

## Research Article



## Evaluation of *In-vivo* Hepatoprotective Activity of *Mimosa rubicaulis* (Lam.) against CCl<sub>4</sub> Induced Liver Toxicity.

Ashpak M. Tamboli\*<sup>1</sup>, Kiran A. Wadkar<sup>2</sup>

1. Department of Pharmaceutical Chemistry, Sahyadri College of Pharmacy, Methwade, Sangola, Solapur, Maharashtra, India.

2. Department of Pharmacognosy, Appasaheb Birnale College of Pharmacy, Sangli, 416416, Sangli, Maharashtra, India.

\*Corresponding author's E-mail: [ashpak.tamboli@gmail.com](mailto:ashpak.tamboli@gmail.com)

Received: 16-04-2021; Revised: 24-06-2021; Accepted: 30-06-2021; Published on: 15-07-2021.

### ABSTRACT

The current study was conducted to assess the hepatoprotective activity of different extracts of *M. rubicaulis* (Lam.) at (200 and 400 mg/kg) doses b.w. against CCl<sub>4</sub> induced liver intoxication in Albino Wistar rats. Male or female Wistar rats about 150 and 200 gm body weight were selected for present study. The animals were divided into thirteen groups, six rats in each group. The extracts and Silymarin-treated animal groups significantly reduced the activities of various biochemical markers such as SGPT, SGOT, ALP, and TB which were elevated by carbon tetrachloride (CCl<sub>4</sub>) intoxication. The extracts of *M. rubicaulis* (Lam.) showed a dose dependent hepatoprotection activity. Among all the extracts, ethanolic extract produced maximum hepatoprotection (SGPT-83.15 %, SGOT-76.82%, ALP-79.33%, TB-80.00%) at a 400mg/kg dose. After CCl<sub>4</sub> administration, the levels of hepatic-antioxidant enzymes such as Glutathione (GSH) and Catalase (CAT) were reduced, whereas the level of hepatic lipid peroxidation (-LPO) increased. These hepatic antioxidant enzymes were also restored to normal levels by extracts and Silymarin treatment. The results of the present investigation indicate that all the extracts of *M. rubicaulis* (Lam.) possess hepatoprotective activity which may be due to the presence of various chemical constituents.

**Keywords:** *M. rubicaulis* (Lam.), Histopathology, Silymarin, CCl<sub>4</sub>, SGPT, SGOT, ALP, TB.

### QUICK RESPONSE CODE →

#### DOI:

10.47583/ijpsrr.2021.v69i01.018



DOI link: <http://dx.doi.org/10.47583/ijpsrr.2021.v69i01.018>

### INTRODUCTION

In vertebrates, the liver is the fundamental organ involve in the vital processes of metabolism and excretion. Various xenobiotic, primarily viral infections, drugs and alcohol can cause hepatic inflammation, disrupts normal hepatic architecture, and results in liver dysfunction, liver failure, or even death. Oxidative stress and immunological liver injuries are the common pathological processes of many liver disorders such as viral and autoimmune hepatitis, and liver cirrhosis<sup>1</sup>. Hepatic fibrosis was evaluated by measurement of liver enzymes. Changes in liver fibrosis and an increase in hepatic enzyme biomarkers were also observed<sup>2</sup>. Liver tissue has also been studied. Histopathological and western blotting tests for the finding of apoptotic gene expression, which is important in hepatotoxicity-induced hepatotoxicity, were performed<sup>3</sup>. In place of synthetic chemical compounds, there is a need for safe protective agents<sup>4</sup>. As a result, there is a strong interest in developing new plant-derived drugs to treat liver diseases. Herbal drugs derived from medicinal plants, according to WHO, are an important part of the traditional medicine system<sup>5</sup>. Recently, in developing countries the utilization of medicinal plants has

gained prominence and popularity because of their safety, efficacy and cost effectiveness<sup>6</sup>. Traditionally *M. rubicaulis* (Lam.) had higher value in treating of various diseases like laxative, leucoderma, leprosy, chronic diarrhea, rheumatism, and anti-diabetic. Treatment of snake bites, Anti-fungal agent. Used for cuts & wounds (had wound healing property). As we know that generally natural origin compounds had fewer side effects and less toxic when compared with synthetic compounds<sup>7</sup>.

### MATERIALS AND METHODS

#### Drugs and chemicals

Enzymatic diagnostic kits were procured from Agape Diagnostics Ltd., Dist. Ernakulam, Kerala, India. CCl<sub>4</sub> and Silymarin were purchased from Research Laboratories, Mumbai. The other chemicals used in this study were all of high analytical quality.

#### Description of plant collection area and preparation of extracts

The plant material was collected from Wildlife Institute of India, Dehradun. The collected plant material leaves were identified and authenticated at BSI, Northern Regional Centre, 192, Kaulagarh Road, Dehradun.

The leaves were dried in the shade at 26 ± 2°C with a mortar and pestle, the dried plant material (500 g) was ground into a powder and then passed through a mesh-sized sieve of 0.3 mm. It was then extracted using various solvents such as PE, CHCl<sub>3</sub>, EA, ETH, and AQ. The extract was refrigerated until it could be used again<sup>8</sup>.



