International Journal of Pharmacy Teaching & Practices 2014, Vol.5, Issue 4, 1574-1581.

Evaluation Of Hepatoprotective Activity Of Whole Plant Extract Of *Chromolaena Odorata King&H.Rob* In Carbon Tetra Chloride And Rifampicin Induced Rats

Palanisamy.P[1]*, R.Margret Chandra[1], B.Jaykar[2], B.S.Venkateshwarlu[1], A.Pasupathi[1]

1. Department Of Pharmacutics,
2. Department Of Pharmaceutical Chemistry, Vinayaka Mission's College Of Pharmacy, Vinayaka Missions University, Salem, Tamil Nadu, India.

Research Article

Please cite this paper as Palanisamy.P[1]*, R.Margret Chandra[1], B.Jaykar[2], B.S.Venkateshwarlu[1], A.Pasupathi[1]. Evaluation Of Hepatoprotective Activity Of Whole Plant Extract Of *Chromolaena Odorata King&H.Rob* In Carbon Tetra Chloride And Rifampicin Induced Rats. IJPTP, 2014, 5(4), 1574-1581.

Corresponding Author:

Mr. PALANISAMY.P * M.Pharm., (Ph.D)
Department of Pharmaceutics,
Vinayaka Mission's College of Pharmacy,
Yercaud Main Road, Kondappanaickenpatty,
Salem (D.T), Tamil Nadu (State),
Pin. Code: 636 008. Mobile. No: 9791735383
Email.ID: palanisamy2907@gmail.com

Abstract

The main objective of this study was to focus on the hepatoprotective activity *Chromolaena odorata King &H. Robinson*, with special reference to its curative and protective role in carbon tetra chloride and rifampicin-induced hepatoprotctive animal model.

To fulfill above object in research envisaged the work was to be complete in following manner. To ccollection and authentication of whole plant of *Chromolaena odorata king & H.Rob.* The dried plant in a soxhlet apparatus by using different solvents in their increasing order of polarity. The extracts were carried out preliminary phytochemical studies, Acute oral toxicity studies and Hepatoprotective activity [Carbon tetra chloride (ccl_4) induced hepatotoxicity in rats model and its Histopathological studies & Rifampicin induced hepatotoxicity in rats and its Histopathological studies. The phytochemical constuients were identified by chemical tests and these tests showed the presence of various phytochemical constituents like carbohydrate, glycoside, fixed oils, phenolic compounds, alkaloids, flavonoids and tannins. The alcoholic and aqueous extract of whole plant of *Chromolaena odorata King & H. Robinson* showed the presence of maximum chemical constituents. In the pharmacological studies alcoholic and aqueous extract of whole plant *Chromolaena odorata King & H.Robinson* showed significant heapatoprotective activity. In last present studies revels that both the alcoholic and aqueous of whole plant of *Chromolaena odorata King & H. Robinson* can be used as effective hepatoprotective activity. Further experiments are required to prove the mechanism and advantage of alcoholic and aqueous extract whole plant of *Chromolaena odorata King & H. Robinson* over other drug.

Keywords: *Chromolaena odorata, carbon tetra chloride, rifampicin and hepatoprotctive activity*

Introduction

A high percentage of useful plant derived drugs were discovered as a result of scientific follow up of well-known plants used in traditional medicine system, and it can be concluded that this is a good approach for discovering other useful drugs from plants. Other approaches, such as phytochemical screening and massive biological screening of randomly collected plants, and phytochemical examination of plants with the aim of identifying new compounds have not proved to be very helpful in discovering new drugs.

The aim of the research is to find out new hepatoprotective and diuretic drug from indigenous plants which are potent and nontoxic agents. *Chromolaena odorata king&H.Rob.* plant is traditional medicinal plant. Their chemical characterization, mode of action and toxicity studies is yet to be established. Present study deals with phytochemical and pharmacological evaluation of whole plant of *Chromolaena odorata king&H.Rob* with special reference to hepatoprotctive activitie in animal models. Normally herbal products are less or free from side effects/adverse effect and they are low cost medicines, which will be beneficial for the poor people of this country.

