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# Evaluation of hepatoprotective potential of *rubia cordifolia* in experimentally (carbon tetrachloride) induced hepatotoxicity in albino rats

**Mr. Aboobecker Siddique P. A**
Assistant Professor, Department of Pharmacology

**Dr. Anand Gaur**
Assistant Professor, Department of Pharmacology, N. C Medical College & Hospital, Israna, Panipat, Haryana

**Dr Anurag Bajpai**
Professor and HOD, Department of Pharmacology, N. C Medical College & Hospital, Israna, Panipat, Haryana

**Dr. Abhay John**
Associate Professor, Department of Pharmacology, Amaltas Institute of Medical Science, Dewas

***Abstract*---**Liver is one of the largest glands of the body and the main site for intense metabolism and excretion. Liver disease is a term for a collection of conditions that adversely affect the cells, tissues, structures, or functions of the liver. Treatment of liver diseses with various drugs develops risk of toxicity. *Rubiacordifolia* is an herbal plant has, been claimed to have hepatoprotective potentials. The present study was conducted to explore the hepatoprotective activity of aqueous extract of *Rubiacordifolia* against carbon tetrachloride induced hepatotoxicity in albino rats. This experimental study was conducted after getting approval from institutional animal ethics committee using, routes of *Rubiacordifolia* in wistar albino rats (150-200g) of either sex. The hepatoprotective activity was evaluated by using histo-pathological examination. Animals were divided in to five groups with six animals in each group. Group-1 was given Normal saline (1ml/kg/day), Group-2 was injected with carbon tetrachloride (1ml/kg) i.p. only once to produce hepatotoxicity. Group-3 and Group-4 were given *Rubiacordifolia* (100mg/kg and 200mg/kg) orally every morning for 21 days followed by carbon tetrachloride (1ml/kg) i.p on 21st day (respectively). Group-5 was given standard drug Liv-

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***Corresponding author**: Dr. Abhay John*
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52(1mg/kg) i.p. for 21 days followed by carbon tetrachloride (1ml/kg) i.p on 21st day. Aqueous extract of roots of *Rubiacordifolia* in oral doses showed a significant effect on Histopathology. Normal hepatic lobule architecture is seen. Hepatocytes and their nuclei are well visible. Only cellular debris is seen and no hepatocytes with nuclei are discernible. It can be concluded that the Aqueous extract of roots of *Rubiacordifolia* is a potent hepatoprotective agent.

***Keywords*---*rubiacordifolia,*** carbon tetrachloride, hepatoprotective, liv-52.

## Introduction

Liver plays an important role in our body. To have a proper understanding of liver ailments, it is important to have a detailed knowledge of hepatic structure and function. [1] Liver disease is a term for a collection of conditions that adversely affect the cells, tissues, structures, or functions of the liver. Symptoms of liver disease may be acute, occurring suddenly, or chronic, developing slowly over a long period of time. Chronic liver disease is much more common than acute. [2] The rates of chronic liver disease for men are two times higher than for women. Liver disease may range from mild to severe depending on the type of disease present. [3]

CCI4 is the classic hepatotoxin, classified as an agent producing direct injury to the liver. Single dose lead promptly to zone 3 necrosis and steatosis, Prolonged administration can lead to cirrhosis and it also causes hepatic carcinoma in rats and mice. $CCI_4$ produces hepatic necrosis and steatosis in a variety of laboratory animals and in humans. [4] The hepatic damage is usually accompanied by lesions of other organs especially kidneys. Necrosis in zone 3 occurs in all species of mammals after administration of the agent by any route except direct injection into the portal venous system; the latter leads to massive necrosis. Hydropic degeneration (i.e. ballooning) and acidophilic degeneration in zone 3 precede the necrosis. These disappear when necrosis is maximal. [5]

The plant is commonly known as'Indian Madder' and sold under the trade name'manjistha'.It is a member of Rubiaceae family,distributed in hilly tracts of India up to 3750 m.It is a perennial, prickly, climbing herb with red rhizomatous base and roots. [6] Leaves variable, arranged four in a whorl, base slightly cordate, petioles are quadrangular, sometimes prickly on the angles, glabrous and shining. Stems is slender, rough, four angled with sharp recurved prickles on the ridges, which are often many yards long, becoming slightly woody at the base. Flowers are in cymes, greenish white. Fruits are didymous or globose,smooth, shining and purplish black when ripe. [7]

**Aim**

Evaluation of hepatoprotective potential of aqueous extract of *Rubia cordifolia* against carbon tetrachloride induced hepatotoxicity in albino rats.

