

Hepatoprotective Activity of Different Extract of *Chromolaena Odorata* Against CCL₄ and Rifampicin–Induced Hepatic Injuries in Rats: A Randomized Controlled Preclinical Trial

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ABSTRACT

Introduction: Now a day's liver disease is a worldwide problem. Modern synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can cause serious side effects.

Objective: This current investigation aims to evaluate the ethanol and aqueous extracts of leaves of *Chromolaena odorata* (L.) R. M. King & H. Rob. (Asteraceae) was investigated against carbon tetrachloride and rifampicin-induced hepatic damage. The plant of *Chromolaena odorata* is a perennial herb and distributed throughout the region of India, Mexico and Asia. In traditionally this plant is used in the treatment of coughs, colds, and skin diseases. Based on the present study, an effort has been made to establish the scientific validity of the hepatoprotective activity against ccl₄ and rifampicin-induced hepatotoxicity.

Methods: To evaluate the hepatoprotective activity by various inducing agents like carbon tetrachloride at the rate of 1 ml/kg and rifampicin 1mg/kg produced liver damage in rats.

Results: The results are manifested by the significant ($P < 0.005$) rise in serum levels of Serum oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), Total protein Total bilirubin and Direct bilirubin compared to respective control values. Histopathological observation also revealed that pretreatment with *Chromolaena odorata* protected the animals from carbon tetrachloride and rifampicin induced liver damage.

Conclusion: From the present work we can strongly conclude that plant of *Chromolaena odorata* is biologically huge potential value. The preliminary chemical test of the samples revealed the presences of high-value phenolic compounds as the major content in the plant of *Chromolaena odorata*.

Key Words: Chromolaena odorata, CCl₄, Rifampicin, Transaminases, Histopathological studies

INTRODUCTION

India is known for its traditional medicinal systems—Ayurveda, Siddha, and Unani. Medical systems are found mentioned even in the ancient Vedas and other scriptures. The Ayurvedic concept appeared and developed between 2500 and 500 BC in India.^{1,2} In India medicinal plants and traditional medicine have been the basis of traditional healthcare especially in remote areas where modern healthcare facilities are inadequate. India is the largest producer of medicinal plants is rightly called the “The botanical garden of the world”.³ The revival of interest in herbal medicines is due to safe, more accessible and more affordable. The plant of *Chromolaena odorata* (L.) R. M. King & H. Rob. family of Asteraceae is a perennial herb and distributed throughout the

region of India, Mexico and Asia.^{4,5} In traditionally this plant is used in the treatment of coughs, colds, and skin diseases.⁶ Now a day's liver disease is a worldwide problem. Modern synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can cause serious side effects.⁷ On the basis of the present study, an effort has been made to establish the scientific validity of the hepatoprotective activity against CCl₄ and rifampicin-induced hepatotoxicity.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Chromolaena odorata* were collected from kaaripatti, Salem (Dt) Tamilnadu. The plant was then au-

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thenticated by the Botanist A. Balasubramaniam, consultant-central Siddha Research, Salem-Tamilnadu.

Preparation of the extract/drug

The fresh leaves of *Chromolaena odorata* dried at under shade, grind into small pieces of crude drugs with help of a mechanical grinder. The crude powder was passed through sieve no.30 and stored in a suitable container. Then the crude drugs were defatted with Ethanol 95% (75-78°C) by using soxhlet apparatus. Then crude drugs were then subjected to cold maceration method by using aqueous water for 72hrs. Then extracts were concentrated by using a rotary vacuum evaporator and kept aside in the desiccators. The extracts were suspended in Tween80 for the presented study. The extract obtained was subjected to various Preliminary Phytochemical Screening tests as per the procedure mentioned in the standard reference books.^{8,9} The extract was used for pharmacological evaluation

Preliminary Phytochemical Screening

The various extracts of *Chromolaena odorata* were then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents, it revealed that the presence of alkaloids, steroids, polyphenolic constituents like flavonoids, saponins, glycosides, tannins, gums and mucilages.

Procurement of experimental animals

Swiss albino mice (20-25gm) and Wister rats (150-200gm) of either sex and approximate same age used in the present studies were procured from listed suppliers of Sri Venkateswara enterprises, Bangaluru, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangaluru) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under the alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of each activity. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC No.-60/2019/IAEC/VMCP.) after scrutinization. The animals received the drug treatment by oral gavage tube.