Material and Method

Plant material

The fresh whole plant of *Chromolaena odorata King & H. Robinson* were collected in the month of January 2014 from Salem district, Tamilnadu, India. The plant was identified and authenticated by the botanist Mr. A Balasubramanian (consultant central siddha research) Executive Director ABS botanical garden, Salem, Tamilnadu.

Animals

Sprague-Dawley rats (150-185g) and Swiss albino mice (20-25 gm) of either sex and of approximately the same age were procured from the animal house of Central Drug Research Institute, Lucknow. They were kept in the departmental animal house at 26 ± 2 °C and relative humidity 44 – 56 % in polypropylene cages. The animals were exposed to alternate 12 hrs of darkness and light each..Animals were provided with standard rodent pellet diet (Dayal, India) and the food was withdrawn 18-24 h before the experiment though water was allowed *ad libitum*. All experiments were performed in the morning according to current guidelines for investigation of experimental pain in conscious animals. The standard orogastric cannula was used for oral drug administration in experimental animals[1].

Preparation of plant extract

Method of extraction: Cold percolation process.

Requirements: Percolater, Shade dried coarse powder of whole plant of *Chromolaena odorata(L) King & H. Robinson*.

Solvents: Soxhlet apparatus.

i. Petroleum ether (60-80^{0}C)
ii. Chloroform
iii. Acetone
iv. Ethanol (95 % v/v)
v. Distilled water with chloroform (0.25%)

The results are shown in table. no: 1

Preliminary Phytochemical Screening

Alcoholic and aqueous extract of *Chromolaena odorata(L) King & H. Robinson* was subjected to qualitative tests for the identification of various active constituents viz. carbohydrate, glycoside, alkaloid, amino acids, flavanoids, fixed oil, tannins, gum and mucilage, phytosterols etc. according to Khandelwal[2]. The results are shown in table. no:2.

Acute toxicity studies (OECD Guideline 423)

This test involves the administration of a simple bolus dose of test substances to faster healthy young adult rodents by oral gavage, observation for upto 15days after dosing and recording of body weight and the necropsy of all the animals. In this method pre-specified fixed doses of the test substances were used ie, 5mg/Kg, 50mg/Kg, 300mg/Kg, 2000mg/Kg and the mortality due to these doses were observed. Generally female animals were used for this study and each dose group should consist of 3 animals[3]. The results are shown in table. no: 3.

Hepatoprotective Studies

Model:1 CCl_4 hepatotoxicity induced rat model

Test compounds

The alcoholic extracts & aqueous extract of *Chromolaena odorata king&H.Rob. whole plant* (200 and 400 mg/kg. body weight) and standard drug silymarin (100 mg/kg body weight) were used.

Chemicals and reagents

The following chemicals were obtained from the indicated commercial grade of Carbon tetrachloride, Silymarin .

Experimental animal

Wister rats (150-200 g) used in the present studies were procured from listed suppliers Sri Venkateswara enterprises, Bangaloru, India.The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water ad libitum. All the animals were acclimatized for a week before use.

Procedure: The rats were divided into 4 groups of 6 animals in each.

Group I : Received vehicle for 7 days, and served as normal control.

Group II : Received Carbon tetrachloride 1ml/kg in 50% v/v olive oil every 72 hrs.

Group III: Received standard drug silymarin (100 mg/kg) for 7 days once daily , simultaneously CCl4 1ml/kg in 50% v/v olive oil every 72 hrs .

Group IV: Received ethanolic extract of *Chromolaena odorata(L)king&H.Rob.* (200 mg/kg) simultaneously CCl4 1ml/kg in 50% v/v olive oil every 72 hrs.

Group V: Received ethanolic extract of *Chromolaena odorata(L)king&H.Rob.* (400 mg/kg) simultaneously CCl4 1ml/kg in 50% v/v olive oil every 72 hrs.