### Selection and procurement of animals

The experiment was performed with the approval of Institutional Animal Ethics Committee (IAEC) following guidelines of CPCSEA. (Approval number: IAEC/ABCP/18/2020-21). The male or female Wistar rats with 150-200 g body weight were selected for study by using CCl<sub>4</sub> induced hepatotoxicity model.

### Housing facilities

The animal selected for experimental purpose maintained with standard procedure of laboratory condition in animal house of A.B.C.P Sangli approved by the Committee for the Purpose of Controlling and Supervision on Experiments on Animal (CPCSEA). All animal was placed in 12 hrs. Light /dark cycle with maintained temperature condition (22±2°C), feed with commercial pellet diet and water *ad libitum*. All animal under experiment were placed in the maintained animal house for at least 5 days before the start of experiment. The experimental protocol for the study was followed according to the norms of Institutional Animal Ethics Committee (IAEC).

### Selection of the dose

CCl<sub>4</sub>: 1ml /kg

Silymarin: 100 mg/kg

Route: Oral.

Sex: Male /female

Model: CCl<sub>4</sub> induced hepatotoxicity.

### Experimental design

Male or female Wistar rats about 150 to 200 gm body weight were selected for present study. The animals was divided into thirteen groups, six rats in each and subjected to the following treatments<sup>9</sup>.

### Acute toxicity testing

The acute toxicity investigation was carried out according to (OECD) guideline 423 for the analysis of substances for acute oral toxicity. A study of the oral acute toxicity for *M. rubicaulis* (Lam.) three animals are used for each step. The starting dose is chosen from one of four fixed levels, which are 5, 50, 300, and 2000 mg/kg body weight.

The test extract was dissolved in DMSO and administered orally. No mortality was observed even at 2000 mg/kg for extracts of *M. rubicaulis* (Lam.) all of the animals were discovered to be healthy and there were no gross behavioral changes like body weight aberrations, no gross findings, till the end of two weeks of observation. From the study, 1/5<sup>th</sup> and 1/10<sup>th</sup> of 2000 mg/kg dose was selected for further pharmacological screenings<sup>10</sup>. Ultimately, 200 and 400 mg/ kg b.w. concentration of the leaf extract was selected as the highest dose for the *in-vivo* hepatoprotective studies<sup>11</sup>.

### Preparation of animal model for *in-vivo* hepatoprotective activity.

Group I - Served as Normal, received saline 1 ml/kg p.o. for 10 days.

Group II - Served as Disease control, received (CCl<sub>4</sub> in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. on 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup> days.

Group III - Served as Standard, received Silymarin 100 mg/kg orally daily for ten days and (CCl<sub>4</sub> in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. on 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup> days.

Group IV - Served as Aqueous extracts of leaves of *M. rubicaulis* (Lam.) 200 mg/kg p.o., for ten days and (CCl<sub>4</sub> in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. on 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup> days.

Group V- Served as Aqueous extracts of leaves of *M. rubicaulis* (Lam.) 400 mg/kg p.o., for ten days and (CCl<sub>4</sub> in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. on 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup> days.

Group VI - Served as Ethanolic extracts of leaves of *M. rubicaulis* (Lam.) 200 mg/kg p.o., for ten days and (CCl<sub>4</sub> in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. on 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup> days.

Group VII- Served as Ethanolic extracts of leaves of *M. rubicaulis* (Lam.) 400mg/kg p.o., for ten days and (CCl<sub>4</sub> in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. on 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup> days.

Group VIII - Served as Ethyl acetate extracts of leaves of *M. rubicaulis* (Lam.) 200 mg/kg p.o., for ten days and (CCl<sub>4</sub> in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. on 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup> days.

Group IX- Served as Ethyl acetate extracts of leaves of *M. rubicaulis* (Lam.) 400mg/kg p.o., for ten days and (CCl<sub>4</sub> in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. on 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup> days.

Group X - Served as Petroleum ether extracts of leaves of *M. rubicaulis* (Lam.) 200 mg/kg p.o., for ten days and (CCl<sub>4</sub> in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. on 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup> days.

Group XI- Served as Petroleum ether extracts of leaves of *M. rubicaulis* (Lam.) 400mg/kg p.o., for ten days and (CCl<sub>4</sub> in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. on 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup> days.

Group XII - Served as Chloroform extracts of leaves of *M. rubicaulis* (Lam.) 200mg/kg p.o., for ten days and (CCl<sub>4</sub> in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. on 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup> days.

Group XIII- Served as Chloroform extracts of leaves of *M. rubicaulis* (Lam.) 400mg/kg p.o., for ten days and (CCl<sub>4</sub> in olive oil 1:1 v/v) in dose of 1 ml/kg p.o. on 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup> days.

On 10<sup>th</sup> day animal will be sacrificed by cervical decapitation and biochemical estimation of serum enzyme level will be done<sup>12, 13</sup>.