Acute toxicity test¹⁰

The ethanol and aqueous extract of *Chromolaena odorata* were subjected for acute toxicity, as per standard method (OECD/OCDE No: 423). Albino female mice weighing 20-28 gm were used in this present study. The animals were fed with standard pellet diet and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under the alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory con-

ditions for 1 week before starting the experiment. The dose of ethanol and aqueous extracts was prepared with suitable saline and was administered by intubations. The acute toxicity was screened up to doses of 5000mg/kg.

Hepatoprotective studies

Carbon tetrachloride [CCl₄] induced hepatotoxicity in rat model^{11,12}

The rats were divided into 7 groups of six animals in each. Group, I served as a control, Group II administered with carbon tetrachloride (1 ml/kg) in 50% v/v olive oil every 72 hrs, Group III was pretreated with the standard drug of silymarin (100mg/kg) orally for 7 days. Simultaneously CCl₄ (1ml/kg) in 50% v/v olive oil every 72 hrs Groups IV, V animals were pretreated with Ethanol extract of *Chromolaena odorata* (200mg/kg, 400mg/kg respectively) for 7 days. Groups VI, VII animals were pretreated with aqueous extract of *Chromolaena odorata* (200mg/kg, 400mg/kg respectively) for 7 days and carbon tetrachloride (1 ml/kg) intraperitoneal every 72 hours for 7 days for test groups. After the experimental period, blood was collected by the retro orbital method and the serum was separated and used for the assay of Serum oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase(SGPT), alkaline phosphatase (ALP), total protein, total &direct bilirubin were estimated respectively. The rats were sacrificed by cervical dislocation method. The liver is removed for histopathological examination

Rifampicin induced model^{13,14}

The rats were divided into 7 groups of six animals in each. Group I served as a control, Group II administered with rifampicin (1gm/kg, p.o) every 72 hrs, Group III were pretreated with the standard drug of silymarin (100mg/kg) orally for 10 days. Simultaneously rifampicin (1 gm/kg) every 72 hrs, Groups IV, V animals were pretreated with Ethanol extract of *Chromolaena odorata* (200 mg/kg, 400mg/kg respectively) for 10 days. Groups VI, VII animals were pretreated with aqueous extract of *Chromolaena odorata* (200mg/kg, 400mg/kg respectively) for 10 days and rifampicin (1gm/kg) intraperitoneal every 72 hours for 10 days for test groups. After the experimental period, blood was collected by the retro-orbital method and the serum was separated and used for the assay of Serum oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase(SGPT), alkaline phosphatase (ALP), total protein, total &direct bilirubin were estimated respectively. The rats were sacrificed by cervical dislocation method. The liver is removed for histopathological examination.

Statistical analysis

The values Mean± standard error means [SEM] are calculated for each parameter. For determining the significant intergroup difference each parameter was analysed separately

and one-way analysis of variance was carried out and the individual comparisons of the group mean values were done using Dunnet's test.¹⁵

RESULTS

Preliminary phytochemical screening revealed the presence of Alkaloids, Steroids, polyphenolic constituents like flavonoids, Saponins, glycosides, tannins, gums and mucilages. Acute toxicity studies of the various extracts of the *Chromolaena odorata* did not exhibit any signs of toxicity up to 5 g/kg body weight. Since there was no mortality of the animals found at the high dose. Hence 200 and 400 mg/kg dose of the extract selected for evaluation of the hepatoprotective activity.

CCl₄ induced hepatotoxicity

A significant reduction was (P<0.05) observed in SGOT, SGPT, ALP, total bilirubin, direct bilirubin, increase in the

total protein levels in the groups treated with 200& 400 mg/kg dose of *Chromolaena odorata*(L)king&H.Rob.extracts. The enzyme levels were almost to the normal. It was observed that the size of the liver was pale reddish-brown and enlarged in CCl₄ intoxicants rat but it was normal in drug-treated groups. A significant reduction (P< 0.05) in liver enzyme level finding table no-1

Rifampicin induced hepatotoxicity

Table 1 summarizes the results obtained in the experimental model of rifampicin-induced hepatotoxicity in rats. The present study was subjected that aqueous extract is indicating dose-dependent hepatoprotective activity while the comparison to ethanolic extract against liver injury induced by rifampicin. A significant reduction (P< 0.05) in liver enzyme level finding table no-2

