EVALUATION OF IN VIVO HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACTS OF ROOTS OF RICINUS COMMUNIS (EE-R-RC) AGAINST CCL 4 INDUCED RAT MODEL

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Abstract:

The liver, an organ only found in vertebrates, detoxifies various metabolites, synthesizes proteins, and produces biochemicals necessary for digestion. The liver is the only human internal organ capable of natural regeneration of lost tissue; as little as 25% of a liver can regenerate into a whole liver. This is, however, not true regeneration but rather compensatory growth in mammals. The lobes that are removed do not regrow and the growth of the liver is a restoration of function, not original form. This contrasts with true regeneration where both original function and form are restored. The main aim and objective of the present research work was the preliminary phytochemical screening of primary and some secondary metabolites present in ethanolic extract of roots of Ricinus communis. The in vivo hepatoprotective activity was performed in Ccl4 induced rat model and histopathological analysis was done by FNAB. The in vivo experimental data displayed that the elevated levels of SGOT, SGPT, ALP and Sr. bilirubin were mainly due to CCl4 intoxication, reduced significantly (*P<0.05) in rats, after treatment with EE-R-RC. Treatment with EE-R-RC at a dose of 100 mg/kg b. w. decreased the SGOT: 11.69%, SGPT: 22.50%, ALP: 3.64%, and Serum bilirubin levels by 32.74%, (significantly) respectively, while at higher dose of 200 mg/kg b. wt. was more effective, causing a reduction of SGOT: 24.30%, SGPT: 41.09%, SALP: 11.97% and Sr. bilirubin: 47.16%, Silymarin is used as standard drug showed a significant reduction of level of SGOT: 53.35%, SGPT: 48.43%, SALP: 47.43% and Sr. bilirubin: 75.35% respectively receiving CCl4 alone.

Key words: Metabolites; hepatoprotective; FNAB; SALP etc.
INTRODUCTION

It is truth that without nature human being life is not possible. The food, clothes and shelter are three basic necessity of human beings and an important one necessity is good health, which provided by plant kingdom. Plant kingdoms are the rich source of organic compounds, many of which have been used for medicinal purposes. In traditional medicine, there are many natural crude drugs that have the potential to treat many disease and disorders one of them is Ricinus communis; Family: Euphorbiaceae popularly known as 'castor plant' and commonly known as 'palm of Christ', Jada (Oriya), Verenda (Bengali), Endi (Hindi), Errandi (Marathi), Diveli (Guajarati) [1]. The plant is widespread throughout tropical regions as ornamental plants.

The castor oil plant is a fast-growing, suckering perennial shrub or occasionally a soft wooded small tree up to 6 meter or more, but it is not hardy in nature. This plants was cultivated for leaf and flower colours and for oil production. Leaves are green or reddish in colour and about 30-60 cm in diameter. The leaves contain 5-12 deep lobes with coarsely toothed segments which are alternate and palmate. The stems are varying in pigmentation. The flowers are monoecious and about 30-60 cm. long [2]. The fruit is a three-celled thorny capsule. The capsule of fruit covered with soft spines like processes and dehiscing in to three 2-valved cocci. The seeds are considerable differences in size and colour. They are oval, somewhat compressed, 8-18 mm long and 4-12 mm broad. The testa is very smooth, thin and brittle. Castor seeds have a warty appendage called the caruncle, which present usually at one end from which runs the raphe to terminate in a slightly raised chalaza at the opposite end of the seed [3]. This plant is common and quite wild in the jungles in India and it is cultivated throughout India, chiefly in the Madras, Bengal and Bombay presidencies. Two varieties of this plant are known A perennial bushy plant with large fruits and large red seeds which yields about 40 P.C of oil. A much smaller annual shrub with small grey (white) seeds having brown spots and yielding 37% of oil.

