

## Antihepatotoxicity of *Ricinus communis* (L.) Against Ketoconazole Induced Hepatic Damage

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**Abstract:** Since chronic hepatic diseases stand as one of the foremost health troubles worldwide, with liver cirrhosis and drug induced liver injury leading to death in western and developing countries. Treatment of liver diseases with plant-derived compounds which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive. The present study was carried out to screen and evaluate the hepatoprotective activity of ethanolic leaf extracts of *Ricinus communis* (L.) at a dose of 100 mg / kg body weight against Ketoconazole (Phytoral) induced liver damage in mice. The degree of protection was measured by performing various liver function tests. Results indicated that ethanolic extracts of *Ricinus communis* (L.) at a dose of 100 mg / kg body weight exhibited relative significant reduction in hepatic enzymes of treated mice. The results of the present investigation confirms the traditional uses of this plant as a potential hepatoprotective agent. The present study revealed that *Ricinus communis* Linn. could be formulated as a drug of choice since this medicinal herb is easily available in southern India.

**Key words:** *Ricinus communis* • Antihepatotoxicity • Ethanolic extract • Liver function tests

### INTRODUCTION

Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. One of the important and well-documented uses of plant-products is their use as hepatoprotective agents. Hence, there is an ever increasing need for safe hepatoprotective agent [1]. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity [2].

Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. Several hundred plants have

been examined for use in a wide variety of liver disorders [3]. The use of herbal medicines in an evidence-or science-based approach to the treatment and prevention of disease is known as phytotherapy flourishing the quest for significant source of significant source of synthetic and herbal drugs [4].

In India, more than 87 plants are used in 33 patented and proprietary multiingredient plant formulations and about 40 polyherbal commercial formulations reputed to have hepatoprotective action are being used. It has been reported that 160 phytoconstituents from 101 plants have hepatoprotective activity [5]. Herbal-based therapeutics for liver disorders has been in use in India for a long time and has been popularized world over by leading pharmaceuticals. Despite the significant popularity of several herbal medicines in general and for liver diseases in particular, they are still unacceptable treatment modalities for liver diseases. The limiting factors that contribute to this eventuality are (i) lack of standardization of the herbal drugs; (ii) lack of identification of active ingredient (s)/principles(s); (iii) lack of randomized controlled clinical trials (RCTs) and (iv) lack of toxicological evaluation [6].

The present study was undertaken due to lack of knowledges on hepatoprotective activity of leaves of *Ricinus communis* (L.).

## MATERIALS AND METHODS

**Collection of Plant:** *Ricinus communis* Linn. were collected in June 2009 from Vattapara, Palakkad and Eachinary, Tamilnadu.

**Preparation of Plant Material:** The collected plants were washed and the leaves were separated. The plant material was dried under sunshade and ground into fine powder using electric blender. The powder was weighed and used for hydro alcoholic extract preparation [7].

### Preparation of Plant Extract

**Hydroalcoholic Extraction:** The *Ricinus communis* Linn. leaf powder was weighed and 100gm was placed in a mixture of 500ml ethanol and 500 ml distilled water for 7 days. Periodic shaking was done for each extract. After 7 days each suspension was filtered twice through sterile muslin cloth. The filtrate thus obtained was filtered once through Whatman No.1 filter paper.

**Hepatoprotective Activity:** The animals purchased from Karpagam University Animal house, were kept one week prior to the experiments, housed in plastic cages fed with standard commercial laboratory diet and water. The body weight and food intake of each group were recorded. The study was conducted after obtaining institutional animal ethical committee clearance [8].

**Experimental Design:** A total of 24 mice aged 5 weeks weighing 65-80 grams were equally divided into four groups each containing three male and three female. The animal feed was stopped 18 hours prior to administration [9].

**Group 1:** Served as normal control and received 2 ml / kg saline daily for four days orally.

**Group 2:** Served as induction control and received 100 mg / kg Ketoconazole (Phytoral) for four days intraperitonally.

**Group 3:** Served as standard and received 100 mg / kg Ketoconazole (Phytoral) followed by 2 ml / kg Liv 52 leaving an interval of 1 hour.

**Group 4:** Served as test and received 100 mg / kg Ketoconazole (phytoral) followed by 100 mg / kg *Ricinus communis* Linn. leaving an interval of 1 hour.

**Collection of Blood:** At the end of four days treatment all experimental animal were denied their feed and water for at least 18 hours before they were again weighed and anesthetized with chloroform. Blood samples were collected by cardiac puncture, allowed to clot and centrifuged at 1000 rpm for 5 minutes to obtain the serum [10].

