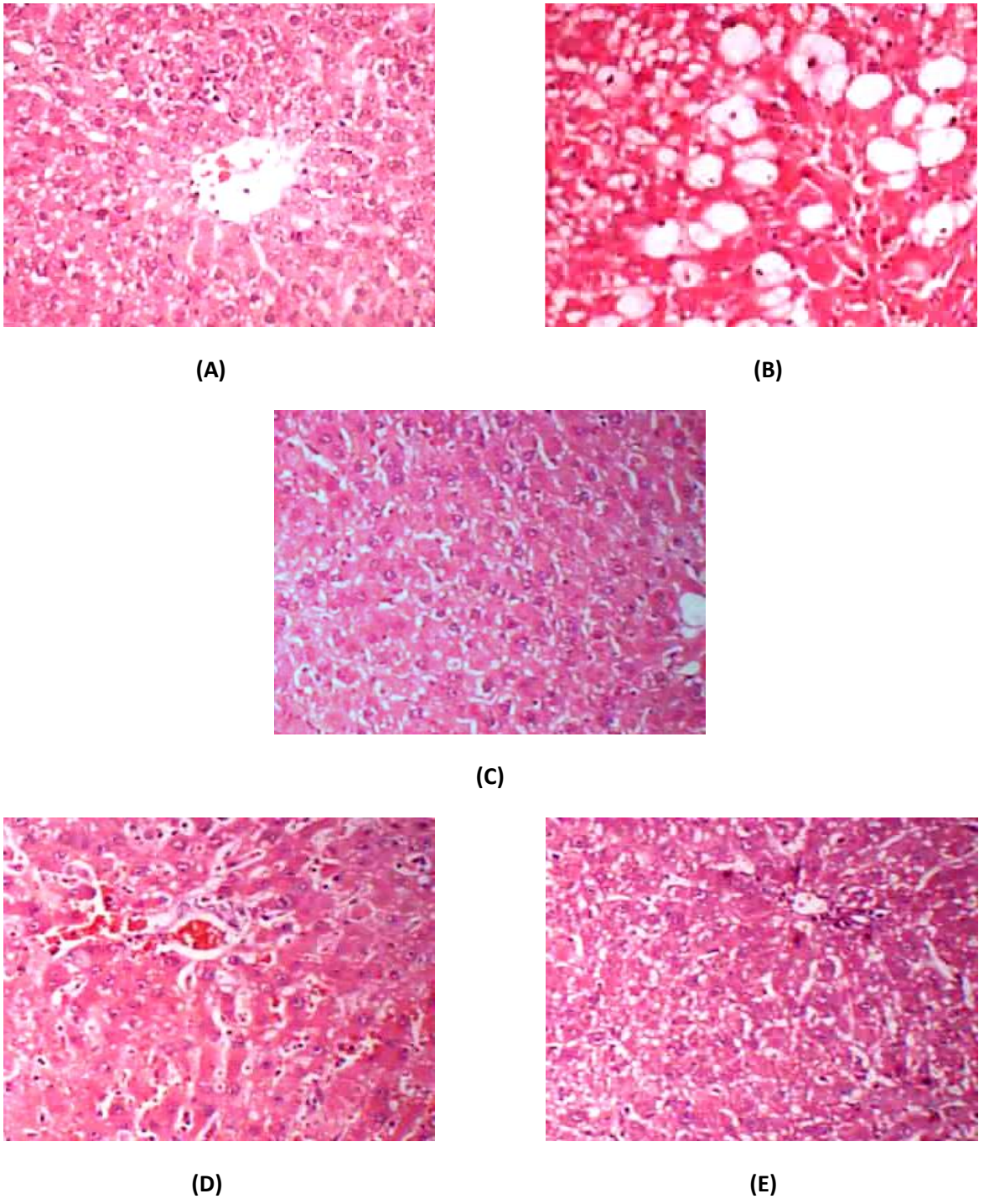
Table 1. Effect of MECG on biochemical parameters in CCl4 induced hepatic toxicity

Group	SGOT(IU/L)	SGPT(IU/L)	ALP (KA units)	TB (mg/dl)
I	97.37±5.89	76±5.0	165.2±7.31	0.03083±0.005
II	420.5±12.28$^{\#\#}$	301±14$^{\#\#\#}$	332.9±0.10$^{\#\#\#}$	0.08217±0.003$^{\#\#\#}$
III	143.6±6.11***	03±6.9***	191.8±6.51***	0.03083±0.004***
IV	327.6±8.16***	229±7.1***	294.4±8.94*	0.04417±0.002***
V	191.9±8.02***	124±7.5***	240.1±10.26***	0.03483±0.002***

SGOT: Serum glutamic oxaloacetic transaminase
SGPT: Serum glutamic-pyruvic transaminase
ALP: Alkaline phosphatase
TB: Total bilirubin
All values are shown as mean ± SEM and n=6.
indicate $p< 0.05$, ## indicate $p<0.01$, ### indicate $p<0.001$ when compared to normal group.
* indicate $p<0.05$, ** indicate $p<0.01$, *** indicate $p<0.001$ when compared to CCl_4 group (One-way ANOVA followed by Tukey's test)



(A) **(B)**

(C)

(D) **(E)**

Figure 1. Histopathological monograph of extract and standard, micrograph of liver of rats, A: Normal, B: CCl_4 (0.55mL.kg^{-1}) alone, C: CCl_4+Silymarin (0.1 ml·kg^{-1}/day +100 mg.kg^{-1}/day), D: CCl_4+MECG (0.1 ml·kg^{-1}/day +200 mg.kg^{-1}/day), E: CCl_4+MECG (0.1 ml·kg^{-1}/day +400 mg.kg^{-1}/day)