Pharmacological profile of R. communis

1. Antioxidant activity: It is concluded that R. communis antioxidant activity by using lipid method and free radical scavenging effect on 2,2 picrylhydrazyl radical (DPPH) and hydroxyl hydrogen peroxide. The high antioxidant activity of the seed of communis at low concentration shows that it could be very useful for...
the treatment of disease resulting from oxidative stress. The responsible chemical constituent of antioxidant activity are Methyl ricinoleate, Ricinoleic acid [4] octadecadienoic acid and methyl ester stem and leave extracts also produce antioxidant activity due to the presence of flavonoids in their extracts

2. Antinociceptive activity: The methanolic leaves extract of antinociceptive activity against formalin induced paw licking and The antinociceptive activity showed due to the presence preliminary Phytoconstituents like saponins, steroids and alkaloids -5-en-3-ol, stigmasterol, Y-sitosterol, fucosterol; essential oil using capillary like α-thujone (31.71%) and 1,8- (12.92%) and 30-Norlupan-3β-ol-20-one are bean [5]. seed extracts produced the per oxidation by ferric thiocyanate 2,2-diphenyl-1- radical generated from R. communis which produce 12- ester [6]. The Ricinus communis extracts [7, 8]. R. communis possesses significant acetic acid induced writhing test, tail immersion methods in mice. Alkaloids [9].

3. Antiasthmatic activity: The ethanolic root extract of R. communis is effective in treatment of asthma because of its antiallergic and mast cell stabilizing potential effect. Saponins has mast cell stabilizing effect and the flavonoids possess smooth muscle relaxant and bronchodilator activity; the apigenin and luteolin like flavonoids were generally inhibit basophil histamine release and neutrophils beta glucuronidase release, and finally shows in-vivo antiallergic activity. The R. communis ethanolic extract decreases milk induced leucocytosis and eosinophilia and possess antiasthmatic activity due to presence of flavonoids or saponins [10].

4. Anti-fertility activity: The methanol extracts of R. communis seed possess positive preliminarily Phytochemical tests for both steroids and alkaloids. The pituitary gland releases gonadotrophins due to Sex hormones by both positive and negative feedback mechanism and also the pituitary gland block the release of luteinizing hormone (LH) and the follicle-stimulating hormone (FSH) because of the effect of combined oestrogen and progesterone in the luteal phase of the menstrual cycle. Finally it helps the inhibition of maturation of the follicle in the ovary and prevents ovulation. The sex hormone being steroidal compound's
(phytosterols) and the presence of steroids in methanol extract of *Ricinus communis* seed produces anti-fertility effects [11, 12].

5. **Antihistaminic Activity:** The ethanol extract of *R. communis* root resulted antihistaminic activity at the dose 100, 125, and 150 mg/kg intraperitoneally by using clonidine induced catalepsy in mice [13].

6. **In vitro immunemodulatory activity:** The plant and animal origin immunemodulatory agents generally increase the immune responsiveness of the human body against pathogens by activating the non-specific immune system. The phagocytosis is the engulfment of microorganism by leucocytes. In last the phagocytosis is the intracellular killing of microorganisms by the neutrophils. The presence of tannins in the leaves of *R. communis* significantly increased the phagocytic function of human neutrophils and resulted produces a possible immunemodulatory effect [14].

