

HEPATOPROTECTIVE ACTIVITY OF *GYMNOSPORIA EMARGINATA* AND *MARSEDENIA VOLUBILIS* AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Objective: The objective of this study was to investigate the hepatoprotective activity of methanolic extracts of *Gymnosporia emarginata* and *Marsdenia volubilis* against CCl₄ induced Hepatotoxicity.

Method: Carbon tetrachloride (CCl₄) is a well-known hepatotoxin and exposure to this chemical is known to induce oxidative stress and causes liver injury by the formation of free radicals. Animals were pretreated with the methanolic extracts of *Gymnosporia emarginata* (300 mg/kg) and *Marsdenia volubilis* (500 mg/kg) for one week and then challenged with CCl₄ (1.5 ml/kg) in olive oil (1:1, v/v) on 7th day. Serum marker enzymes (ALP, AST, ALT and Total bilirubin) were estimated in all the study groups. Alteration in the levels of biochemical markers of hepatic damage like AST, ALT, ALP and Total bilirubin were tested in both CCl₄ treated and extract treated groups. CCl₄ has decreased the serum levels of ALP, AST, ALT and Total bilirubin.

Results: Treatment of methanolic extracts of *Gymnosporia emarginata* (300 mg/kg) and *Marsdenia volubilis* (500 mg/kg) exhibited a significant protective effect by altering the serum levels of AST, ALT, ALP and Total bilirubin. These biochemical observations were supported by histopathological study of liver sections.

Conclusion: From this preliminary study it has been concluded that among the two extracts tested, the methanolic extract of *Marsdenia volubilis* found to possess significant protective effect against CCl₄ induced hepatotoxicity.

Keywords: *Gymnosporia emarginata*, *Marsdenia volubilis*, CCl₄, Hepatoprotective.

INTRODUCTION

Liver - a major metabolic organ affected by various chemicals and toxins daily and identification of a successful hepatoprotective agent will provide a useful tool for the treatment of hepatic diseases. In absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders and quite often claimed to offer significant relief [1]. Exposure to various organic compounds including a number of environmental pollutants and drugs can cause cellular damages through metabolic activation of those compounds to highly reactive substances such as reactive oxygen species (ROS). Carbon tetrachloride (CCl₄) is a well-known hepatotoxin and exposure to this chemical is known to induce oxidative stress and causes liver injury by the formation of free radicals [2].

Gymnosporia emarginata (Celastraceae) commonly known as "Thorny staff tree" a small shrub, 1-3m tall, branches gray-brown, shallowly longitudinally fluted, unarmed. The leaves are simple and alternate or opposite; stipules are small and caduceous or absent. The crude plant extracts of this family in traditional medicine and agriculture is astonishing, and includes stimulant, restorative, male contraceptive, anti tumor, anti leukemic, anti bacterial, insecticidal and insect repellent activities. Traditionally species of *Gymnosporia* has been used for Fever, Asthma, Rheumatism and Gastro intestinal disorders. Recently some biomolecules from Mayentus species have been reported to be active against HIV protease [3], Carcinoma and leukemia [4], ulcers [5] and MDR (multi drug resistance) [6]. *Marsdenia volubilis* belong to the family Asclepiadaceae commonly called as "Green wax flower", "Sneezing silk cotton", "cotton milk plant" which is a tall woody climber. It is widely used in Indian traditional medicines and the leaf paste to treat Rheumatic pain, cough, Fever, and severe cold, leaf paste is taken along with pepper to treat Dyspepsia, bark paste mixed with hot milk is used internally for treating Urinary troubles and leaf powder is taken orally along with cow milk have anti diabetic activity. The root is applied to snake bites and given to women to cure headache after child birth and the leaves are applied to boils and abscesses to promote suppuration. It is emetic, diaphoretic and diuretic. The juice of the plant is used as sternutatory [7-11].

MATERIALS AND METHODS

Plant Materials

The fresh leaves of *Gymnosporia emarginata* and *Marsdenia volubilis* were collected from Dr. K. Madhava Chetty, Assistant professor, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh, India, in June 2010 [12]. The plant was identified by a Botanist, and voucher specimen was deposited in Sri Venkateswara University, Department of Botany and a copy has been preserved for the future reference at the herbarium of the institute TRRC. After authentication, the leaves were cleaned and shade dried and milled into coarse powder by a mechanical pulverizer.