### ***In-vivo* hepatoprotective activity against hepatotoxicity caused by carbon tetrachloride**

For different extracts *In-vivo* hepatoprotective activity of *M. rubicaulis* (Lam.) was tested in contrast to carbon tetrachloride (CCl<sub>4</sub>) induced hepatotoxicity in the current study by measuring biochemical enzymes (SGPT, SGOT, ALP and TB) a rise in the enzymes. The concentrations of these biochemical parameters are a sensitive indicator of hepatic damage. The animals in both the control and test groups were given a dose of 100 mg/kg of Silymarin and 200, 400 mg/kg doses of different extracts of *M. rubicaulis* (Lam.) for ten days. On 10<sup>th</sup> day, 1 h after treatment with standard drug and selected plant extracts, the animals were intoxicated with CCl<sub>4</sub> in olive oil (1:1 v/v of CCl<sub>4</sub> /kg, p.o.). Serum were separated by centrifugation at 37°C and used for estimation of various biochemical markers. Biochemical markers such as SGPT, SGOT, ALP, and TB were determined using commercial reagent kits and an auto analyzer. (RM4000, Biochemical systems International, Italy)<sup>14, 15, 16</sup>.

### **Hepatic antioxidant activities**

#### ***Lipid peroxidation (LPO)***

Animal's liver tissue (900 mg) was washed in saline solution and dripping in filter paper. The tissues were mixed in (0.15 M) 3.0 ml of Tris-HCl buffer (pH 7.4) and centrifuged for 1 hour at 3000 rpm at 4°C. The upper supernatant were collected and analysed for LPO. LPO was find out by measuring the levels of malondialdehyde (MDA) produced, which was represented as nM/g liver tissue homogenate<sup>17</sup>.

### ***Glutathione (GSH) content***

In 0.15 M Tris-HCl buffer (pH 7.4), homogenized liver (30% m/v) was prepared and tri-chloroacetic acid was used to precipitate proteins. The samples centrifuged for 1 hour at 15000 rpm at 4 °C. The upper supernatant was used to determine the GSH content, which was expressed in M/mg liver tissue homogenate<sup>18</sup>.

### ***Catalase (CAT) activity***

Admixture of 900 mg of liver tissue in M/15 buffer (pH 7.0) on ice was followed separation by centrifugation for 1 hour at 3000 rpm at 4°C, with the resulting upper supernatant collected and CAT activity measured. A 10 L aliquant of the collected liver upper supernatant was placed in a cuvette, and the reaction was started by adding freshly prepared 30 mM H<sub>2</sub>O<sub>2</sub> in Fifty mM phosphate buffer having pH 7.0. The rate of H<sub>2</sub>O<sub>2</sub> breakdown was measured by the absorbance at 240 nm during 120s using a UV-Vis spectrophotometer. CAT activity were denoted in U/mg liver tissue homogenate. 1U of CAT activity was defined as the amount of extracts needed to decompose 1 μmol of H<sub>2</sub>O<sub>2</sub> per min<sup>18</sup>.

### **Statistical analysis**

Results were shown as (Mean ± SEM.) (n=6), Data was analyzed using one-way ANOVA followed by Dunnet's multiple comparison test by using Graph Pad Prism 8.4.3

### **Histopathological examination by light microscopy**

Liver tissues from the respective groups have been preserved in buffered formalin. Following dehydration, tissue samples were embedded in paraffin to form microtome blocks. Tissues were cut 4–5 mm thick with a microtome, stained with Hematoxylin-Eosin (H&E), and examined at 40X under a light microscope (DIALUX 20 EB)<sup>19</sup>.

## **RESULTS AND DISCUSSION**

**Table 1:** Effect of different extracts of leaves of *M. rubicaulis* (Lam.) on serum level of the CCl<sub>4</sub>-induced hepatotoxicity in rats.

Group	Treatment	SGPT (U/L)	SGOT (U/L)	ALP (U/L)	TB (mg/dL)
I.	NC (d.w 1ml/kg p.o.,)	73.8±1.080	76.53± 0.62	208.01± 2.51	0.49± 0.03
II.	CCl <sub>4</sub> + olive oil (1 ml/kg p.o.)	166.89±1.71	264.79±2.09	329.08±1.01	2.94± 0.01
III.	Silymarin (100mg/kg)	78.21±1.30***	79.15±1.34***	217.92 ±2.27***	0.54± 0.01***
IV.	PE extract (200mg/kg)	136.98±2.06	184.56±2.12	293.69±1.57	1.98±0.01
V.	PE extract(400mg/kg)	126.23±1.59	160.23 ±1.03	281.32 ±1.33	1.90 ±0.01
VI.	CHCl <sub>3</sub> extract(200mg/kg)	119.56±0.86	158.93±0.51	275.89±1.30	1.76±0.02
VII.	CHCl <sub>3</sub> extract(400mg/kg)	108.1±0.87*	149.28±1.22*	264.14± 1.23*	1.65±0.04*
VIII.	EA extract(200mg/kg)	105.98±1.09*	141.65±1.69*	256.91±1.34*	1.3±0.01*
IX.	EA extract(400mg/kg)	101.72±1.00**	135.63±1.19**	248.26±1.55**	1.24±0.045**
X.	ETH extract(200mg/kg)	100.21±1.21**	134.65±1.55**	246.56±1.10**	1.22±0.01**
XI.	ETH extract(400mg/kg)	89.51 ±1.84**	120.16 ±1.69**	233.03 ± 0.44**	0.98±0.03**
XII.	AQ extract(200mg/kg)	122.56±1.75*	156.32±1.11*	278.95±1.92*	1.97±0.01*
XIII.	AQ extract(400mg/kg)	115.56±1.74*	142.6 ± 0.86*	265.18 ±1.07*	1.89±0.01*

Values are expressed as Mean ± SEM (n=6), Data was analyzed using one-way ANOVA followed by Dunnet's multiple comparison test by using Graph Pad Prism 8.4.3 for Windows. CCl<sub>4</sub> induced vs. Treated Groups: (\* p<0.05, \*\* p<0.01, \*\*\* p<0.001).