### Liver Functions Tests

**Estimation of Total Bilurubin:** Total Bilurubin were determined using the kit of Thermo Electron Corporation by Acid Diazo method. The total Bilurubin reagent is a modification of the Pearlman and Lee method in which a surfactant is used as a solubiliser. Conjugated and solubilised unconjugated Bilurubin react with diazotized sulphanic acid to produce an acid azobilurubin, the absorbance of which is proportional to the concentration of Bilurubin in the sample and can be measured at 550nm [11].

**Estimation of Total Protein:** Total protein was estimated using the kit of Biosystems reagents and instruments by biuret method. Protein in the sample reacts with copper (II) ion in alkaline medium forming a coloured complex that can be measured by spectrophotometry [12].

**Estimation of Albumin:** Albumin was estimated using the kit of Thermo Electron Corporation by BCG method. The method is based on [13] in which Albumin binds with BCG causing a shift in the absorption spectra of the dye. The dye- Albumin complex formed has an absorbance peak at 625nm which is proportional to the concentration of albumin in the sample.

**Estimation of Globulin:** Globulin content in serum was estimated using the following calculation:

Globulin content = Total protein content- Albumin content [12].

**Estimation of A/G Ratio:** A /G ration in serum was estimated using the following calculation:

A/G ratio = Albumin content/Globulin content [13].

**Estimation of Aspartate Aminotransferase (AST/GOT):**

Aspartate aminotransferase was assayed using the kit of Biosystems reagents and instruments by Kinetic method. Aspartate aminotransferase catalyses the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxaloacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340nm by means of the malate dehydrogenase (MDH) coupled reaction [14].

**Estimation of Alanine Aminotransferase (ALT/GPT):**

Alanine aminotransferase was assayed using the kit of Biosystems reagents and instruments by Kinetic method. Alanine aminotransferase catalyses the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340nm by means of the lactate dehydrogenase (LDH) coupled reaction [14].

**Estimation of Alkaline Phosphatase (ALP):** Alkaline phosphatase was assayed using the kit of Diasys-Diagnostic systems international by Kinetic method. Kinetic photometric test was carried out according to the international federation of clinical chemistry and laboratory medicine (IFCC) [15].

**Estimation of Gamma- Glutamyl Transferase ( $\gamma$ -GT):**

Gamma- glutamyl transferase was assayed using the kit of Biosystems reagents and instruments by Kinetic method.

Gamma- glutamyl transferase catalyses the transfer of the  $\gamma$ - glutamyl group from  $\gamma$ - glutamyl- 3- carboxy- 4- nitroanilide to glycylglycin, liberating 3- carboxy- 4- nitroanilide. The catalytic concentration is determined from the 3- carboxy- 4- nitroanilide formation [16].

**Statistical Analysis:** The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The values are expressed as mean  $\pm$  SEM and  $p < 0.05$  was considered significant [17].

**RESULTS AND DISCUSSION**

Ketoconazole is used to treat fungal infections of the mouth, skin or nails, urinary tract and blood. Damage to the liver is reported in taking over dose or long term dose of Ketoconazole(200-400 mg/day).

The Liver function tests were showed in Table 1 & 2 and overviewed in Fig. 1, 2, 3, 4 & 5. Group 1 was maintained as normal control and was administered with saline. Here, neither *Ricinus communis* Linn. (Test control) nor Ketoconazole (Induction control) were administered and the values indicated as of healthy mice which could be compared with the other groups (Figure 1). Group 2 was administered with Ketoconazole intraperitoneally and its liver function test values were plotted. The levels of enzymes were increased significantly and the levels of proteins vice versa which indicated the significant liver damage (Figure 2).