Preparation of Extract

The coarse powder of plant material was defatted with petroleum ether (60-80°C) in a soxhlet extraction apparatus and marc was extracted with methanol (1000 ml). Overnight, at room temperature with constant stirring. The extract was filtered and the filtrate was concentrated at 30°C under reduced pressure in a rotary evaporator. Extract was dried in dessicator. The crude extract was suspended in 1% Tween-80 to required concentrations and used for the experiments.

Phytochemical screening

The methanolic extract obtained was subjected to preliminary Phytochemical screening, to identify the chemical constituents [13]. The phytoconstituents in the extract was found to contain alkaloid, flavonoides, glycosides, steroids and tannins.

Formulation: Suspensions were formulated of required concentrations 300 mg/kg and 500 mg/kg by using 1% Tween-80 and double distilled water. The formulated suspensions were compared for various evaluation parameters.

Pharmacological studies

Animals

Male Wistar rats weighing between 140-180 gm were used for this study. The animals were obtained from NIN, Hyderabad, India. The animals were placed in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature

of $24 \pm 2^\circ\text{C}$ and relative humidity of 30-70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted diet. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the CPCSEA (No. 1447/PO/a/11/CPCSEA).

Hepatoprotective Activity

Animals were divided into 5 groups of six rats each. Group- I and II served as normal and toxic control, and received only the vehicle (1% Tween-80; 1 ml/kg; p.o). Group- III animals were treated with standard silymarin at an oral dose of 100 mg/kg and group- IV and group- V received the *Gymnosporia emarginata* extract and *Marsdenia volubilis* extract at an oral dose of 300 mg/kg and 500 mg/kg respectively, as a fine suspension of 1% Tween-80. The treatment was continued for 7 days, once daily. On the day of 7 for groups II - V, 30 min post-dose of extract administration animals received CCl_4 at the dose of 1.5 ml/kg (1:1 v/v of CCl_4 in olive oil) orally [14,15,16].

RESULTS

Table 1: Effect of *Gymnosporia emarginata* and *Marsdenia volubilis* on serum marker enzymes (ALT, AST, ALP) and Total bilirubin on CCl_4 induced hepatotoxicity in rats.

Groups	ALT(μL)	AST(μL)	ALP(μL)	TB(μL)
Normal Control	57 ± 1.065	52.83 ± 1.5	34 ± 1.183	0.3915 ± 0.0006
CCl_4 Control	$111.2 \pm 1.352^*$	$100.5 \pm 0.7638^*$	$102.3 \pm 0.9189^*$	$2.117 \pm 0.04869^*$
MEGE	$83.00 \pm 0.3651^{**}$	$80.83 \pm 0.6009^{**}$	$58.83 \pm 0.6009^{**}$	$0.626 \pm 0.0092^{**}$
MEMV	$70.50 \pm 0.4282^{**}$	$68.33 \pm 0.5578^{**}$	$50.33 \pm 0.8819^{**}$	$0.493 \pm 0.0073^{**}$
Silymarin	63.17 ± 0.79	62.50 ± 0.76	40.83 ± 0.60	0.40 ± 0.003

MEGE, Methanolic Extract of *Gymnosporia emarginata*;

MEMV, Methanolic Extract of *Marsdenia volubilis*;

Values are expressed as mean \pm SEM for six rats in each group. * $P \leq 0.01$ when compared to control. * $P \leq 0.01$ when compared to CCl_4 . * $P \leq 0.01$ when compared to silymarin.

Biochemical parameters

The results of hepatoprotective activity of methanolic extract of *Gymnosporia emarginata* and *Marsdenia volubilis* on CCl_4 treated rats are shown in Table 1. The hepatic enzymes ALT (111.2 ± 1.352), AST (100.5 ± 0.7638), ALP (102.3 ± 0.91) and bilirubin (2.117 ± 0.04869) in serum was significantly increased in CCl_4 treated animals when compared to control. Among the methanolic extract of *Gymnosporia emarginata* and *Marsdenia volubilis* treatments, reduced the levels of ALT (83.00 ± 0.3651 ; 70.50 ± 0.4282), AST (80.83 ± 0.6009 ; $68.33 \pm 0.5578^*$), ALP (58.83 ± 0.6009 ; 50.33 ± 0.8819) and bilirubin (0.626 ± 0.0092 ; 0.493 ± 0.0073) when compared to CCl_4 alone treated rats. among the two extracts *Marsdenia* reduced the level to the higher degree than that of *Gymnosporia*. Silymarin (25mg/kg) treated animals also showed significant decrease in ALT (63.17 ± 0.79), AST (62.50 ± 0.76), ALP (40.83 ± 0.60) and bilirubin (0.40 ± 0.003) levels when compared to CCl_4 alone treated rats.