As shown in Table 1, there has been a significant rise in the levels observed SGPT (73.8±1.0805 to 166.89±1.7133), SGOT (76.53±0.6238 to 264.79±2.0919), ALP (208.01±2.5101 to 329.08±1.0168) & TB (0.49±0.0324 to 2.94±0.0115) in the toxicant group which are treated with (1ml/kg p.o.) CCl<sub>4</sub> indicative of acute hepatocellular

damage and biliary obstruction. Pretreatment with *M. rubicaulis* (Lam.) extracts and Silymarin in test groups and standard group respectively, daily for ten days showed significant (\*\*p<0.01) protective effect when compared to toxicant group against CCl<sub>4</sub> induced hepatotoxicity.

**Table 2:** Percentage protection in Biochemical Markers due to treatment with different leaves extracts of *Mimosa rubicaulis* (Lam.)

Sr. No.	Treatment	Percentage protection			
		SGPT	SGOT	ALP	TB
1	Silymarin (100mg/kg)	95.26%	98.60 %	91.81 %	97.96 %
2	PE extract (200mg/kg)	32.13%	42.61%	29.23%	39.18%
3	PE extract(400mg/kg)	43.67%	55.54 %	39.44 %	42.44%
4	CHCl <sub>3</sub> extract (200mg/kg)	50.84%	56.23%	43.93%	48.16%
5	CHCl <sub>3</sub> extract (400mg/kg)	63.25%	61.35 %	52.77 %	52.65 %
6	EA extract(200mg/kg)	65.43%	65.40%	59.61%	66.93%
7	EA extract(400mg/kg)	70.00%	68.60 %	66.75 %	69.38 %
8	ETH extract(200mg/kg)	71.62%	69.12%	68.15%	70.20%
9	ETH extract(400mg/kg)	83.51 %	76.82 %	79.33%	80.00 %
10	AQ extract(200mg/kg)	47.62%	57.61%	41.40%	39.59%
11	AQ extract(400mg/kg)	55.14 %	64.90 %	52.77%	42.86 %

#### Abbreviations

**SGOT:** Serum Glutamic Oxaloacetic Transaminase; **SGPT:** Serum Glutamic Pyruvic Transaminase; **ALP:** Alkaline

Phosphatase; **TB:** Total Bilirubin. ***M. rubicaulis:*** *Mimosa rubicaulis* (Lam.), **NC:** Normal Control, **PE:** Petroleum ether, **CHCl<sub>3</sub>:** Chloroform, **EA:** Ethyl acetate, **ETH:** Ethanol, **AQ:** Aqueous, **CCl<sub>4</sub>:** Carbon tetrachloride.

#### *In vivo* Hepatoprotective activity of test substance is calculated as follows

$$\text{Percentage protection} = \frac{[\text{Toxicant group}] - [\text{Drug + Toxicant group}]}{[\text{Toxicant group}] - [\text{Before Treatment}]} \times 100$$

As Shown in Table 2 it was observed that the percentage protection in Silymarin pretreated group (100 mg/kg b.w) the biochemical parameters found to be SGPT (95.26%), SGOT (98.60%), ALKP (91.81%), and TB (97.96 %).

Whereas highly significant percentage protection was found by *M. rubicaulis* (Lam.) ethanolic extract (200, 400 mg/kg b.w) the biochemical parameters in the pretreated group were discovered to be SGPT (71.62%, 83.51%), SGOT (69.12%, 76.82%), ALKP (68.15%, 79.33%), TB (70.20%, 80.00%).

Whereas *M. rubicaulis* (Lam.) Petroleum ether (200mg/kg, 400mg/kg b.w.) extract showed very less significant Percentage Protection when compared to other extracts, biological parameters are as follows SGPT (32.13%, 43.67%), SGOT (42.61%, 55.54 %), ALKP (29.23%, 39.44%), TB (39.18%, 42.44%).

Chloroform extract of *M. rubicaulis* (Lam.) (200, 400mg/kg b.w.) pretreated group exhibited significant (p<0.01)

percentage protection in biochemical parameters, SGPT (50.84 %, 63.25%), SGOT (56.23%, 61.35%), ALKP (43.93%, 52.77%), TB (48.16%, 52.65%).

Ethyl acetate extract of *M. rubicaulis* (Lam.) (200, 400mg/kg b.w.) pretreated group exhibited moderate significant (p<0.01) Percentage Protection in biochemical parameters, SGPT (65.43%, 70.00%), SGOT (65.40%, 68.60 %), ALKP (59.61%, 66.75 %), TB (66.93%, 69.38 %).