7. **Hepatoprotective activity:** *Ricinus communis* leaves ethanolic extract 250/500mg/kg body weight possesses hepatoprotective activity due to their inhibitory activities of an increase in the activities of serum transaminases and the level of liver lipid per oxidation, protein, glycogen and the activities of acid and alkaline phosphatase in liver induced by carbon tetrachloride (CCL4). The *R. communis* ethanolic extract 250/500mg/kg body weight also treated the depletion of glutathione level and adenosine triphosphatase activity which was observed in the CCl4-induced rat liver. The presence of flavonoids in ethanol extract of *R. communis* produces beneficial effect the flavonoids have the membrane stabilizing and antiperoxidative effects. Hence the *R. communis* increase the regenerative and reparative capacity of the liver due to the presence of flavonoids and tannins. The anticholestatic and hepatoprotective activity was seen against paracetamol-induced hepatic damage due to the presence of N-demethyl ricinine isolated from the leaves of *Ricinus communis* Linn. The whole leaves of *Ricinus communis* showed the protective effect against liver necrosis as well as fatty changes induced by CCL4 while the glycoside and cold aqueous extract provide protection only against liver necrosis and fatty changes respectively, [15, 16, 17].
8. **Anti-inflammatory activity:** Anti-inflammatory activities of the leaves and root extract were studied in Wistar albino rats in acute and chronic inflammatory models. The study indicated that the paw edema formation due to sub plantar administration of carrageenan, characterizing the cellular events of acute inflammation. The 250 and 500 mg/kg dose of *R. communis* methanolic leaves extract possess protective effect in prevention of cellular events during edema formation and in all the stages of acute inflammation. The anti-inflammatory activity of *R. communis* methanol extract was due to the presence of flavonoids because the flavonoids have the protective effect against carrageenan-induced paw edema in rats [18, 19, 20].

9. **Antimicrobial activity:** The antimicrobial activities of *Ricinus communis* were good against dermatophytic and pathogenic bacterial strains *Streptococcus progenies, Staphylococcus aureus* as well as *Klebsiella pneumonia, Escherichia coli*. The result showed that the petroleum ether and acetone extracts possess good zone of inhibition where as ethanolic extract having anti bacterial activity only on higher concentration [21]. The different solvent extracts of roots of *Ricinus communis* (200 mg/ml) possess antimicrobial activity by using well diffusion method against pathogenic microorganisms such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Proteus vulgaris, Bacillus subtilis, Candida albicans* and *Aspergillus niger*. The hexane and methanol extracts showed maximum antimicrobial activity where the aqueous extracts has no significant antimicrobial properties [22].

10. **Antidiabetic activity:** The ethanolic extract of roots of *Ricinus communis* (RCRE) was investigated along with its bioassay-guided purification. By Administration of the effective dose (500mg/kg b. w) of RCRE to the diabetic rats for 20 days possess favorable effects not only on fasting blood glucose, but also on total lipid profile and liver and kidney functions. Amongst all fractions the R-18 fraction suggests the significant antihyperglycemic activity. RCRE showed no significant difference in alkaline phosphatase, serum bilirubin, creatinine, serum glutamate oxaloacetate transaminases, serum glutamate pyruvate transaminases and total protein which was observed even after the administration of the
extract at a dose of 10 g/kg b.wt. Thus *R. communis* is a potent phytomedicine for diabetes [23].

11. **Wound healing activity:** The *Ricinus communis* possess wound healing activity due to the active constituent of castor oil which produce antioxidant activity and inhibit lipid per oxidation. Those agents whose inhibits lipid per oxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibres, increasing the circulation, preventing the cell damage and by promoting the DNA synthesis. The study of wound healing activity of castor oil was in terms of scar area, % closure of scar area and epithelization in excision wound model. Due to the astringent and antimicrobial property the tannins, flavonoids, triterpenoids and sesquiterpenes promotes the woundhealing process, which are responsible for wound contraction and increased rate of epithelialisation. The study resulted that the Castor oil showed wound healing activity by reducing the scar area and also the epithelization time in excision wound model. The comparison study of two different concentrations (5%w/w and 10%w/w) of castor oil was resulted that the 10 % w/w Castor oil ointment possesses better wound-healing property [24].