The animals were sacrificed 36hr after administration of acute dose of CCl_4 . The blood was collected by retro orbital artery bleeding. Blood samples were centrifuged for 10 min at 3000 rpm to separate the serum. Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP) and Total Bilirubin (TB) levels were estimated from the serum by using standard kits [17].

Histopathological studies

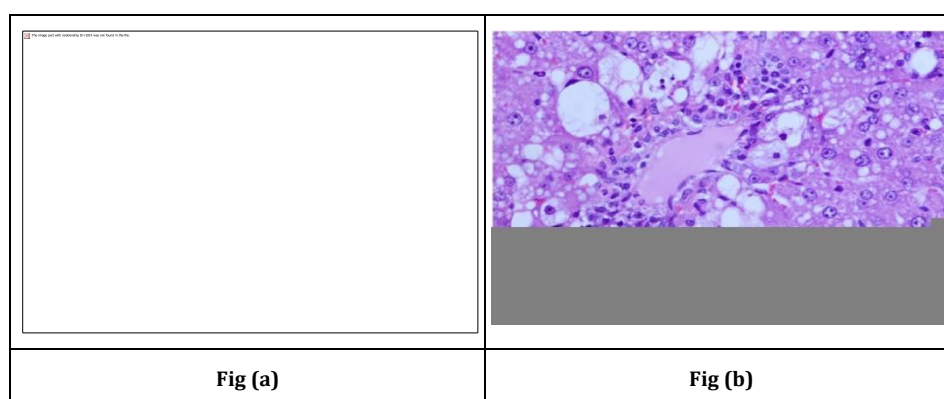
The livers were excised quickly and fixed in 10% formalin and stained with haematoxylin and eosin and then observed under microscope for degeneration, fatty changes, necrotic changes and evidence of hepatotoxicity if any [18].

Statistical analysis

The results were shown in the Table No.1. The values were expressed as mean \pm SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' - test. P values < 0.01 were considered significant.

Histopathological studies

Histopathological examination of liver sections of control group showed normal hepatocytes, multiple FAN is seen with mild portal tract infiltration with lymphocytes (Fig. d). In CCl_4 treated animals, expanded portal tracts with lymphocyte infiltration and perivascular and periportal vacuolated and degenerated hepatocytes are seen, and extensive vacuolated (micro vesicular) hepatocytes seen with perivascular inflammatory infiltrate of lymphocytes and neutrophils (Fig. a). The liver sections of the rats treated with *Gymnosporia emarginata* followed by CCl_4 showed multiple FAN with portal tract and perivascular lymphocyte infiltrate with perivascular and periportal degenerated and vacuolated hepatocytes. Perivascular and periportal small vacuolar change in hepatocytes is also seen (Fig. b); whereas *Marsdenia volubilis* and Silymarin followed by CCl_4 showed a sign of protection as it was evident by multiple small FAN seen with mild to moderate portal tract infiltration with lymphocytes (Fig. c, e).