Table 1: Hepatoprotective activity of *chromolaena odorata*(L) King & H. Rob. against carbon tetrachloride (CCl₄) induced hepatotoxicity

Treatment	SGOT	SGPT	T.Protein	T.Bilirubin	D.Bilirubin	ALP
Control	164.3333± 1.05	111.8333 ±1.35	6.961667 ±0.01	0.391667 ±0.04	0.273333 ±0.07	220.3333 ±1.58
Ccl4 induced	320.33±1.35###	265.66±3.51###	4.66±0.13###	0.98±0.01###	0.88±0.01###	389.5±2.65###
Silymarin treated	181.66±1.22**	152.33±0.98**	6.53±0.01**	0.52±0.01**	0.39±0.01**	242.16±1.81**
Ethanol Extract- 200mg/kg	271.5±1.38***	239.66±0.88***	5.25±0.06***	0.71±0.01***	0.63±0.01***	306.16±2.22***
Ethanol Extract -400mg/kg	232±1.41**	190±5.48**	6.03±0.01**	0.64±0.01**	0.51±0.01**	283.33±1.05**
Aqueous extract -200mg/kg	242.16±1.42**	202.83±1.13**	5.88±0.01**	0.66±0.07**	0.52±0.01**	284.33±1.02**
Aqueous extract -400mg/kg	199±1.18**	172±0.85**	6.32±0.01**	0.57±0.01**	0.42±0.01**	259.5±0.88**

- Values are expressed as mean ± S.E.M. (n=6),
- #, normal control group compare with ccl4 treated group
- ### = p< 0.001, **=p< 0.01, *=p< 0.05
- *, compare with ccl4 treated group
- ***= p< 0.001, ## = p<0.01, # = p< 0.05

Table 2: Hepatoprotective activity of *chromolaena odorata*(L)King&H.Rob. against rifampicin induced hepatotoxicity

Treatment	SGOT	SGPT	T.Protein	T.Bilirubin	D.Bilirubin	ALP
Control	112±0.96	80.16±0.47	6.33±0.08	0.51±0.01	0.41±0.01	271.6±0.88
Rifampicin induced	184.16±1.01###	147.83±0.79###	4.98±0.02###	0.89±0.01###	0.77±0.01###	380.5±0.76###
Silymarin treated	121.66±0.88**	89.83±1.04**	5.9±0.05**	0.51±0.01**	0.46±0.01**	286.83±0.6**
Ethanol Extract -200mg/kg	162.83±0.94**	131±0.93**	5.33±0.01**	0.76±0.01**	0.66±0.01**	340.83±1.24**
Ethanol Extract -400mg/kg	145.33±0.76***	117.5±0.76***	5.49±0.01***	0.60±0.01***	0.54±0.01***	305.83±1.27***

Table 2: (Continued)

Treatment	SGOT	SGPT	T.Protein	T.Bilirubin	D.Bilirubin	ALP
Aqueous extract -200mg/kg	154.83±0.47***	122.16±0.6***	5.44±0.01***	0.62±0.01***	0.57±0.01***	312.5±1.11***
Aqueous extract -400mg/kg	130.5±0.56***	101.33±0.98***	5.6±0.04***	0.59±0.01***	0.52±0.01***	296±0.57***

- Values are expressed as mean ± S.E.M. (n=6),
- #, normal control group compare with ccl₄ treated group
- ### = p< 0.001, **=p< 0.01, *=p< 0.05
- *, compare with ccl₄ treated group
- ***= p< 0.001, ## = p<0.01, # = p< 0.05