Table 1: Liver function tests(estimation of enzymes) of Group 1 to 4

Groups (n=6)	Bilirubin mg/dl	SGOT IU/L	SGPT IU/L	ALP IU/L	$\gamma$ GT IU/L
Group 1 <sup>a</sup>	0.2 $\pm$ 0.00 <sup>a</sup>	429 $\pm$ 6.99 <sup>a</sup>	95.83 $\pm$ 2.32 <sup>a</sup>	64.33 $\pm$ 5.35 <sup>a</sup>	2 $\pm$ 0.00 <sup>a</sup>
Group 2 <sup>b</sup>	0.25 $\pm$ 0.05 <sup>b</sup>	1033.67 $\pm$ 251.30 <sup>b</sup>	109.67 $\pm$ 19.58 <sup>b</sup>	69.5 $\pm$ 17.66 <sup>b</sup>	4.33 $\pm$ 0.52 <sup>b</sup>
Group 3 <sup>c</sup>	0.1 $\pm$ 0.00 <sup>c</sup>	415.17 $\pm$ 52.50 <sup>a</sup>	59.83 $\pm$ 5.56 <sup>c</sup>	36.83 $\pm$ 10.46 <sup>c</sup>	1.5 $\pm$ 0.55 <sup>c</sup>
Group 4 <sup>d</sup>	0.1 $\pm$ 0.00 <sup>c</sup>	325.33 $\pm$ 23.60 <sup>a</sup>	47.5 $\pm$ 5.75 <sup>c</sup>	37 $\pm$ 4.38 <sup>c</sup>	1.33 $\pm$ 0.52 <sup>c</sup>
P<0.05	0.043	178.32	15.26	5.67	0.427

Note: SGOT- Aspartate aminotransferase; SGPT- Alanine aminotransferase; ALP- Alkaline phosphatase;  $\gamma$  GT- Gamma glutamyl transferase; Group 1- Saline, Group 2- Induction; Group 3- Standard; Group 4- Test. Values are mean $\pm$ Standard deviation of six samples Means, followed by a common superscript are not significant at 5 % level by using DMRT analysis ( $p < 0.05$ )

Table 2: Liver function tests(estimation of protein) of Group 1 to 4

Groups (n=6)	Total protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	A / G ratio (gm/dl)
Group 1 <sup>a</sup>	6.33 $\pm$ 0.12 <sup>a</sup>	3.55 $\pm$ 0.14 <sup>a</sup>	2.72 $\pm$ 0.04 <sup>a</sup>	1.43 $\pm$ 0.05 <sup>a</sup>
Group 2 <sup>b</sup>	5.87 $\pm$ 0.28 <sup>b</sup>	3.52 $\pm$ 0.12 <sup>b</sup>	2.33 $\pm$ 0.04 <sup>b</sup>	1.53 $\pm$ 0.05 <sup>b</sup>
Group 3 <sup>c</sup>	5.68 $\pm$ 0.08 <sup>b</sup>	3.45 $\pm$ 0.05 <sup>c</sup>	2.23 $\pm$ 0.12 <sup>c</sup>	1.58 $\pm$ 0.12 <sup>c</sup>
Group 4 <sup>d</sup>	5.71 $\pm$ 0.16 <sup>b</sup>	3.57 $\pm$ 0.05 <sup>a</sup>	2.12 $\pm$ 0.15 <sup>c</sup>	1.7 $\pm$ 0.11 <sup>d</sup>
P<0.05	0.278	0.012	0.08	0.035

Note: Group 1- Saline, Group 2- Induction; Group 3- Standard; Group 4- Test. Values are mean  $\pm$  Standard deviation of six samples Means, followed by a common superscript are not significant at 5 % level by using DMRT analysis ( $p < 0.05$ )

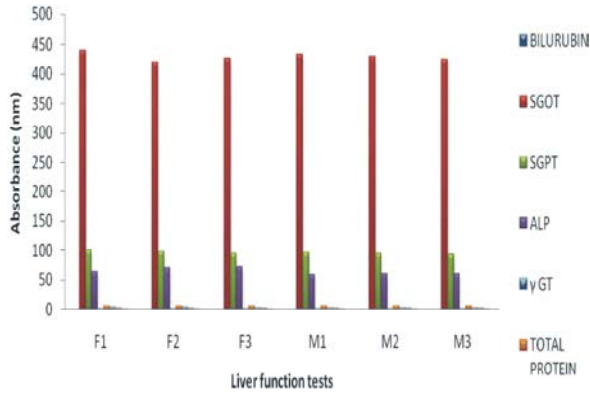


Fig.1. Liver function tests of Group 1 (Normal control)

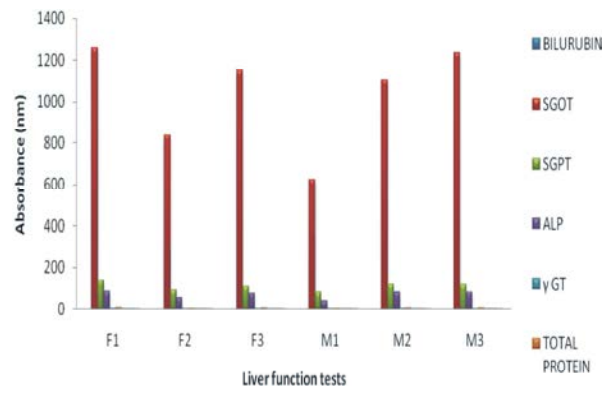


Fig. 2: Liver function tests of Group 2 (Induction control)