Group VI: Received aqueous extract of *Chromolaena odorata(L)king&H.Rob.* (200 mg/kg) simultaneously CCl4 1ml/kg in 50% v/v olive oil every 72 hrs.

Group VII: Received aqueous extract of *Chromolaena odorata(L)king&H.Rob.* (400 mg/kg) simultaneously CCl4 1ml/kg in 50% v/v olive oil every 72 hrs.

On the 7th day food and water were withdrawn after giving the last doses of alcoholic & aqueous extracts. After 36 hours the blood samples were collected and allowed to clot and serum was separated by centrifuge at 2500rpm for 15 min and analyzed for various biochemical parameters[4-5]. The results are shown in table. no: 4-5 and Fig. no: 1-6.

Model:2 Rifampicin Induced Hepatotoxicity in rats

Test compounds

The alcoholic extracts & aqueous extract of *Chromolaena odorata(L)king&H.Rob.*whole plant (200 and 400 mg/kg. body weight) and standard drug silymarin (100 mg/kg body weight) were used.

Chemicals and reagents

The following chemicals were obtained form the indicated commercial Rifampicin(R-cin,Lupin.Ltd,Aurangabad,Maharashtra), Silymarin

Experimental animal

Wister rats (150-200 g) used in the present studies were procured from listed suppliers Sri Venkateswara enterprises, Bangaloru, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad*

libitum. All the animals were acclimatized for a week before use.

Procedure:

The rats were divided into 7 groups of 6 animals in each.

Group I: Received vehicle for 10 days, and served as normal control.

Group II :Received Rifampicin control (1gm/kg p.o) every 72 hrs.

Group III: Received standard drug silymarin (25 mg/kg) for 10 days once daily, simultaneously Rifampicin 1gm/kg body weight every 72 hrs .

Group IV: Receivedal ethanolic extract of *Chromolaena odorata(L)king&H.Rob.* (200 mg/kg) simultaneously Rifampicin 1gm/kg body weight every 72 hrs .

Group V: Received ethanolic extract of *Chromolaena odorata(L)king&H.Rob.* (400 mg/kg) simultaneously Rifampicin 1gm/kg body weight every 72 hrs .

Group VI: Received aqueous extract of *Chromolaena odorata(L)king&H.Rob.* (200 mg/kg) simultaneously Rifampicin 1gm/kg body weight every 72 hrs .

Group VII: Received aqueous extract of *Chromolaena odorata(L)king&H.Rob.* (400mg/kg) simultaneously Rifampicin 1gm/kg body weight every 72 hrs .

On the 10th day food and water were withdrawn after giving the last doses of alcoholic & aqueous extracts. After 36 hours the blood samples were collected and allowed to clot and serum was separated by centrifuge at 2500rpm for 15 min and analyzed for various biochemical parameters[6]. The results are shown in table. no: 6-7 and Fig. no: 7-12.

Assessment of Hepatoprotective activity:

1. Morphological Parameters:

These parameters were studied by recording the liver weight and volume. In damaged liver due to the fatty changes the volume and weight of the liver is increased as compared with control group.

2. Biochemical parameters:

Biochemical parameters i.e., serum gultamic pyruvate transaminase (SGPT), Serum gultamic oxaloacetic transaminase (SGOT), Alkaline phosphatase (ALP),Billirubin, Total protein, were analyzed according to reported methods.

3. Liver Histopathology:

After withdrawing the blood from the animals they were sacrificed and the liver was removed, fixed in 10% formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin, the studies were read by pathologist who was not aware of the treatments.

Effect on biochemical parameters:

The effect on biochemical parameters was studied against one hepatotoxin (carbon tetrachloride and Rifampicin) according to Schedule of treatment.