12. **Lipolytic activity:** The ricin produces the lipolytic activity by using the various substrates: (i) one analogue of triacylglycerol, BAL-TC4; (ii) various chromogenic substrates such as p-NP esters of aliphatic short to medium chain acids, and (iii) monomolecular films of a pure natural diacylglycerol, DC10 in emulsion and in a Membrane-like model. The study concluded that ricin from *R. communis* act as a lipase and has the capability of hydrolyzing different lipid classes. Ricin also hydrolyses phospholipids which are the major components of cellular membranes. The lipolytic activities are maximal at pH 7.0 in the presence of 0.2 M galactose. The action of ricin on membrane phospholipids could occur through a phospholipase A1 activity which is very often a minor activity of lipases [25]

13. **Molluscicidal, Insecticidal and Larvicidal activity:** The leaf extract of *R. communis* possess molluscicidal activity against *Lymnaea acuminata* and the seed extracts showed better insecticidal and insectistatic activity than the leaf extracts against *S. frugiperda* due to the active ingredients like castor oil and ricinine[26, 27, 28]. The aqueous leaves extracts of *R. communis* possess
suitable Larvicidal activity against *Anopheles arabiensis*, *Callosobruchus chinensis* and *Culex Quinquefasciatus* mosquitoes [29].

14. Antiulcer activity: The castor oil of *R. communis* seed possess significant antiulcer properties at a dose of 500 mg/kg and 1000 mg/kg, but at the dose 1000 mg/kg was more potent against the ulceration caused by pylorus ligation, aspirin and ethanol in rats. The result showed that the antiulcer activity of *R. communis* is due to the cytoprotective action of the drug or strengthening of gastric mucosa and thus enhancing the mucosal defence [30, 31].

*R. communis* or castor plant is a widely traditionally used and potent medicinal plant amongst all the thousands of medicinal plants. The pharmacological activities reported in the present review confirm that the therapeutic value of *R. communis* is much more. It is an important source of compounds with theirs chemical structures as well as pharmacological properties. The presence of phytochemical constituents and pharmacological activities proved that the plant has a leading capacity for the development of new good efficacy drugs in future.

EXPERIMENTAL PHYTOCHEMISTRY

Materials and method

**Drugs and chemicals:** The standard drug silymarin purchased from Local Retail Pharmacy Shop and solvents and other chemicals used for the extraction and phytochemical screening were provided by Institutional Store and were of LR and AR grade.

**Methodology for Soxhlet extraction:** First the dried roots of *R. communis* (R-RC) are triturate to make fine powder and the powered material is placed into the thimble made of stout filter paper and the apparatus is fitted up. The flask containing suitable solvent like ethanol is heated on a water bath or on a heating mental. As the solvent boil, its vaporise rise through the side tube up into the water condenser. The condensed liquid drops on the solid in the thimble, dissolves the organic substances present in the powdered material and filters out into the space between the thimble and the glass cylinder. As the level of liquid here rises, the solution flows through the siphon back into the boiling flask. The solvent is once again vaporized, leaving behind the extracted substance in the flask. In this way a continuous stream of pure solvent drops on the
solid material, extract the soluble substance and returns to the flask. At the end of the operation the solvent in the boiling flask is distilled off, leaving the organic substance behind [32]. Afterwards the ethanolic extract of roots of R. communis (EE-R-RC) transfer in a clean and dried beaker and are concentrated by placing on a water bath and then cool, keep it in a freeze. From this concentrated extract the preliminary phytochemical screening has to be carried out.

**Phytochemical screening [33-36]:** Preliminary phytochemical screening of EE-R-RC have shown the presence of diverse bioactive molecules such as: carbohydrates, proteins and amino acids polyphenols, carotenoids, phytosterols and alkaloids which are confirmed by their specific qualitative confirmatory chemical tests.

**EXPERIMENTAL PHARMACOLOGY**

**Animals:** Swiss albino rats (120-150 gm)

**Protocol for the study of acute oral toxicity of EE-R-RC:** In the present study the acute oral toxicity of EE-R-RC was performed by acute toxic class method. In this method the toxicity of the extract was planned to test using step wise procedure, each step using three Wister rats. The rats were fasted prior to dosing (food but not water should be withheld) for three to four hrs. Following the period of fasting the animals were weighed and the extract was administered orally at a dose of 2000 mg/Kg b. w. Animals were observed individually after dosing at least once during the first 30 min; periodically the surveillance was carried out for the first 24 hrs with special attention given during the first 4 hrs and daily thereafter, for a total of 14 days [37]. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of CPCSEA: IAEC/XXIX/01/2017.