Histopathological Studies

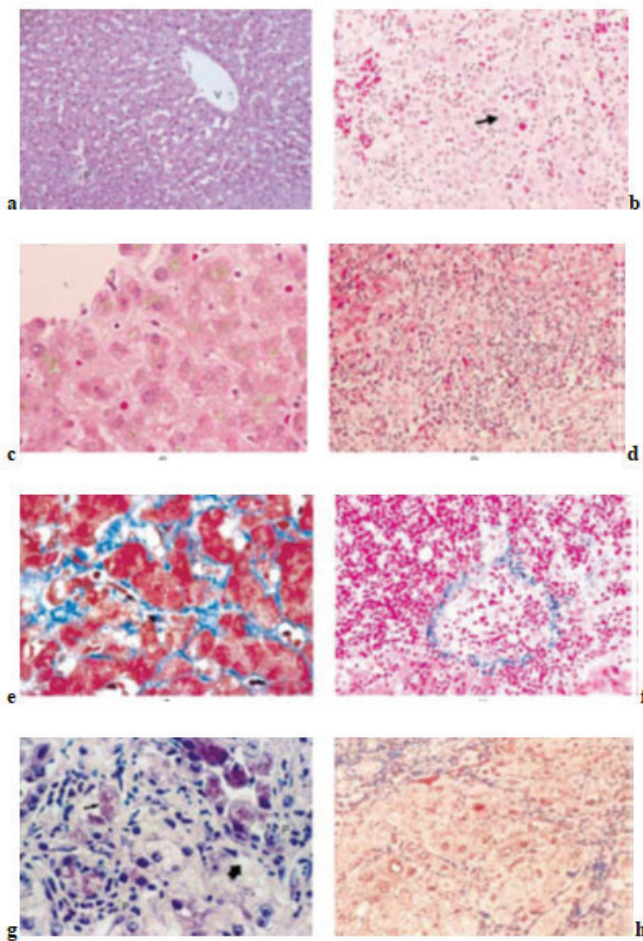


Figure 1: (a) liver of normal control rat; (b) Negative control (CCl₄ iinuced); (c) standard drug treated; (CCl₄ model); (d) standard drug treated (Rifampicin model) (e) Effect of ethanolic extract on CCl₄ induced hepatotoxicity in rat; (f) Effect of aqueous extract on CCl₄ induced hepatotoxicity in rat; (g) Effect of ethanolic extract on rifampicin induced hepatotoxicity in rat; (h) Effect of aqueous extract on rifampicin induced hepatotoxicity in rat.

DISCUSSION

Literature review revealed that various chemical and biological investigations were carried out with these plants. Phytochemical screening revealed the presence of Alkaloids, Steroids, polyphenolic constituents like flavonoids, Saponins, glycosides, tannins, gums and mucilages. Carbon tetrachloride (CCl₄) is potent hepatotoxin producing centrilobular hepatic necrosis. It is accumulated in hepatic parenchyma cells and metabolized to CCl₃ by liver Cytochrome P450-dependent monooxygenase its enzymatic transformation by CYP2E1 into a highly reactive free radical (CCl₃•) and subsequent derivatives (Cl₃COO) Those free radicals initiate and promote the propagations of lipid peroxidation.^{16,17} Administration of carbon tetrachloride showed significantly elevated levels of, SGOT, SGPT, ALP, TP and TB, due to its enzymatic activation of CCl₃ free radical, which in turn alters the structure and function of liver cells. Treatment with aqueous extract of *Chromolaena odorata(L) king & H.Rob.* showed dose-dependent protection against the injurious effects of CCl₄ when compared to ethanol extract. Rifampin (also referred to as rifampicin) is a macrocyclic antibiotic with major activity against mycobacteria, commonly used in combination with other agents as therapy of tuberculosis. Rifampicin extensively metabolized by the liver and induces multiple hepatic enzymes including Cytochrome [CYP 3A4 and Adenosine binding cassette C2 [CYP 3A4 and ABC C2], Multidrug resistance-associated protein-2 [MRP2]. Thus, the cause of injury is likely to be due to idiosyncratic metabolic products that are either directly toxic or induce an immunologic reaction. The rise in direct and total bilirubin in rare patients receiving rifampin may relate to gene defects in [MRP2, ABC C2] The major bilirubin glucuronide transporter in hepatocytes that is known to be abnormal in the Dubin-Johnson syndrome. Patients with pre-existing liver disease and cirrhosis are particularly likely to develop jaundice on rifampin therapy.^{18,19}

CONCLUSION

The present study concludes that the ethanol and aqueous extracts of the leaves of *Chromolaena odorata* possesses

significant hepatoprotective activity in Carbon tetrachloride (CCl₄) induced rats at a dose of 1 ml/kg/i.p and rifampicin at a dose of 1mg/kg. Thus, it has been scientifically proved that the traditional knowledge obtained from the tribal people of South India is true and the extracts have enough potential as a hepatoprotective agent and hence worth investigative. Further study needs to be isolated, identify the active compounds and formulation.

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