Aqueous extract of *M. rubicaulis* (Lam.) (200, 400mg/kg b.w.) pretreated group exhibited less significant (p<0.01) percentage protection in biochemical parameters, SGPT (47.62%, 55.14 %), SGOT (57.61%, 64.90%), ALKP (41.40 %, 52.77 %), TB (39.59%, 42.86 %).

The decreasing order of activity of extract is:

Ethanol > Ethyl acetate > Chloroform > Aqueous > Petroleum ether.

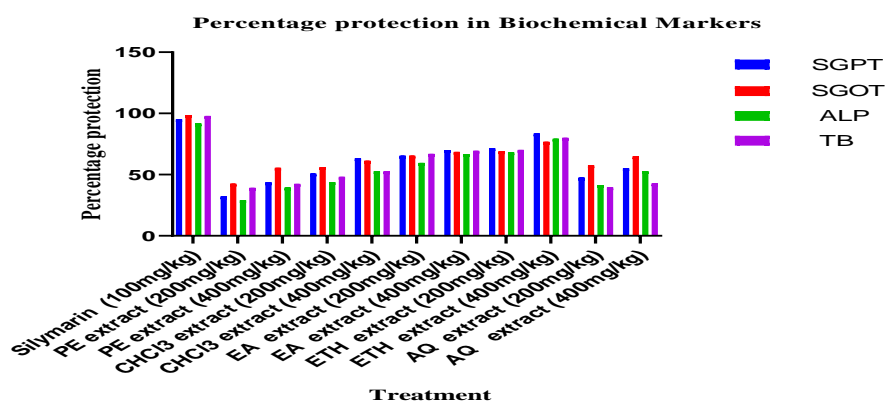


Figure 1: Percentage protection in Biochemical Markers.

Table 3: Effect of different leaves extracts of *M. rubicaulis* (Lam.) on Antioxidant enzymes in CCl<sub>4</sub> induced hepatotoxicity in rats.

Sr. No.	Treatment	GSH $\mu\text{g}/\text{min}/\text{mg}$	CAT $\mu\text{m}/\text{min}/\text{mg}$	MDA $\text{Mm}/100\text{g}$
1.	Normal Control (d.w 1ml/kg p.o.,)	15.26 $\pm$ 1.02	34.18 $\pm$ 1.41	118.72 $\pm$ 1.88
2.	CCl <sub>4</sub> + olive oil (1 ml/kg p.o.)	12.62 $\pm$ 1.41	16.23 $\pm$ 1.55	186.34 $\pm$ 1.43
3.	Silymarin (100mg/kg)	14.46 $\pm$ 1.01***	22.31 $\pm$ 1.57***	146.33 $\pm$ 0.83***
4.	PE extract(200mg/kg)	8.45 $\pm$ 1.18*	17.30 $\pm$ 1.75*	184.20 $\pm$ 1.08*
5.	PE extract(400mg/kg)	9.27 $\pm$ 1.03*	18.41 $\pm$ 1.53*	178.74 $\pm$ 1.21*
6.	CHCl <sub>3</sub> extract(200mg/kg)	9.78 $\pm$ 1.47*	18.83 $\pm$ 1.59*	180.37 $\pm$ 1.20*
7.	CHCl <sub>3</sub> extract(400mg/kg)	10.37 $\pm$ 1.18*	19.24 $\pm$ 1.36*	176.45 $\pm$ 1.63*
8.	EA extract (200mg/kg)	10.18 $\pm$ 1.26*	18.69 $\pm$ 1.59*	174.04 $\pm$ 1.57*
9.	EA extract (400mg/kg)	10.67 $\pm$ 1.27**	19.45 $\pm$ 1.72**	169.47 $\pm$ 1.48**
10.	ETH extract (200mg/kg)	12.73 $\pm$ 1.32*	20.93 $\pm$ 1.24*	163.28 $\pm$ 0.92*
11.	ETH extract (400mg/kg)	13.43 $\pm$ 1.57**	21.73 $\pm$ 1.08**	152.14 $\pm$ 1.30**
12.	AQ extract (200mg/kg)	8.22 $\pm$ 0.78*	17.54 $\pm$ 1.18*	185.21 $\pm$ 1.13*
13.	AQ extract (400mg/kg)	9.18 $\pm$ 0.94*	18.16 $\pm$ 1.36*	179.28 $\pm$ 1.13*

Values are expressed as Mean  $\pm$  SEM (n=6), Data was analyzed using one-way ANOVA followed by Dunnett’s multiple comparison test by using Graph Pad Prism 8.4.3 for Windows. Disease Control vs. Treated Groups: (\* p<0.05, \*\* p<0.01, \*\*\* p<0.001).

