

Role of *Emblica officinalis* (amla) in the prophylaxis of hepatic injury by carbon tetrachloride (CCl₄) in albino rats

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ABSTRACT

Background: The objective of the study was to evaluate the hepatoprotective activity of fruits extract of *Emblica officinalis* on carbon tetrachloride induced hepatotoxicity in Albino rats. Liver is vulnerable to wide variety of metabolic, toxic, microbial, circulatory and neoplastic insults. The dominant primary diseases of the liver are viral hepatitis, alcoholic liver disease and hepatocellular carcinoma. Due to absence of reliable drugs management of liver ailments in the modern medicine, plants and natural products are providing to be good hepatoprotectants as evident from voluminous published work on their hepatoprotective potentials.

Methods: The required amount of fresh fruits of *Emblica officinalis* was collected and dried at room temperature. The dried fruit ground into powdered and was prepared by percolation method using 95% ethanol. Acute oral toxicity test of the fruits extract was performed as per OECD 423 guidelines. Carbon tetrachloride was used as the hepatotoxin. Silymarin suspension 100mg was used as a standard drug in this study.

Results: The study was carried out with an attempt to evaluate the hepatoprotective activity of *Emblica officinalis* in carbon tetrachloride induced hepatic injury in albino rats. The results were analysed by using one way ANOVA followed by Dunnett's test and (p <0.05) was considered significant.

Conclusions: The present study shows that the *Emblica officinalis* has significant hepatoprotective activity in rats with carbon tetrachloride induced liver injury. *Emblica officinalis* is a commonly used traditional herb, which is safe, cost effective and grows widely in India and used by different ethnic groups for various diseases. That is why *Emblica officinalis*, a commonly used natural herb, deserve further consideration in order to establish its potential as safe economical hepatoprotective agent.

Keywords: Air tight container, Carbon tetrachloride, Drier table, Fruit extract of *Emblica officinalis*, 95% ethyl alcohol, Percolator, Silymarin

INTRODUCTION

Xenobiotic can produce a diverse group of hepatic lesions that mimic virtually any form of hepatic disease, from viral hepatitis and cirrhosis to vascular occlusion and portal hypertension. It is estimated that 5% of cases of jaundice are due to drugs, and the therapeutic agents cause 25% fulminant hepatitis.¹ *Emblica officinalis* is one of the most celebrated herbs in Indian traditional system, Ayurveda. It is found natively in India. It has been used as valuable ingredient of various medicines in India and abroad.

Emblica officinalis is a small to medium sized tree, deciduous; branchlets feathery with distichously leaves, resembling a pinnate leaf. Bark greyish or light brownish, exfoliating in irregular plates; blaze pinkish brown to reddish green at the edge, 0.25-0.5 inch thick.²

Plant extract

The plant was authenticated by Prof. M. Islam, Dept. of Life Sciences, Dibrugarh University, Assam.

The required amount of fresh fruits of *Emblica officinalis* were collected and dried in a drier table at room temperature. The dried fruit ground into a powder. Sufficient amount of powdered drug was moistened with 95% ethyl alcohol and allowed to remain for 6 hours in tightly covered container. Then they were packed in a percolator and add enough menstrum to saturate the powders. When the liquid begins to drop from the percolator, the lower orifice is closed and the percolator macerate is covered for 48 hours. Percolator was then allowed slowly with sufficient menstrum, until the drug is exhausted. Then, the residue obtained from percolation was put in vacuum desiccators.³

Animals

All the animals used in the study were procured from Central Animal House, Assam Medical College and Hospital, Dibrugarh, Assam. The study was conducted in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals) guidelines. They were fed with standard diet and water *ad libitum* was provided. Experimental animals used were healthy albino rats of the species *Rattus norvegicus* of either sex weighing 150-200gm.

Acute toxicity study

Acute toxicity test was done for the ethanolic extract of *Emblica officinalis* following OECD 425 guidelines.⁴ An arbitrary dose 200mg/kg was selected for the study, as the extract was found safe even at doses more than 2000mg/kg without any sign of toxicity or mortality.

METHODS

Silymarin tablets were collected and crushed into powder. The stock solution was prepared by dissolving 100mg of Silymarin in 5ml of normal saline and used as a standard drug in doses of 100mg/kg body weight/day.