Blood Biochemistry

Blood samples were collected at the time as specified in table under "Schedule of treatment" just before sacrificing the animals in glass tubes from orbital sinus (retino orbital plexus). The blood was allowed to clot for 30 minutes at room temperature and then the clot was gently detached from the wall of the test tubes by a very thin glass rod. These tubes were then centrifuged for 10 minutes at 2500 rpm to obtain haemolysis free clear serum for the analysis of following parameters.[7]

Biochemical Parameters Studied[8]:

Different parameters studied in all the four models are described as below.

1) Serum Bilirubin
2) SGOT (AST)
3) SGPT (ALT)
4) ALP (Alkaline phosphate)
5) Total Protein Content

Statistical Analysis

All the values were expressed as mean ± SEM (standard error mean) for six rats. Statistical analysis was carried out by using PRISM software package (version 3.0). Statistical significance of differences between the control and experimental groups was assessed by One-way ANOVA followed by Newman-Keuls Multiple Comparison Test. The value of probability less than 5% ($P < 0.05$) was considered statically significant.

Histopathological Studies

Section of the pancreas and kidney tissues were made, stained with Haematoxylin and Eosin reagent and observed under low and high power objective for histopathological changes. The alteration and changes in the histology of pancreas and kidney were shown in vide plate. The results are shown Fig. no: 13-14.

Results and Discussion

The phytoconstituents were extracted by using different solvent of increasing polarity like Petroleum ether, chloroform, acetone, Ethanol and aqueous .The extractive values were presented in table no.1

Table No 1. Percentage yield of various extracts of whole plant of *Chromolaena odorata King & H. Robinson* .

Plant Name	Part used	Method of extraction	Percentage yield				
			Petroleum ether	Chloroform	Acetone	Ethanol	Aqueous
Chromolaena odorata King & H. Robinson	Whole plant	successive solvent extraction	2.4g	2.6g	3.1g	3.8g	4.2g

Table No. 2. Preliminary phytochemical screening

S. no.	Constituents	Petroleum Ether Extract	Chloroform Extract	Acetone Extract	Ethanol Extract	Aqueous Extract
01	Alkaloids	-	-	-	-	-
02	Sterols	-	-	-	-	-
03	Glycosides	-	-	-	+	+
04	Fixed oil and fats	+	+	-	-	-
05	Phenolic compounds	+	+	+	+	+
06	Protein and amino acids	+	+	+	+	+
07	Tannins	-	-	-	+	-
08	Gum & mucilage	-	-	-	-	-
09	Flavonoids	-	-	+	+	+
10	Carbohydrates	-	-	-	+	+
11	Saponins	-	-	-	-	-

Where,+ = Presence, − = Absence

The phytochemical evaluation shows the presence of flavonoid, phenolic compounds, tannins, glycosides, saponins, and carbohydrate in the ethanolic extact.

Table no: 3 Acute toxicity study of aqueous and alcoholic extracts of whole plant of *Chromolaena odorata(L)king&H.Rob.* based on OECD guidelines.

S. No	Number of animals	Dose in mg/kg	Report
1	3	5mg/kg	No death
2	3	50mg/kg	No death
3	3	300mg/kg	No death
4	3	2000mg/kg	No death

From the observation the alcoholic and aqueous extract of whole plant of *Chromolaena odorata(L)king&H.Rob.* were screened for acute toxicity study by OECD guidelines for determining the $LD_{50.}$ The results showed that LD50 was found to be 2000mg/kg. Therefore its ED50 was found to be 200mg/kg.

Table no. – 4 Hepatoprotective activity of *Chromolaena odorata(L)king&H.Rob.* against carbon tetrachloride ($CCl_4$1ml/kg i.p.) induced hepatotoxicity Liver weight .

Treatment	Liver weight (in gm)
Control	5.70 ± 0.06
CCl_4	6.42 ± 0.14$^{\#\#}$
Silymarin(100mg/kg)	5.76 ± 0.04**
Ethanolic extract of *Chromolaena odorata(L)king&H.Rob.* (200mg/kg)	5.82 ± 0.22**
Ethanolic extract of *Chromolaena odorata(L)king&H.Rob.* (400mg/kg)	5.78 ±0.04***
Aqueous extract of *Chromolaena odorata(L)king&H.Rob.* (200mg/kg)	5.73 ± 0.01***
Aqueous extract of *Chromolaena odorata(L)king&H.Rob.* (400mg/kg)	5.69 ± 0.06***

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

Fig no- 1 Effect of Ethanol & Aqueous extract of *Chromolaena odorata king&H.Robi.* on SGOT level in ccl4 induced Liver damage in rats.