**Evaluation of in vivo Hepatoprotective activity [38]:** A total of 30 rats were taken and divided into 6 groups:

**Treatment Groups**

(A) Group I: Normal Control Group (only the vehicle (1 ml/kg/day of 1% CMC; p. o.)

(B) Group II: Negative Control CCl₄ 1 ml / kg (1:1 of CCl₄ in olive oil) I. p.
(C) Group III: Low Dose Group [CCl₄ 1 ml / kg (1:1 of CCl₄ in olive oil) I. p + EE-R-RC (100 mg/ kg b. w. p. o.)]. Treatment was given daily for seven days orally.

(D) Group IV: High Dose Group [CCl₄ 1 ml / kg (1:1 of CCl₄ in olive oil) I. p + EE-R-RC (200 mg/ kg b. w. p. o.)]

(E) Group V: Standard Group [CCl₄ 1 ml/kg (1:1 of CCl₄ in olive oil) I. p. + Standard Silymarin 50 mg/kg orally (p. o.) for 7 days].

Biochemical analysis: On the 8th day, blood was collected by retro orbital puncture, under mild ether anaesthesia after 8 hr fasting. Blood samples were centrifuged at 3000 rpm for 20 minutes. Serum was separated and stored at –20 °C until biochemical estimations. All the biochemical parameters were determined by spectrocolorimetrically. The Serum samples were analyzed for SGPT; SGOT; Sr. ALP and serum bilirubin

Histopathological analysis: Histopathological study was carried out by Fine needle aspiration biopsy of rat liver (FANB) [39] and the smear was stained by H and E-stained technique. The whole assessment was carried out at V.H.S Hospital in Chennai.

Statistical analysis: The data (Table:1 and 2) were expressed as mean ± SD. Statistical differences at *P < 0.05 between the groups were analyzed by one-way ANOVA followed by Dunnett’s Multiple Comparison Test using Graph Pad Prism 5.04 Instate software package. The data’s were compared with group II i. e. Negative Control group.

RESULTS AND DISCUSSIONS

Biochemical analysis

The present study displayed that EE-R-RC possessed a significant hepatoprotective activity. The declining of plasma enzyme level is a prognostic status of the hepatoprotective action of the drug. Protection of hepatic damage caused by carbon tetrachloride administration was observed by recording SGOT, SGPT, SALP and Serum bilirubin levels in different groups [40]. The transport function of the hepatocytes is disturbed in hepatic injury, causing the leakage of enzymes due to altered membrane permeability [41].

The in vivo experimental data displayed that the elevated levels of SGOT, SGPT, ALP and Sr. bilirubin were mainly due to CCl₄ intoxication, reduced significantly (*P<0.05) in
rats, after treatment with EE-R-RC. Treatment with EE-R-RC at a dose of 100 mg/kg b. w. decreased the SGOT: 11.69%, SGPT: 22.50%, ALP: 3.64%, and Serum bilirubin levels by 32.74%, (significantly) respectively, while at higher dose of 200 mg/kg b. wt. was more effective, causing a reduction of SGOT: 24.30%, SGPT: 41.09%, SALP: 11.97% and Sr. bilirubin: 47.16%, Silymarin is used as standard drug showed a significant reduction of level of SGOT: 53.35%, SGPT: 48.43%, SALP: 47.43% and Sr. bilirubin: 75.35% respectively receiving CCl₄ alone.