**Materials and Methods**

This was an experiment study conducted on wistar rats in Department of Pharmacology, Index Medical College over a period of 2 year.

Healthy Albino wistar rats of either gender, weighing 150- 200g were obtained from CPCSEA approved Central Animal House of Medical College. The selected rats were housed in polpropylene cages under controlled conditions of temperature (25 $^0$C) and alternating periods of light and darkness of 12 hours each. The rats had free access to standard rat pellet diet and tap water *ad libitum*. After one week of acclimatization, the animals were rendered suitable for study. Pregnant female rats were not included in the study.

**Test compound**

1. To induce hepatotoxicity, commercially available preparation of injectable Carbon tetrachloride was used.
2. Aqueous extract of *Rubia cordifolia,* to evaluvate hepatoprotevtive activity.

**Rubia cordifolia**

Roots of *Rubia cordifolia* were dried under shade and ground to make a fine powder in a herb grinder. One gram of finely powdered plant material was extracted with 50 ml of water. The solution was filtered using Whatman filter paper no 1. Solution was evaporated under vacuum in a rotary evaporator to make the extract dry. This dried extract was stored at 0- 4$^o$C and dissolved in water to make a solution of 1% concentration when ever required.

The animals were randomly divided into seven groups of six animals each. The groups are described as follows:

- Group- I: control group was administered 0.9% NaCl solution in a single oral dose of 1ml/kg body wt for 21 days.
- Group -II: In addition to pellet diet and tap water ad libitum,this group was injected with toxin CCl4 (1ml/kg) i.p only once to produce hepatotoxicity on 21stday.
- Group-III: This group was given Rubia cordifolia orally (100 mg/kg) (Gilani AH et al.,1995). as a single dose per orally every morning for 21days followed by an injection of CCl4 (1 ml/kg i.p.) on 21st day.
- Group –IV: This group was received Rubia cordifolia orally (200 mg/kg) as a single dose per orally every morning for 21days followed by an injection of CCl4 (1 ml/kg i.p.) on 21st day.
- Group-V: This group received Liv.52 (1ml/kg) orally for 21 days followed by an injection CCl4 (1 ml/kg i.p.) on 21$^{st}$ day.

Liv.52 and the test compound (*Rubia cordifolia*) were administered by gavage method with animals fasted 3-4 hours prior to and 1 hour after administration of test drugs to ensure proper absorption. After administration of carbon tetrachloride, animals of all the groups were fasted for 24 hours although which time water remained freely available during this period, Thereafter animals were sacrificed under Ketamine (75 mg/kg) and Diazepam (10 mg/kg) anaesthesia given intraperitoneally.

The liver was excised from the animals and washed with the normal saline. About one cm piece was cut and fixed in 10% neutral formalin for 12-24 hours. It was then dehydrated and cleared with ethanol and xylene respectively followed by embedding in paraffin wax from which blocks were prepared. Sections of 5m thickness were taken from the blocks using a microtome . These were processed in alcohol-xylene series and were stained with Harris haematoxylin and eosin stain [ and subjected to histopathological examination.

**Statistical Analysis**

The data thus obtained was appropriately organized and analyzed by suitable statistical methods. i.e ; ANOVA.

**Results**

Aqueous extract of *Rubia cordifolia* showed effect on biochemical parameters.

Table 1 : Effect of Liv-52, *Rubia cordiafolia* in their respective doses on carbon tetrachloride induced changesin serum Alanine Transaminase (n=6).

| TREATMENT (mg/kg) | Alanine transaminase(IU/L)(mean±SE) |
|---|---|
| Normal saline (1ml) | 29.5±3.35 |
| $CCl_4$ (1) | 433.5±48.67^ |
| LIV.52 (1) | 140.7±8.1^ |
| *Rubia cordifolia* (100) | 281±18.33* |
| *Rubia codifolia*(200) | 177.5±12.73* |

£P< 0.05 as compare to $ccl_4$ treated group.