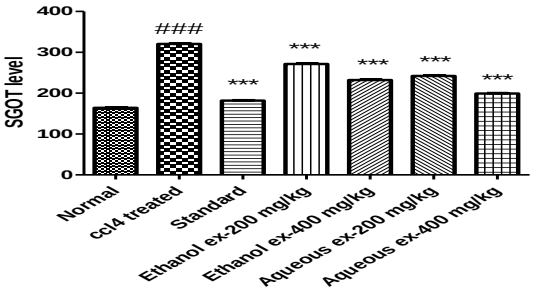


Fig no- 2 Effect of Ethanol & Aqueous extract of *Chromolaena odorata king&H.Robi.* on SGPT level in ccl4 induced Liver damage in rats.

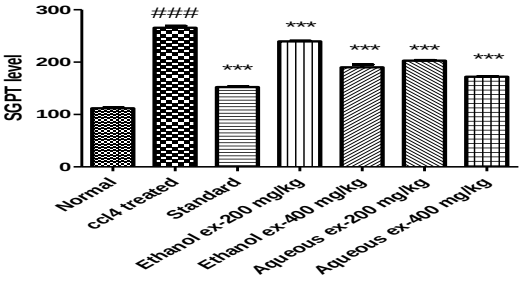


Fig no- 3 Effect of Ethanol & Aqueous extract of *Chromolaena odorata king&H.Robi.* on T.Protein level in ccl4 induced Liver damage in rats.

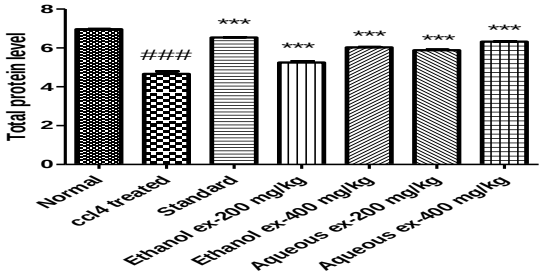


Table no-5 Hepatoprotective activity of *Chromolaena odorata(L)king&H.Rob*. against carbon tetrachloride ($CCl_4$1ml/kg i.p) induced hepatotoxicity.

Treatment	SGOT	SGPT	T.Protein	T.Billirubin	D.Billirubin	ALP
Control	164.33±1.05	111.83±1.35	6.9616±0.01	0.391±0.04	0.2733±0.07	220.33±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 treated-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 treated-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 treated-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 treated-200mg/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

Fig no- 4 Effect of Ethanol & Aqueous extract of *Chromolaena odorata king&H.Robi.* on T.Bilirubin level in ccl4 induced Liver damage in rats.

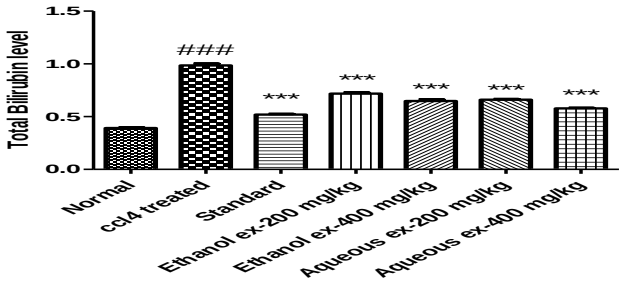


Fig no- 5 Effect of Ethanol & Aqueous extract of *Chromolaena odorata king&H.Robi.* on D.Bilirubin level in ccl4 induced Liver damage in rats.