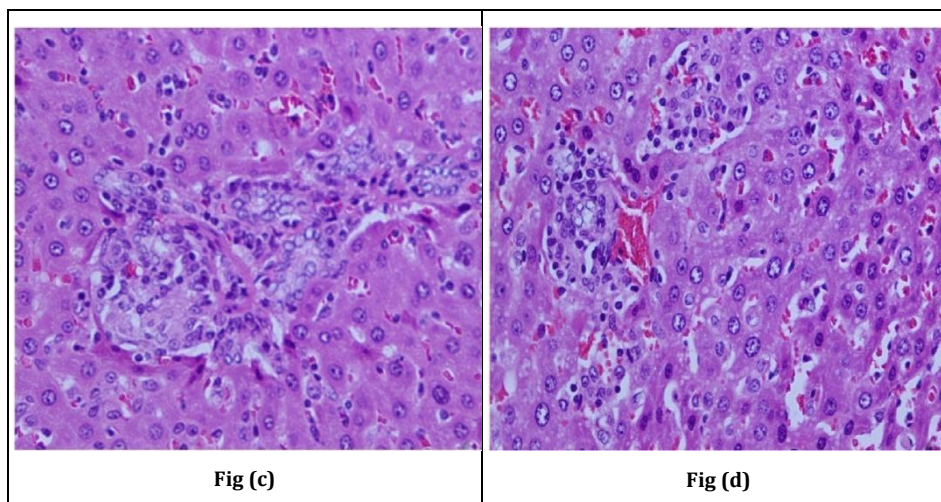


Fig. 5: Histology of Liver sections

(a) Section of the liver tissue of animal treated with CCl_4 ; (b) Section of liver tissue of methanolic extract of *Gymnosporia emarginata* treated animal; (c) Section of liver tissue of methanolic extract of *Marsedenia volubilis* treated animal; (d) Section of the liver tissue of control animal

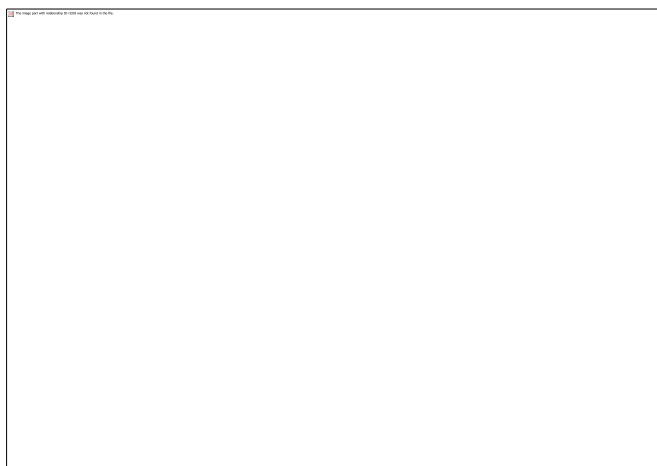


Fig. 5: Histology of Liver sections- Section of the liver tissue of Silymarin treated animal

DISCUSSION

A number of chemicals including various environmental toxicants and clinically useful drugs can cause severe cellular damages in different organs of our body through the metabolic activation to highly reactive substances such as free radicals. CCl_4 is one of such extensively studied environmental toxicant [19]. Up to the present time, the etiology and treatment of most liver diseases are not known. The liver is the commonest site affected during the toxic manifestation of many drugs. Toxicity in liver due to CCl_4 and other chemicals is attributed to the toxic metabolites formed, responsible for the initiation of CCl_4 dependent lipid peroxidation, the nature of which is not yet unambiguously determined. The most likely candidate is the trichloromethyl radical [20]. In the liver, CCl_4 is metabolized by the cytochrome P450-dependent monooxygenase systems to produce the Trichloromethyl free radicals, which in turn covalently binds to cell membrane and organelles to elicit lipid peroxidation [21]. It has been evident that several phytoconstituents have the ability to induce microsomal enzymes either by accelerating the excretion of ccl_4 or by inhibition of lipid peroxidation induced by ccl_4 [22].

Present study was conducted to evaluate the protective effect of the *Gymnosporia emarginata* and *Marsedenia volubilis* against CCl_4 induced hepatic damage in rat. Results suggest that the extract possesses protective action against hepatic dysfunction induced by

the potent toxin CCl_4 . Both biochemical and histopathological data showed that there was no difference in extract treatment when compared with standard drug silymarin. Extensive evidence demonstrated that trichloromethyl radical are formed as a result of the metabolic activation of CCl_4 , which in turn, initiate lipid peroxidation process. A known potent antioxidant, vitamin E, could protect CCl_4 induced liver injury indicating that oxidative stress is responsible for CCl_4 induced hepatic disorder in this particular model [23,24]. *Marsedenia volubilis* and silymarin treated groups significantly protect organ against CCl_4 induced hepatic damage. Our results suggest that among the two methanolic extract tested, *Marsedenia volubilis* possesses comparatively more hepatoprotective activity than *Gymnosporia emarginata*.

CONCLUSION

From the above preliminary study, among the two extracts tested, we conclude that the methanolic extract of *Marsedenia volubilis* found to possess significant protective effect against CCl_4 induced hepatotoxicity. Further studies are recommended.

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