*p< 0.01 as compare to $ccl_4$ treated group.

^P< 0.001 as compare to normal saline treated group.

ALT level in normal saline treated group was 29.5±3.35 IU/L. It was found to be significantly increased (p<0.001) with administration of $CCl_4$ to 433.5±48.67 IU/L. Pretreatment with known hepatoprotective preparation Liv.52 significantly (p<0.001) limited the rise in ALT levels after $CCl_4$ adminstration to 140.7±8.1 IU/L. With *Rubia cordifolia* there is dose depended limitation of ALT rise after $CCl_4$ administration. Although the dose of 100mg/kg for 21 days showed a significant limitation (p<0.01) of ALT rise (281±18.33) when compared to $CCl_4$ treated group but it did not match the efficacy of Liv. 52 treated group. However , in dose of 200 mg/kg for 21 days the *Rubia cordifolia* extract had much efficacy, in limiting the ALT rise after $CCl_4$ administration, to 177.5±12.73 IU/L, which was significant (p<0.01).

Table 2 : Effect of Liv-5, *Rubia cordiafolia* in their respective doses on carbon tetrachloride inducedchanges in Alkaline Phosphatase (n=6).

| TREATMENT (mg/kg) | Alkaline phosphatase (IU/L)(mean±SE) |
|---|---|
| Normal saline (1ml) | 73.9±4.63 |
| $CCl_4$ (1) | 600.42±52.9^ |
| LIV.52 (1) | 198.9±11.6^ |
| *Rubia cordifolia* (100) | 401±45.26£ |
| *Rubia codifolia*(200) | 245.1±21.25* |

£P< 0.05 as compare to $ccl_4$ treated group.

*p< 0.01 as compare to $ccl_4$ treated group.

^*P< 0.001 as compare to normal saline treated group.*

ALP level in normal saline treated group was 73.9±4.63 IU/L. It was found to be significantly increased (p<0.001) with administration of $CCl_4$ to 600.42±52.9 IU/L. Pretreatment with known hepatoprotective preparation Liv.52 significantly (p<0.001) limited the rise in ALP levels levels after $CCl_4$adminstration to 198.9±11.6 IU/L. With *Rubia cordifolia* there is dose depended limitation of ALP rise after $CCl_4$ administration. Although the dose of 100mg/kg for 21 days showed a significant limitation (p<0.05) of ALP rise (401±45.26) when compare to $CCl_4$ treated group but it did not match the efficacy of Liv. 52 treated group. However , in dose of 200 mg/kg for 21 days the *Rubia cordifolia* extract had much efficacy, in limiting the ALT rise after $CCl_4$ administration, to 245.1±21.25 IU/L, which was significant (p<0.01).

Table 3: Effect of Liv-52, *Rubia cordiafolia* in their respective doses on carbon tetrachloride induced changes in total serum bilirubin (n=6)

| TREATMENT (mg/kg) | Total bilirubin (IU/L) (mean±SE) |
|---|---|
| Normal saline (1ml) | 0.25±0.95 |
| $CCl_4$ (1) | 2.03±0.86^ |
| LIV.52 (1) | 0.46±0.42^ |
| *Rubia cordifolia* (100) | 0.93±0.53* |
| *Rubia codifolia* (200) | 0.83±0.13* |

£P< 0.05 as compare to $ccl_4$ treated group.

*p< 0.01 as compare to $ccl_4$ treated group.

^P< 0.001 as compare to normal saline treated group.