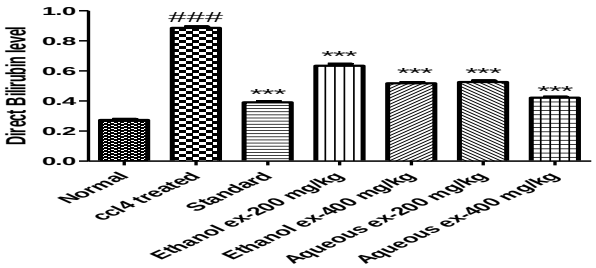


Fig no- 6 Effect of Ethanol & Aqueous extract of *Chromolaena odorata king&H.Robi.* on ALP level in ccl4 induced Liver damage in rats.

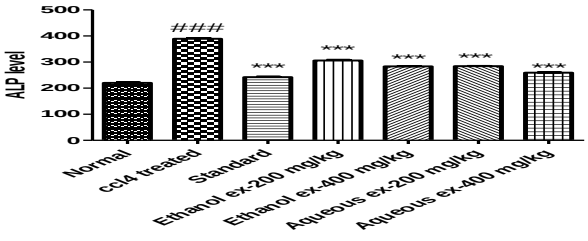


Table no. – 6 Hepatoprotective activity of *Chromolaena odorata(L)king&H.Rob.* Against Rifampicin induced hepatotoxicity Liver weight.

Treatment	Liver weight (in gm)
Control	5.43±0.12
CCl_4	6.86 ± 0.14##
Silymarin(100mg/kg)	5.81 ± 0.04**
Ethanolic extract of *Chromolaena odorata(L)king&H.Rob.* (200mg/kg)	6.01 ± 0.22**
Ethanolic extract of *Chromolaena odorata(L)king&H.Rob.* (400mg/kg)	5.46±0.04***
Aqueous extract of *Chromolaena odorata(L)king&H.Rob.* (200mg/kg)	5.78 ± 0.01***
Aqueous extract of *Chromolaena odorata(L)king&H.Rob.* (400mg/kg)	5.52 ± 0.06***

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

Fig no- 7 Effect of Ethanol & Aqueous extract of *Chromolaena odorata king&H.Robi.* on SGOT level in Rifampicin induced Liver damage in rats.

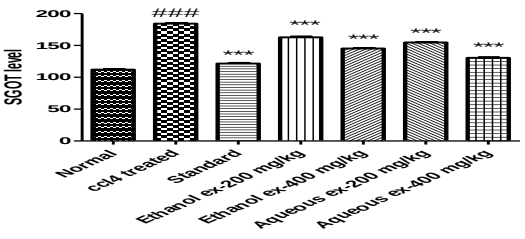


Table.no-7 Hepatoprotective activity of*Chromolaena odorata(L)king&H.Rob*. against Rifampicin (1gm/kg) induced hepatotoxicity**.**

Treatment	SGOT	SGPT	T.Protein	T.Billirubin	D.Billirubin	ALP
Control	112±0.96	80.166±0.47	6.333± 0.08	0.518± 0.01	0.416±0.01	271.666± 0.88
Ccl4 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 treated-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 treated-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***
Aqueous extract treated-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 treated-200mg/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

Fig no- 8 Effect of Ethanol & Aqueous extract of *Chromolaena odorata king&H.Robi.* on SGPT level in Rifampicin induced Liver damage in rats.

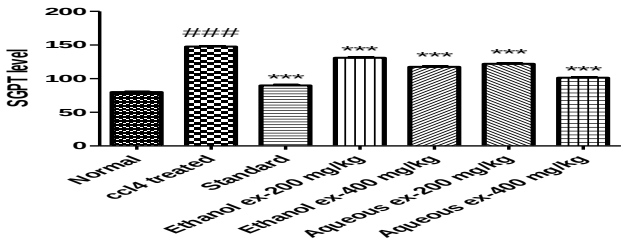


Fig no- 9 Effect of Ethanol & Aqueous extract of *Chromolaena odorata king&H.Robi.* on T.Protein level in Rifampicin induced Liver damage in rats.