LP- Lipid peroxidation (mmoles of malondialdehyde (MDA) formed /mg, GSH- Glutathione reductase ( $\mu\text{g}$  of glutathione consumed/  $\mu\text{g}/\text{min}/\text{mg}$ ), CAT-Catalase (unit/mm/mg protein)

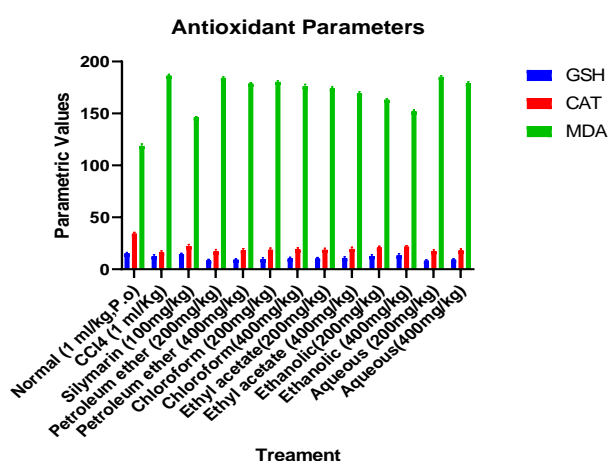


Figure 2: Effect of leaves extracts of *M. rubicaulis* (Lam.) on Antioxidant Parameters.

Effects of extracts on antioxidant enzymes (LPO, GSH and CAT)

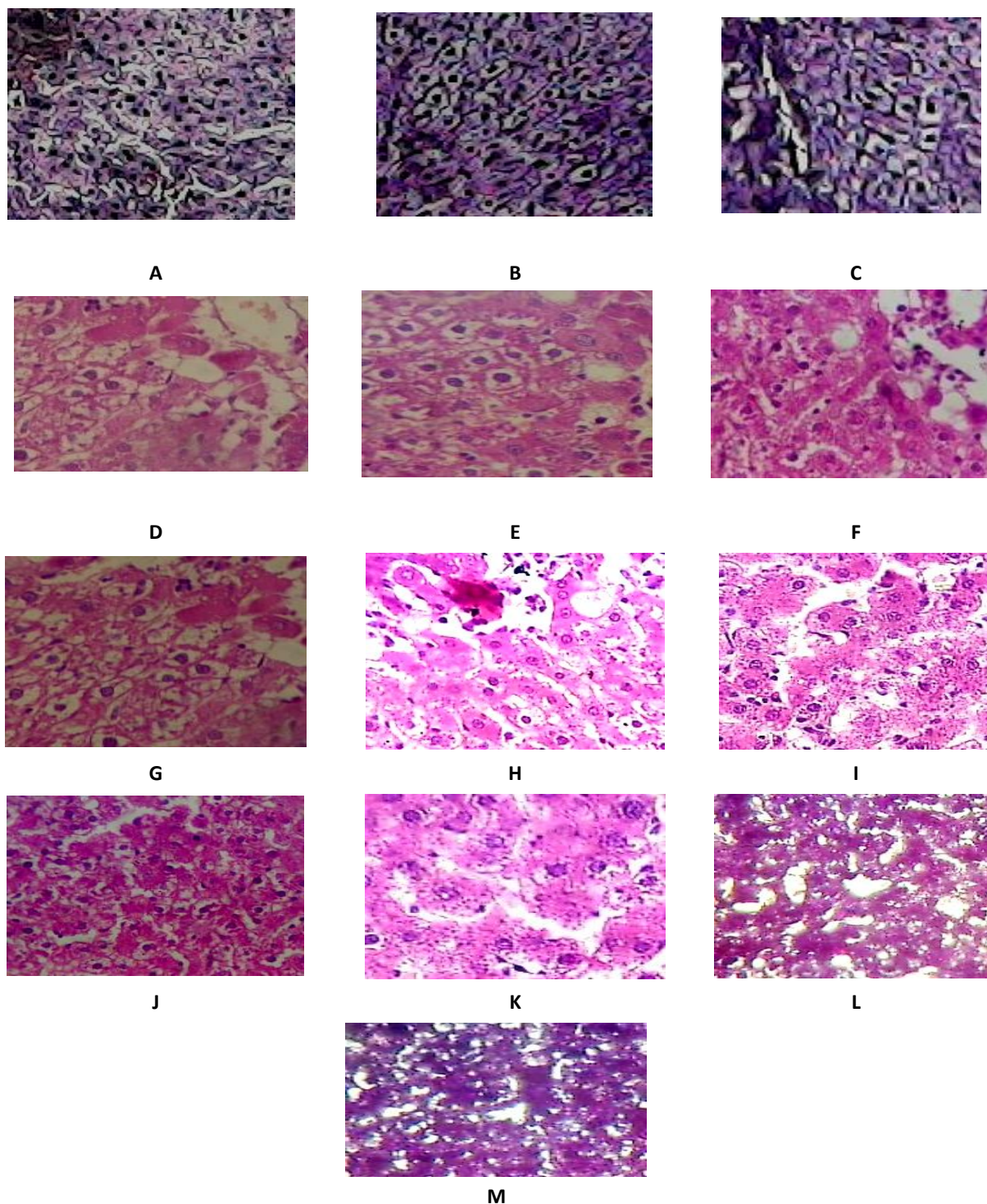
The result showed that there was an increase in level of LPO in CCl<sub>4</sub> intoxicated group. Treatment of the animals with extracts at doses of (200 and 400 mg/kg), p.o., and Silymarin (100 mg/kg, p.o.) significantly (\*\*p < 0.01) reduce the level of LPO compared to CCl<sub>4</sub> intoxicated group. The levels of GSH and CAT decreased in CCl<sub>4</sub> intoxicated group when compared to control group but after treatment of animals with extracts (200 and 400 mg/kg, p.o.) or Silymarin (100 mg/kg, p.o.) there was a significant rise in levels of GSH and CAT compared to CCl<sub>4</sub> intoxicated group<sup>20</sup>.