**Histopathological analysis**

The results of FANB of liver cells of rats of control, CCl₄ treated and treated with EE-R-RC were represented in fig. FANB of liver cells of rats revealed that the liver treated with CCl₄ showed a high degree of damage characterized by piecemeal necrosis and portal tract necrosis, interface hepatitis due to expanded portal tract by infiltration of lymphocytes, plasma cells and macrophages, fulminant necrosis which is characterized by sub massive and massive necrosis.

**Table-1: for the assessment of serum biochemical parameters**

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT IU/L</th>
<th>SGPT IU/L</th>
<th>SALP IU/L</th>
<th>Sr. bilirubin mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>49.21±0.352</td>
<td>47.11±0.1539</td>
<td>46.91±0.1536</td>
<td>0.696±0.00173</td>
</tr>
<tr>
<td>Group II</td>
<td>198.3±0.203***</td>
<td>204.4±0.1045***</td>
<td>290.5±1.398***</td>
<td>6.172±0.1012***</td>
</tr>
<tr>
<td>Group III</td>
<td>175.1±0.1432***</td>
<td>158.4±0.0821***</td>
<td>279.9±0.1887***</td>
<td>4.151±0.04351***</td>
</tr>
<tr>
<td>Group IV</td>
<td>150.1±0.0514***</td>
<td>120.4±0.0632***</td>
<td>255.7±0.0756***</td>
<td>3.261±0.05351***</td>
</tr>
<tr>
<td>Group V</td>
<td>92.49±0.1401***</td>
<td>105.4±0.1443***</td>
<td>152.7±0.1328***</td>
<td>1.521±0.222***</td>
</tr>
</tbody>
</table>

**Table-2: for the decrease percentage (%) of serum biochemical parameters**

<table>
<thead>
<tr>
<th>Group</th>
<th>SGOT (%)</th>
<th>SGPT (%)</th>
<th>SALP (%)</th>
<th>Sr. bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group III</td>
<td>11.69</td>
<td>22.50</td>
<td>3.64</td>
<td>32.74</td>
</tr>
<tr>
<td>Group IV</td>
<td>24.30</td>
<td>41.09</td>
<td>11.97</td>
<td>47.16</td>
</tr>
<tr>
<td>Group V</td>
<td>53.35</td>
<td>48.43</td>
<td>47.43</td>
<td>75.35</td>
</tr>
</tbody>
</table>
EVALUATION OF IN VIVO HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF ROOTS OF RICINUS COMMUNIS

Fig-1: Reduction of serum enzyme profile in treatment groups

Fig-2: Reduction of serum bilirubin in treatment groups
Fig-3: Percentage reduction of serum enzymes and bilirubin level in treatment groups

Fig-3: FNAB of the rat liver stained with H and E: (A) control rats, (B) carbon tetrachloride-intoxicated rats showing focal areas with massive degeneration, necrosis and inflammatory cellular infiltration, (C) carbon tetrachloride-intoxicated rats treated with EE-R-RC(100 mg/kg) alone revealing marked improvement of hepatocellular degeneration, (D) carbon tetrachloride-intoxicated rats treated with EE-R-RC(200 mg/kg) displayed few areas of little hepatic cells degeneration and focal areas of cellular infiltration improvement,
but still there are scattered areas of degeneration and (E) carbon tetrachloride intoxicated rats treated with silymarin (50 mg/kg) showing moderate improvement of hepatocellular degeneration.

CONCLUSION

From the above experimental data, here we concluded that the EE-R-RC contained various bioactive molecules which were confirmed by their qualitative confirmatory chemical tests and displayed the potential ability to restore as well as regenerate hepatocytes which were intoxicated with CCl₄ in rat model.

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How to cite this article:
Asish Bhaumik, Ch. Ram Naresh, P. Kalyani, K. Krishnamachary; Evaluation of in vivo hepatoprotective activity of ethanolic extract of roots of Ricinus Communis (EE-R-RC) against CCL4 induced rat model; Panacea Journal of Pharmacy and Pharmaceutical Sciences 2018:7(3);83-98.