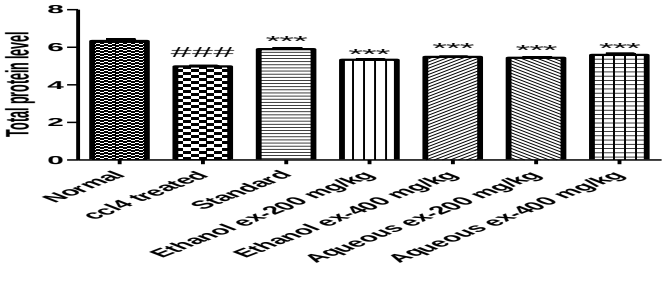


Fig no- 10 Effect of Ethanol & Aqueous extract of *Chromolaena odorata king&H.Robi.* on T.Bilirubin level in Rifampicin induced Liver damage in rats.

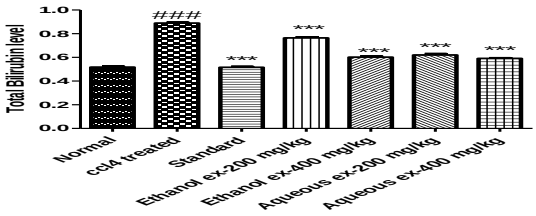


Fig no- 11 Effect of Ethanol & Aqueous extract of *Chromolaena odorata king&H.Robi.* on D. Bilirubin level in Rifampicin induced Liver damage in rats.

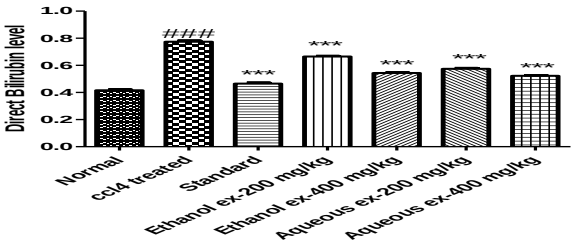
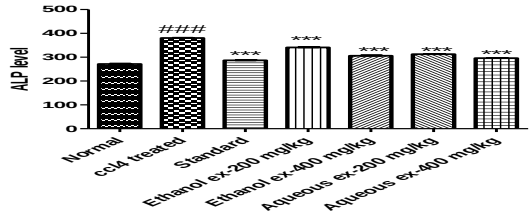


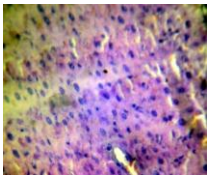
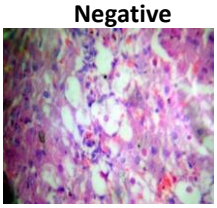
Fig no- 12 Effect of Ethanol & Aqueous extract of *Chromolaena odorata king&H.Robi.* on ALP level in Rifampicin induced Liver damage in rats.



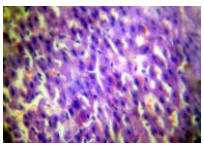
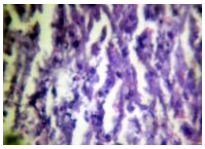
HISTOPATHOLOGICAL STUDIES OF LIVER BY CCL_4 INDUCED RATS

Fig. no: 13 HISTOPATHOLOGY OF RATS LIVER (CCL_4 induced)

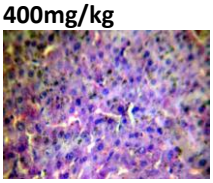
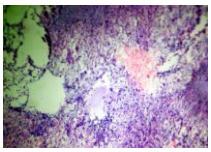
Normal **Negative**