As shown in table 3 among all the extracts, the ethanolic leaves extracts of *M. rubicaulis* (Lam.) at 400 mg/kg showed significant (\*\*p<0.01) CCl<sub>4</sub>-protective effect against

induced toxicity and increases the GSH level ( $13.43 \pm 1.57$ ) and at 200 mg/kg ( $12.73 \pm 1.32$ ).

The ethanolic extracts of leaves of *M. rubicaulis* (Lam.) at 400 mg/kg showed significant (\*\* $p < 0.01$ ) CCl<sub>4</sub>-protective effect against induced toxicity and increases the Catalase level ( $21.73 \pm 1.08^{**}$ ) and at 200 mg/kg ( $20.93 \pm 1.24^*$ ) also increases the Catalase level (\*\* $p < 0.01$ ).

The ethanolic extracts of leaves of *M. rubicaulis* (Lam.) at 400 mg/kg showed significant (\*\* $p < 0.01$ ) CCl<sub>4</sub>-protective effect against induced toxicity and reduced MDA level ( $152.14 \pm 1.30^{**}$ ) and at 200 mg/kg ( $163.28 \pm 0.92^*$ ) also reduced the MDA level (\*\* $p < 0.01$ ).



**Figure: 3** Photomicrographs of rat liver treated with different leaves extract, Normal distilled water, induced CCl<sub>4</sub> and Standard (Silymarin).

**Notes:** (A) Negative control (D.W 1ml/kg only), (B) Toxicant control (administered with CCl<sub>4</sub> only), (C) Positive control (Silymarin (100mg/kg) + CCl<sub>4</sub>), (D) PE 200+CCl<sub>4</sub>, (E) PE 400+CCl<sub>4</sub>, (F) CHCl<sub>3</sub> 200 + CCl<sub>4</sub>, (G) CHCl<sub>3</sub> 400 + CCl<sub>4</sub>, (H) EA 200+CCl<sub>4</sub>, (I) EA 400+CCl<sub>4</sub>, (J) ETH 200+CCl<sub>4</sub>, (K) ETH 400+CCl<sub>4</sub>, (L) AQ 200 + CCl<sub>4</sub>, (M) AQ 400 + CCl<sub>4</sub>.

### Histopathological observations

(A) Displayed Normal hepatic lobule structures are visible. (B) Displayed Massive fatty changes are observed, as well as necrosis, ballooning, degeneration, and the loss of cellular boundaries. (C) Liver sections revealed only mild sinusoidal dilatation in the centri-zonal areas. (D) Mild hepatotoxicity with typical lobular arrangement and minimal centrilobular necrosis. (E) A slight sinusoidal dilation with a prominent centrilobular fatty change. No evidence of periportal inflammation. (F) The portal triads exhibit mild peri-portal lymphocyte inflammation. (G) Moderate sinusoidal dilatation in the vicinity of the central vein. Scattered hepatocytes show fine vacuolar change as well. (H) A liver abscess is typically solitary, with an irregular cell wall and (ballooning) necrosis. (I) Hepatocytes has moderate cytoplasm and moderately enlarged pleomorphic and hyperchromatic nuclei. The portal triads have mild peri-portal lymphocyte inflammation, and the central veins are normal. (J) Normal cell architecture with irregular necrotic ballooning cell and toxicant-protective effect. (K) Significantly reduced peri-portal inflammation and sinusoidal dilatation. The compound also demonstrated good recovery with no necrosis, and both the central vein and the portal vein were clearly visible. (L) The majority of hepatocytes are enlarged with large lipid vacuoles, peripherally displaced nuclei, and necrosis. (M) Normal architecture with mild necrosis.

The goal of this study was to discover *M. rubicaulis* (Lam.) hepatoprotective properties. In biological systems, abnormal free radical production causes cell necrosis and tissue damage<sup>21</sup>. CCl<sub>4</sub> is one of the most commonly used hepatotoxins in the experimental study of liver diseases, and it is an excellent experimental model for studying acute hepatic injury<sup>22</sup>. CCl<sub>4</sub> induced liver damage is mainly attributed to the toxic metabolites, mainly trichloromethyl radical, for the initiation of CCl<sub>4</sub> dependent lipid peroxidation. These free radicals can then interact with O<sub>2</sub> to form trichloromethyl peroxy radicals, which can then attack lipids on the endoplasmic reticulum membrane, causing necrosis and cell death<sup>23</sup>. In the *in-vivo* hepatoprotective studies we conducted, animals were administered with CCl<sub>4</sub>, which produces •CCl<sub>3</sub> and •OCCl<sub>3</sub> free radicals. These radicals attack membrane lipids in the endoplasmic reticulum, thereby raising the level of SGOT, SGPT, ALP, TB This may be due to release of these enzymes from the cytoplasm, into the blood stream rapidly after rupture of the plasma membrane and cellular damage. Anti-oxidant parameters from liver tissue like Catalase (CAT), Malondialdehyde (MDA) and reduced glutathione (GSH) decreasing metabolic functions (synthesis of proteins and storage of cholesterol and sugars) in blood serum.