Note: (Figure 1 & 2): SGOT- Aspartate aminotransferase; SGPT- Alanine aminotransferase; ALP- Alkaline phosphatase;  $\gamma$  GT- Gamma glutamyl transferase; F1- Female 1; F2- Female 2; F3- Female 3; M1- Male 1; M2- Male 2; M3- Male 3

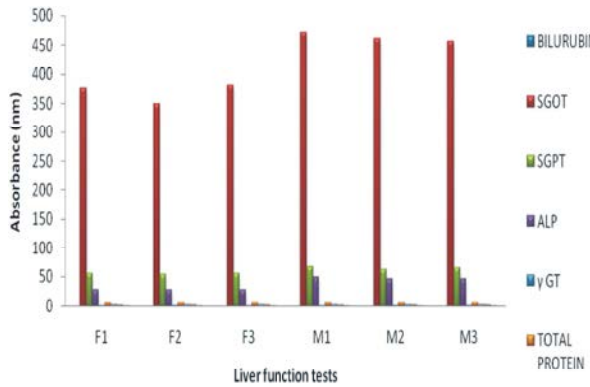


Fig. 3: Liver function tests of Group 3 (Standard)

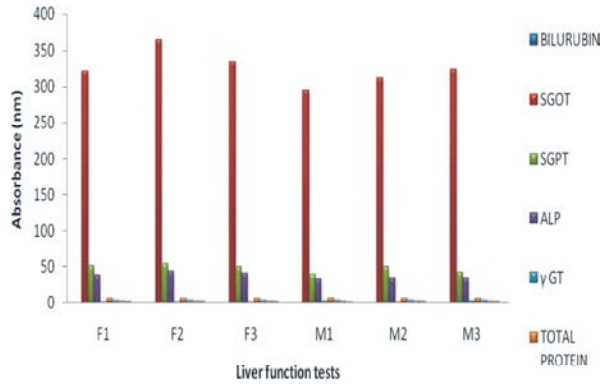


Fig. 4: Liver function tests of Group 4 (Test)

Note: (Figure 3 & 4): SGOT- Aspartate aminotransferase; SGPT- Alanine aminotransferase; ALP- Alkaline phosphatase;  $\gamma$  GT- Gamma glutamyl transferase; F1- Female 1; F2- Female 2; F3- Female 3; M1- Male 1; M2- Male 2; M3- Male 3

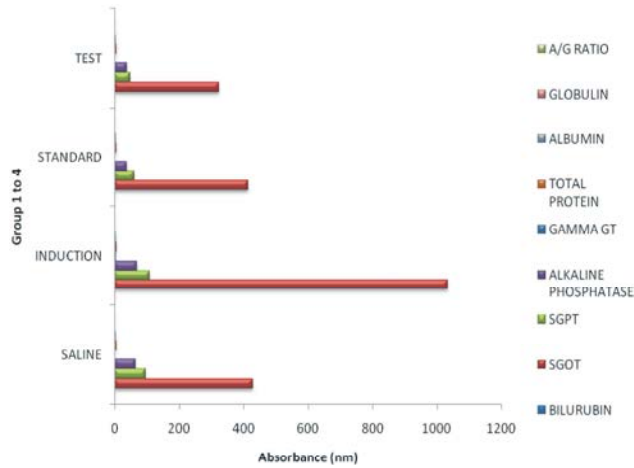


Fig. 5: Comparison of Liver function tests from Group 1 to 4 (average)

Note: (Figure 5): SGOT- Aspartate aminotransferase; SGPT- Alanine aminotransferase;  $\gamma$  GT- Gamma glutamyl transferase; A/G-Albumin/Globulin

Group 3 was administered with Ketoconazole and then with Liv-52 (standard hepatoprotective herbal tonic) which resulted in significant decrease in the enzyme levels and increase in the protein levels. The values observed were similar to the values observed in normal control (Group 1) which indicated the hepatoprotective activity of Liv-52 (Figure 3).

[18] reported that Liv-52 has shown more significant hepatoprotective activity against CCL 4 -induced hepatotoxicity in rats in comparison to Livomyn. The hepatoprotective activity of two marketed formulations, Liv-52 and Livomyn were studied. The formulations showed significant hepatoprotective effect by reducing elevated serum enzyme levels such as glutamate pyruvate transaminase (SGPT), glutamate oxaloacetate transaminase (SGOT), bilirubin content (direct and total) and total protein.