Total Bilirubin level in normal saline treated group was 0.25±0.95 IU/L. it was found to be significantly increased (p<0.001) with administration of $CCl_4$ to 2.03±0.86 IU/L. Pretreatment with known hepatoprotective preparation Liv.52 significantly (p<0.001) limited the rise in total bilirubin levels after $CCl_4$adminstration to 0.46±0.42IU/L. With *Rubia cordifolia* there is dose depended limitation of Total Bilirubin rise after $CCl_4$ administration. Although the dose of 100mg/kg for 21 days showed a significant limitation (p<0.01) of Total Bilirubin rise (0.93±0.53) when compared to $CCl_4$ treated group but it did not match the efficacy of Liv. 52 treated group. However, in dose of 200 mg/kg for 21 days the *Rubia cordifolia* extract had much efficacy, in limiting the Total Bilirubin rise after $CCl_4$ administration, to 0.83±0.13 IU/L, which was significant (p<0.01).

Table 4: Effect of Liv-52, *Rubia cordiafolia* in their respective doses on carbon tetrachloride induced changes in serum Albumin(mean± SE) (n=6)

| TREATMENT (mg/kg) | Albumin (gm/dl) (mean±SE) |
|---|---|
| Normal saline (1ml) | 4.6±0.82 |
| $CCl_4$ (1) | 5.3±0.92 |
| LIV.52 (1) | 5.2±0.31 |
| *Rubia cordifolia* (100) | 5.4±1.14 |
| *Rubia codifolia* (200) | 5.04±0.94 |

There was no such difference in serum Albumin levels of any of groups and all the measurement recorded were in normal range. The Albumin levels varied from 4.2gm/dl to 5.4gm/dl showed no correlation. which was insignificant ($p>0.05$) also.

**Effect on Histology**

Histology of livers of normal saline treated group showed normal liver architecture. The hepatic cords and the sinusoids were well visible. (fig-1) Classical centrizonal necrosis was seen in the $CCl_4$ treated group. The hepatocytes around the central vein were necrosed with no distinguishable nuclei (fig-2).

Liv.52 treated group revealed vary mild signs of liver injury. Only difference from the normal saline treated group was the presence of inflammatory cells and constricted sinusoids indicating apperent hepatocyte swelling (fig-3).

Group treated with *Rubia cordifolia* with dose 100mg/kg showed feathery degeneration in the centrizonal area which is predominant histilogical feature, and necrosis was absent. However at dose 200mg/kg showed normal hepatic lobule architecture was seen with mild fatty changes and no necrosis was seen (fig.4-5).

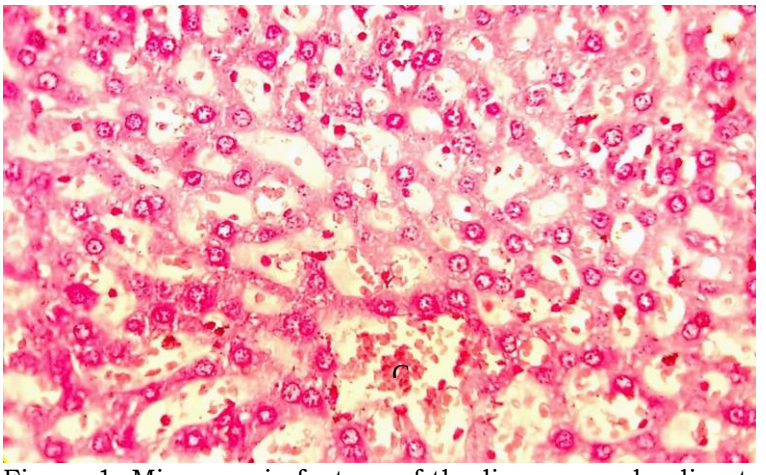

Figure 1: Microscopic feature of the liver normal saline treated group. Normal hepatic lobule architecture is seen. Hepatocytes and theirnuclei are well visible. (H & E Stain x 400)