Carbon tetrachloride 0.5ml/kg/body weight with olive oil 1:1 volume was prepared and was used as the hepatotoxin. Liver damage was induced in Albino rats with 1:1 (v/v)

mixture of carbon tetrachloride in olive oil, administered at the dose of 0.5ml/kg/body weight subcutaneously.

The experiment was carried out for a period of 14 days. For the experiment, the animals were weighted, recorded, numbered and randomly divided into 4 groups of 6 animals each.

- **Group I:** Received only normal saline 5ml/kg orally 7 days.
- **Group II:** Received carbon tetrachloride 0.5ml/kg/body weight with olive oil 1:1 volume subcutaneously for 7 days + normal saline for 7 days.
- **Group III:** Received carbon tetrachloride 0.5ml/kg/body weight with olive oil 1:1 volume subcutaneously + *Emblica officinalis* extract (EOE) 200mg/kg/ body weight orally for 7 days.
- **Group IV:** Received carbon tetrachloride 0.5ml/kg/body weight with olive oil 1:1 volume subcutaneously + Silymarin suspension 100mg/kg/body weight orally for 7 days.

RESULTS

The study was carried out with an attempt to evaluate the hepatoprotective activity of *Emblica officinalis* in the prophylaxis of hepatic injury by carbon tetrachloride in albino rats. In the study, the efficacy of *Emblica officinalis* was compared with standard known hepatoprotective agent, Silymarin. The statistical significance between groups was analysed using one way ANOVA followed by dunnett's and bonferroni test. The significance was expressed by 'p' values as mentioned in the tables. P value of <0.05 was considered significant. Table 1 shows all the parameters are not significantly increased in prophylactically treated group (group III) in comparison to carbon tetrachloride treated group (group II). But there is no significant difference between group III and group IV.

Table 1 shows all the parameters are not significantly increased in prophylactically treated group (group III) in comparison to carbon tetrachloride treated group (group II). But there is no significant difference between group III and group IV.

Table 1: One way ANOVA followed by Dunnett's test and Bonferroni test.

Group	Serum alkaline phosphatase	AST	ALT	Total protein	Albumin globulin ratio
I (control)	12.6±0.09	28±1.66	12±1.53	6.4±0.18	1.5±0.08
II CCl ₄	22.6±0.49 ^a	60±1.41 ^a	40±1.15 ^a	50±0.02 ^a	0.3±0.09 ^a
III(CCl ₄ +EO)	14.0±0.93 ^b	24±0.93 ^b	17±0.95 ^b	5.6±0.09 ^b	0.6±0.24 ^b
IV (CCl ₄ +sily)	13.4±0.09 ^b	26±1.56 ^b	16±1.56 ^b	5.8±0.12 ^b	1.3±0.09 ^b
ANOVA	F10.39 df3, 20 P<0.05	F387.59 df3, 20 P<0.05	F 67.40 df3, 20 P<0.05	F26.25 df3, 20 P<0.05	F 29.08 df3, 20 P<0.05

Values are expressed as SEM (n=6)

^ap <0.05, when compared to the Normal Control Group

^bp <0.05, when compared to the Experimental Control Group

Table 1 shows the Serum Alkaline Phosphatase level in different groups. The Serum Alkaline Phosphatase level of rat exposed to CCL₄ group II on 7th day was 22.6±0.49 (P <0.05). The Serum Alkaline Phosphatase level of group III (CCL₄ with EO) for 7 days was 14±0.93 (P <0.05). The Serum Alkaline Phosphatase level of group IV (CCL₄ with Silymarin) for 7 days was 13.3±0.09 (P <0.05). The AST level in different groups. The AST level of rat exposed to CCL₄ group II on 7th day was 60±1.41 (P <0.05). The AST level of group III (CCL₄ with EO) for 7 days was 24±0.93 (P <0.05). The AST level of group IV (CCL₄ with Silymarin) for 7 days was 26±1.56 (P <0.05). The ALT level in different groups. The ALT level of rat exposed to CCL₄ group II on 7th day was 40±1.15 (P <0.05). The ALT level of group III (CCL₄ with EO) for 7 days was 27±0.95 (P <0.05). The ALT level of group IV (CCL₄ with Silymarin) for 7 days was 16±1.56 (P <0.05). The Albumin Globulin Ratio in different groups. The Total Protein level in different groups. The Total Protein level of rat exposed to CCL₄ group II on 7th day was 50±0.02 (P <0.05). The Total Protein level of group III (CCL₄ with EO) for 7 days was 5.6±0.09 (P <0.05). The Total Protein level of group IV (CCL₄ with Silymarin) for 7 days was 5.8±0.12 (P <0.05). The Albumin Globulin Ratio in different groups. The Albumin Globulin Ratio level of rat exposed to CCL₄ group II on 7th day was 0.3±0.09 (P <0.05). The Albumin Globulin Ratio level of group III (CCL₄ with EO) for 7 days was 0.6±0.24 (P <0.05). The Albumin Globulin Ratio of group IV (CCL₄ with Silymarin) for 7 days was 1.3±0.09 (P <0.05).