Group 4 was administered with Ketoconazole and then with *Ricinus communis* Linn. When treated with ethanolic extract of *Ricinus communis* Linn. the level of aspartate aminotransferases, alanine aminotransferases, bilirubin and alkaline phosphatase got significantly reduced; total protein and albumin vice versa. This signifies that the enzymes and protein of the *Ricinus communis* Linn. administered liver has been brought back to normal values with liver recovery (Figure 4).

Similar observations were reported by [19] that several hundred plants have been examined for use in a wide variety of liver disorders. Just handfuls have been fairly well researched *Ricinus communis* Linn. has been shown to influence several metabolic as well as histochemical activities in the human body [20]. *Ricinus communis* Linn. (leaf extract) was evaluated for hepatoprotective, choleric and anticholestatic activity. In a preliminary test with albino rats, an ethanol extract showed significant protection against galactosamine-induced hepatic damage [21].

The average of the values observed in each group and liver function tests were plotted in a line chart which clearly indicated that there were no significant difference in the enzyme and protein content of normal control (Group 1), Standard (Group 3) and Test (Group 4) when compared to Induction (Group 2). This clearly showed that *Ricinus communis* Linn. and Liv-52 were hepatoprotective against the hepatotoxicity of the Ketoconazole (Figure 5).

From the statistical analysis of the Liver function tests of all groups, it is clear that the mean values obtained from Bilirubin, Aspartate aminotransferase,

Alanine aminotransferase, Alkaline phosphatase, gamma glutamyl transferase, Albumin and Globulin of Group 1 (Normal control) is having significant difference with Group 2 (Induction), Group 3 (Standard) and Group 4 (Test); also Group 3 and 4 are having significant difference with Group 2. In the mean values obtained from Total protein, there was a significant difference for Group 1 with Group 2, 3 and 4; also Group 2, 3 and 4 were not having significant difference within themselves. In the mean value of A/ G ratio, there was a significant difference within all the groups. When Liver function tests are considered together, Group 2 had a high significance with Group 1, 3 and 4 concluding Induction of Liver damage in this group. Group 3 and 4 were not having high significance that concludes *Ricinus communis* Linn. is hepatoprotective as that of the standard Liv-52 (Table 1 & 2).

The present study, hepatoprotective effect of *Ricinus communis* Linn. is evidenced by the improvement ALT, AST and ALP levels. Previous studies have reported elevations of transaminases after APAP treatment [22]. The increase is time dependent with significant elevation noted after 48 h ( $p < 0.05$ ) suggesting severe hepatocellular damage caused by leakage of these enzymes into circulation that is normally cytoplasmic in location [23].

Since in this present investigation, there was significant hepatoprotective activity for *Ricinus communis* Linn. Leaf extracts, purification of the bioactive compounds of the plant extracts might be further carried out and could be formulated as a drug of choice with genetic engineering strategies to increase hepatoprotective activity of *Ricinus communis* Linn. and to remove the toxic biocompounds present in it. Further the drug can be formulated in combination of *Phyllanthus amarus*. In southern India, the availability and handling convenience of both the plants is more. *Phyllanthus amarus* are used in the Ayurvedic and other traditional medical systems of India for treating jaundice [24].

The Indian Traditional Medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. The association of medicinal plants with other plants in their habitat also influences their medicinal values in some cases. One of the important and well-documented uses of plant-products is their use as hepatoprotective agents. Hence, there is an ever increasing need for safe hepatoprotective agent [25].

The use of natural remedies for the treatment of liver diseases has a long history, starting with the Ayurvedic treatment and extending to the Chinese, European and other systems of traditional medicines.

The 21st century has seen a paradigm shift towards therapeutic evaluation of herbal products in liver disease models by carefully synergizing the strengths of the traditional systems of medicine with that of the modern concept of evidence-based medicinal evaluation, standardization and randomized placebo controlled clinical trials to support clinical efficacy [26]. In spite of the tremendous advances made, no significant and safe hepatoprotective agents is available in modern therapeutics. The available synthetic drugs to treat liver disorders also cause further damage to the liver. Therefore, due importance has been given globally to develop plant based hepatoprotective drugs effective against a variety of liver disorders. Hence, Herbal drugs have become increasingly popular and their use is widespread. Herbal medicines have been used in the treatment of liver diseases for a long time. A number of herbal preparations are available in the market. Many infectious microorganisms are resistant to synthetic drugs; hence an alternative therapy is very much needed. Various medicinal plants have been used for years in daily life to treat diseases all over the world [27].

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