CV – Central vein

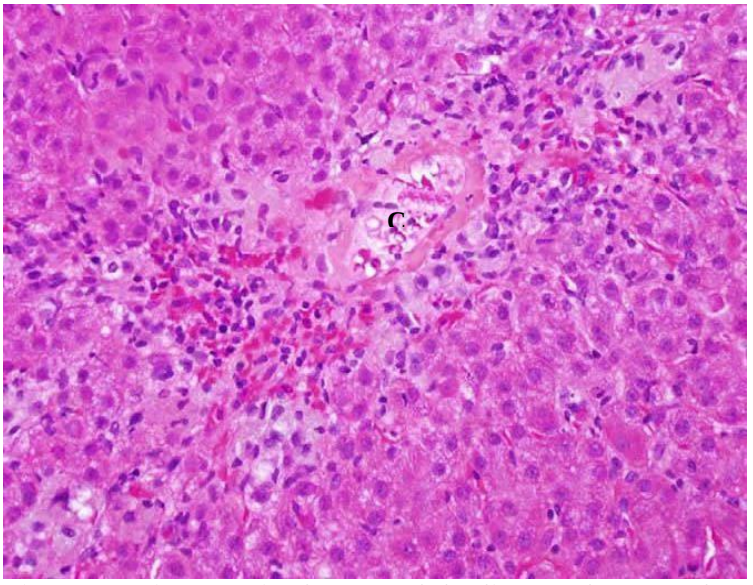


Figure 2: Microscopic feature of the liver CCl4 treated group.
Extensive centrizonal necrosis is seen. Only cellular debris is seen and no hepatocytes with nuclei are discernible. (H & E Stain x 400)

CV – Central vein

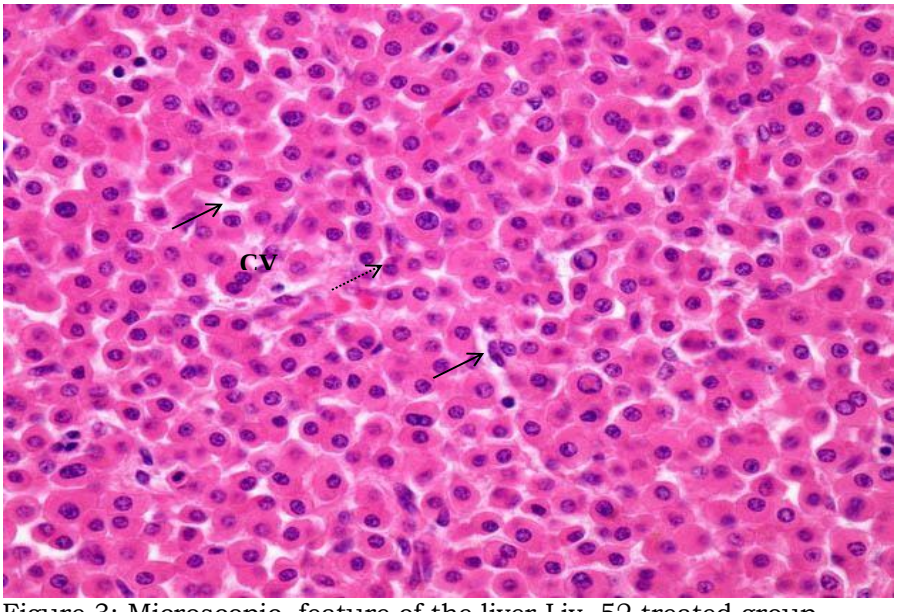


Figure 3: Microscopic feature of the liver Liv. 52 treated group.
Mild hepatocyte swelling is present as indicated by constructed sinusoids. Inflammatroy cells are seen mostly arround central vein.(H & E Stain x 400)