Standard **Ethanol extract 400mg/kg**

Aqueous extract 400mg/kg **Ethanol extract 200mg/kg**

Aqueous extract 200mg/kg

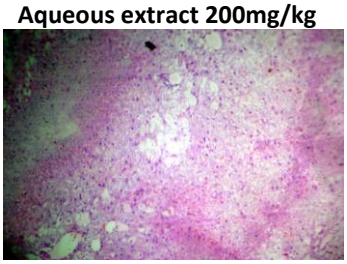
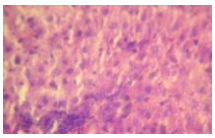
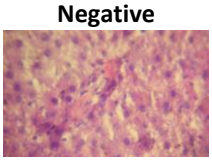
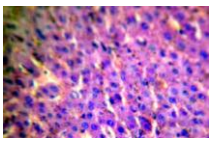
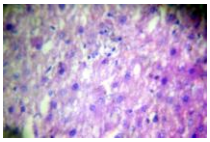


Fig. no: 14 HISTOPATHOLOGY OF RATS LIVER (Rifampicin induced)

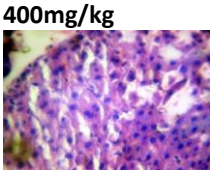
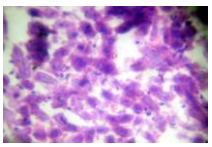
Normal **Negative**

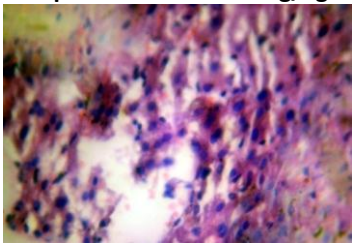
Standard control **Ethanol extract 400mg/kg**

Aqueous extract 400mg/kg **Ethanol 200mg/kg**

Aqueous extract 200mg/kg



Conclusion:

The main objective of this study was to focus on the hepatoprotective activity *Chromolaena odorata King &H. Robinson*, with special reference to its curative and protective role in carbon tetra chloride and rifampicin-induced hepatoprotctive animal model.

The phytochemical constituents were extracted by successive solvent extraction. The phytochemical constuients were identified by chemical tests and these tests showed the presence of various phytochemical constituents like carbohydrate, glycoside, fixed oils, phenolic compounds, alkaloids, flavonoids and tannins.

The alcoholic and aqueous extract of whole plant *Chromolaena odorata(L) King & H. Robinson* .showed the presence of maximum chemical constituents.

In the pharmacological studies alcoholic and aqueous extract of whole plant of *Chromolaenaodorata(L)King & H.Robinson.* showed significant heapatoprotective activity.

In last present studies revels that both the alcoholic and aqueous whole plant of *Chromolaena odorata(L) King &H. Robinson.* can be used as effective hepatoprotective activity. Further experiments are required to prove the mechanism and advantage of alcoholic and aqueous extract whole plant of *Chromolaena odorata(L) King & H.Robinson.* over other drug

ACKNOWLEDGEMENT

Authors are thankful to Prof (Dr.). B. Jaykar, Principal Vinayaka Mission's College of Pharmacy, Salem, Tamil nadu and providing all the facilities for this research project.

References

1. Khandelwal, K.R., Practical Pharmacognosy technique and experiments. 2nd ed.Pune : Nirali Prakashan, 149-56, 2000.
2. Zimmerman. M., Ethical guidelines for investigations of experimental pain in conscious animals, *Pain*, 16: 109-110, 1983.
3. Medicinal uses and pharmacological actions of five commonly used indian medicinal plants: A-mini review. Muniappan ayyanar, Savarimuthu ignacimuthu.in indian journal of pharmacology & therapeutics,471,589.
4. N ghosh . Fundamental of experimental pharmacology, 2nd edition ; 190
5. Gerhard vogel h. Drug discovery & evaluation pharmacological assay, isbn 3- 540-42396-6 springer verlay berlin heidelberg: newyork;2002.

6. Screening methods in pharmacology by N.S. Parmar, Shiv Prakash,451.
7. Kulkarni s.k., "hand book of experimental pharmacology", vallabh prakashan, delhi, 1987, 1st ed., 70.
8. Turner r.a., "screening methods in pharmacology", academic press, new york, 1972, 166, 189.

AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.