The animals of group II, which received only CCl<sub>4</sub>, were found to develop significant hepatic damage as was observed from elevated levels of SGPT, SGOT, ALP and TB as shown in table-1. Pretreatment with Silymarine Group-III (Standard group) showed significant (p<0.01) protective

effect contrary to CCl<sub>4</sub> induced hepatotoxicity when compared to toxicant group. The treatment with different leaves extract at dose of 200 and 400 mg/kg, for 10 days resulted in groups IV to XIII significant (p<0.05) reduction of CCl<sub>4</sub>-induced elevation of serum enzyme markers and Percentage decrease in Biochemical parameters in extracts (Shown in Table: 1 and 2). From the above observation, ethanolic leaf extract of *M. rubicaulis* (Lam.) significantly reduced the levels of these marker enzymes and decreased the percentage of biochemical parameters in CCl<sub>4</sub> treated rats. This means that the extract tends to prevent damage to the liver, suppresses enzyme leakage through cell membranes, maintains the integrity of plasma membranes, and thus restores enzyme levels this is consistent with the widely held belief that serum transaminase levels return to normal with hepatic parenchymal healing and hepatocyte regeneration<sup>24</sup>.

Animals treated with *M. rubicaulis* (Lam.) leaves extracts have lower levels of enzymatic properties, which could be due to hepatic cell membrane stabilization and inhibition of lipid peroxidation<sup>25</sup>. The defensive nature of the *M. rubicaulis* (Lam.) leaf extracts could be due to increased antioxidant properties due to the presence of unique secondary metabolites such as flavonoids, polyphenols, and alkaloids. These compounds are known to have antioxidant properties and can be used to treat liver diseases<sup>26, 27, 28</sup>.

*M. rubicaulis* (Lam.) hepatoprotection is provided at various dose levels, but 400 mg/kg is more effective and comparable to the standard drug Silymarin (100 mg/kg). The histopathological examination of liver sections confirmed that CCl<sub>4</sub> treatment caused damage to normal liver cellular architecture. However, liver sections from rats treated with *M. rubicaulis* (Lam.) revealed that the reduction in histopathological scores as well as cellular damage in ethanolic extract were proven by biochemical and histopathological analysis. (shown in Fig: 3.)

The MR has shown dose dependent activity among which at the dose level of 400 mg/kg, shows significant activity (shown in Figure. 1)

In this study, we also looked at how *M. rubicaulis* (Lam.) affected hepatic antioxidant enzymes like MDA, GSH and CAT whose levels were depleted due to oxidative stress caused by CCl<sub>4</sub>. Excessive ROS production is inhibited, which is an important mechanism in the protection against CCl<sub>4</sub>-induced liver damage. MDA is widely used as a lipid peroxidation marker and a key parameter in the status of oxidative stress<sup>29</sup>. MDA levels in the liver rise under the enrichment of oxidative stress in a rodent model<sup>30</sup>. In our study, rats treated with CCl<sub>4</sub> had a significant increase in MDA levels when compared to the control group. Treatment with ethanolic extracts of leaves of *M. rubicaulis* (Lam.) reduced the CCl<sub>4</sub>-induced hepatic MDA elevation. Thus ethanolic extracts of leaves of In terms of preventing lipid peroxide formation and blocking the oxidative chain reaction, *M. rubicaulis* (Lam.) protects against CCl<sub>4</sub>-induced liver damage. Small molecules like



GSH and antioxidant enzymes are also important components of the body's defence system<sup>31</sup>. CCl<sub>4</sub> administration to rats reduced the activities of hepatic antioxidants such as GSH and CAT, as previously reported<sup>32</sup>. Our findings show that treatment with ethanolic extracts of *M. rubicaulis* (Lam.) leaves improved the rat liver's anti-oxidative defence system, as evidenced by the restoration of enzymatic activities. (shown in Figure 2 and Table 3)

## CONCLUSIONS

We conclude that the *M. rubicaulis* (Lam.) leaves collected from Wildlife Institute of India, Dehradun, has protective activity against carbon tetrachloride-induced hepatotoxicity in Albino Wistar rats. By comparative study of different extracts indicate that the ethanolic leaves extract of *M. rubicaulis* (Lam.) exhibits hepatoprotective activity by reducing elevated levels of serum biochemical enzymes and significant reduction in the MDA and elevation in CAT and GSH when compared with CCl<sub>4</sub>-treated rats. More research into the extract's active components and the underlying molecular mechanisms this hepatoprotection is highly recommended. This research has resulted in a thorough examination of the plant untapped potential as a traditional medicine that provides protection against various liver parameters, as demonstrated by biochemical serum enzyme levels and histopathological analysis. The dose-dependent activity of *M. rubicaulis* (Lam.) showed increased activity at a dose level of 400 mg/kg which is comparable with the control and standard groups.

**Acknowledgments:** The authors thank the authorities of Wildlife Institute of India Dehradun and BSI, Northern Regional Centre, 192, Kaulagarh Road, Dehradun for providing plant material for work.

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**Source of Support:** The author(s) received no financial support for the research, authorship, and/or publication of this article.

**Conflict of Interest:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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