CV – Central vein
Solid Arrows : Sinusoids
Dotted Arrows : Inflammatory cells

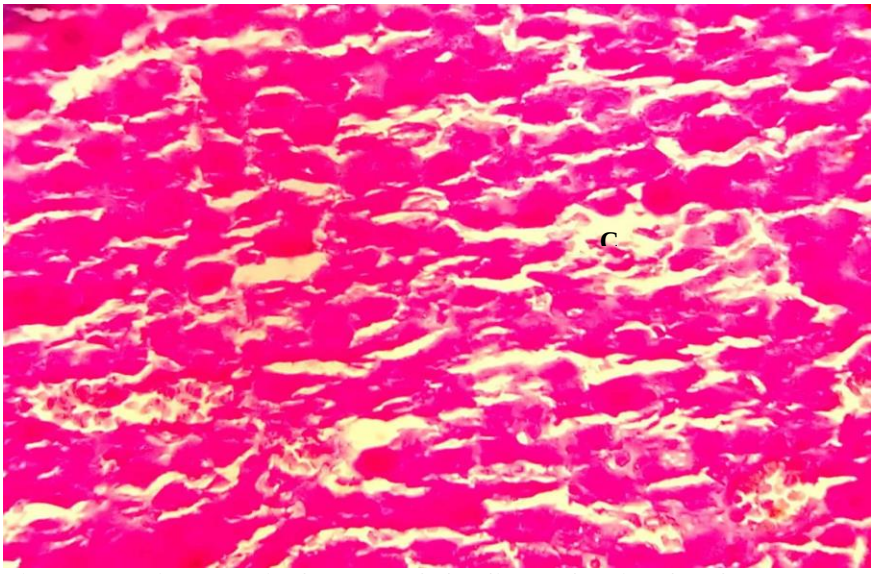

Figure 4: Microscopic feature of the liver of animals of group treated with Rubia Cordifolia (100 mg/kg) .
Remarkable Feathery degeneration of haepatocyte is seen arround the central vein. Further away from the central vein cell with fattychanges are seen. (H & E Stain x 400)
CV – Central vein

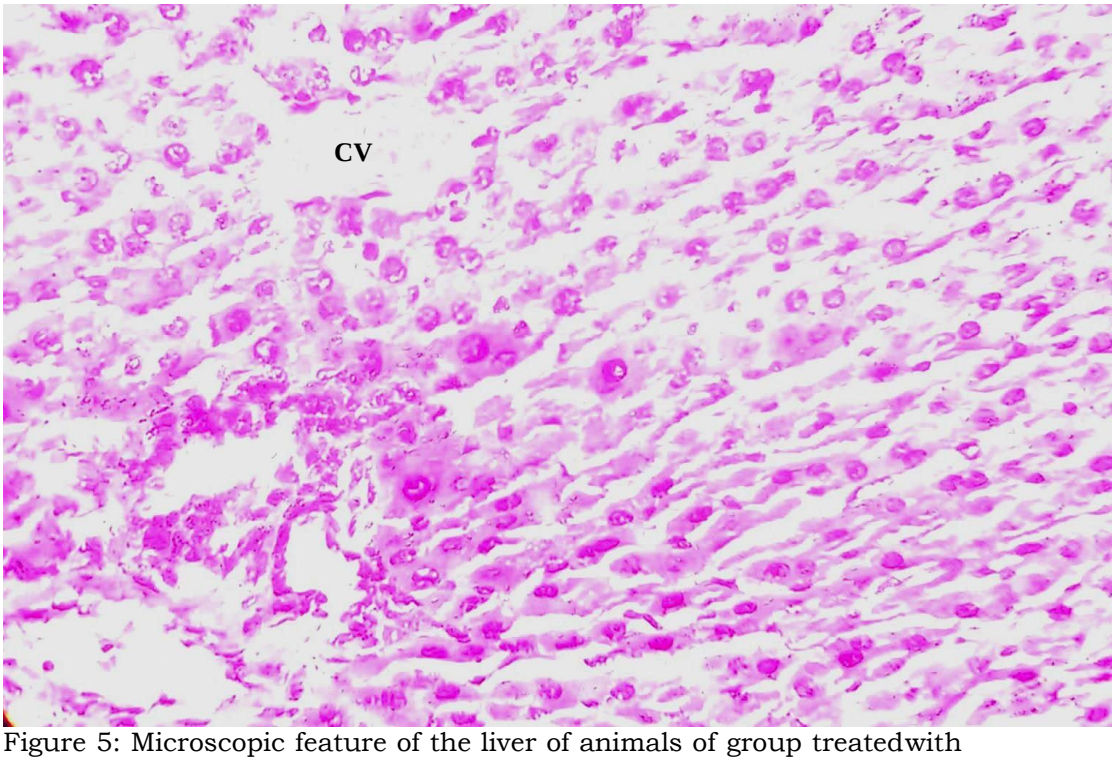


Figure 5: Microscopic feature of the liver of animals of group treatedwith Rubia Cordifolia (200 mg/kg) .

Mimimal feathery degeneration around central vein. Cells with fattychanges are seen. Necrosis is not seen. (H & E Stain x 400)

CV – Central vein.

**Discussion**

Liver diseases are major problems throughout the world. Many environmental toxins cause liver diseases in human being. Despite new advances in hepatology, treatment of liver diseases caused by toxin has not been resolved as yet. Furthermore, despite the need for agents to protect the liver from damage, modern medicine lacks a reliable liver protective drug. [8] Therefore, there has been a considerable interest in the role of complementary and alternative medicines for the treatment of liver disease. [9] Recently so called healthy foods including functional food and dietary supplements are being increasingly used worldwide on part of complementary and supplementary medicine or self medication. [10]

The aqueous extract of *Rubia cordifolia* exhibited dose dependant hepatoprotection, both biochemically and histologically. Same dose for same duration also gave good protection from increasing ALP as compared to liv.52 and slightly less protection from increasing Total Bilirubin as compare to Liv.52. No significant change was seen with albumin levels.

The hepatoprotective effect of *Rubia cordifolia* may be related to glutathione-mediated detoxification. Glutathione (g-glutamylcysteinyl glycine, GSH) is a sulfhydryl (SH) antioxidant, antitoxin, and enzyme cofactor. Glutathione is found mainly in the cell cytosol and other aqueous phases of the living system. [11] Glutathione exist in two form: the antioxidant – reduced glutathione‖ tripeptide is conventionally called glutathione and abbreviated GSH; the oxidized form is a sulfur-sulfur linked compound, known as glutathione disulfide or GSSG. The GSSG/GSH ratio may be a sensitive indicator of oxidative stress. GSH has potent electron-donating capacity, as indicated by the high negative redox potential of the GSH/GSSH -redox couple (E‘0=-0.33v). [12] Its high redox potential renders GSH both as a potent antioxidants and a convenient cofactor for enzymatic reactions that require readily available electron pairs 1. [13] Intracellular GSH status is a sensitiveindicator of the cells overall health, and of its ability to resist toxicchallenge. *Rubia cordifolia* and could also enhance the GSH in cells thereby may protect hepatic cells from toxic damages. [14]

Since formation of free radicals by cytochrome P450 after metabolism of $CCl_4$ has been implicated for lipid peroxidation mediated hepatocyte injury, the hepatoprotective mechanisms of aqueous extract of *Rubia cordifolia* could be an inhibitory effect on the microsomal enzymes (cytochrome P450) so that generation of free radicals is limited.

Like silymarin, *Rubia cordifolia* and *Withania somnifera* may also act as free radical scavengers and has antioxidant property as it may have an inhibitory effect on lipid peroxidation, Silymarin which is a well known hepatoprotective

agent. The hepatoprotective effects of silymarin are mainly attributable to its antioxidant and free radical scavengingproperties. [15]

Hepatocytes have a practically unlimited capacity for proliferation, with full regeneration. Possible mechanism for regeneration of liver are changes in gene expression, an array of transcription factors (NF-kB, STAT3, fos and jun) and various hepatic mitogenes which promote hepatocyte regeneration. Like silymarin, a proved hepatoprotective agent (acts through entry inside the nucleus and stimulate protein synthesis), may enhance *Rubia cordifolia* the activity of one or more of the above factors to stimulate the hepatic regeneration. [16]

**Conclusion**

Aqueous extract *Rubia cordifolia* is a potent hepatoprotective agent. Further studies with its hydroalcoholic extract can be expected to provide still better results. It is suggestible that the dosage form of *Rubia cordifolia* aqueous extract can be changed from the current *Rubia cordifolia* aqueous extract form, to some other form like hydroalcoholic extract or extraction of some specific component which is involved in hepatoprotective action. Also the safety profile of *Rubia cordifolia* aqueous extract has been very encouraging in this study. Direct human studies can be undertaken without any risk, which will help to determine the exact status of *Rubia cordifolia*. Furtherstudies of extract of *Rubia cordifolia* could be extended for the isolation and structure determination of the hepatoprotective principle(s) and better and detailed explanations of mechanisms by which *Rubia cordifolia* acts.

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