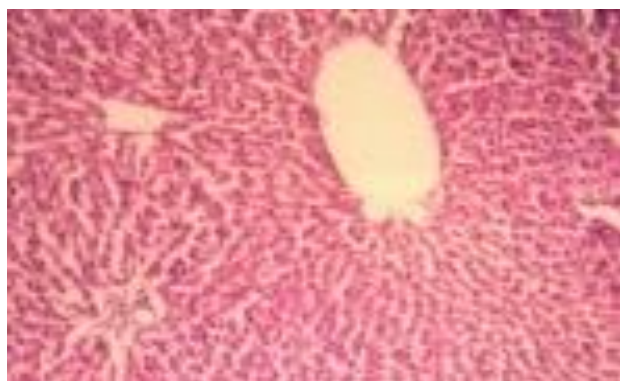


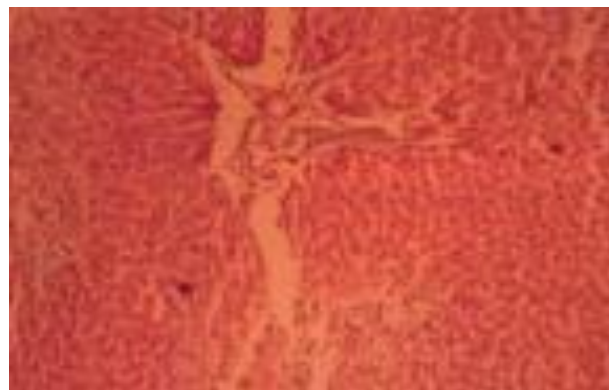
Figure 1: Normal hepatic cells.



Figure 2: CCl₄ Treated hepatic cells.

Figure 1 shows normal hepatic cells. Figure 2 shows carbontetrachloride (CCl₄) treated areas of hepatic injury.

Figure 3 shows carbontetrachloride (CCl₄) and *Emblia officinalis* treated group showing less hepatic injury than carbontetrachloride (CCl₄) treated group.



CCl₄+ *Emblia officinalis* treated shows less hepatic injury with regenerative areas

Figure 3: CCl₄+ *Emblia officinalis*.

Figure 4 shows carbontetrachloride (CCl₄) and Silymarin treated group showing less hepatic injury than only carbontetrachloride treated group (CCl₄). Both groups (CCl₄+ *Emblia officinalis* and CCl₄+Silymarin) showing less hepatic injury with some regenerative areas of hepatic cells.



CCl₄+SILYMARIN treated shows less hepatic injury with regenerative areas

Figure 4: CCl₄+Silymarin.

DISCUSSION

The purpose of the study was to evaluate the hepatoprotective activity of the alcoholic extract of the fruit of *Emblia officinalis* to a standard drug Silymarin on carbon tetrachloride induced hepatotoxicity in Albino rats.

The statistical analysis was done by using one way ANOVA test followed by Dunnet's test. Values of <0.05 were considered significant.

Carbon tetrachloride (CCl₄) is a synthetic compound also known as tetrachlormethane, is a synthetic compound formerly used in fire extinguishers and refrigeration, but now largely abandoned due to its toxicity.

The experimental intoxication induced by carbon tetrachloride (CCl₄) is widely used for modelling liver injury in rats. Hepatotoxicity is connected with severe impairment of cell protection mechanisms. The location of the liver injury is defined mainly by biotransformation of CCl₄, which is cytochrome P₄₅₀ dependent. Free radicals initiate the process of lipid peroxidation, which is generally caused of inhibition of enzyme activity.⁵

In this study liver damage produced by carbon tetrachloride was confirmed by analysis of different levels of enzymes and histopathological examination of liver when compared with control group.

The histological profile of control animal showed normal hepatocytes, the section of the liver of the group II carbon tetrachloride treated group exhibited severe intense centrilobular necrosis, vascuolization and macro vesicular fatty changes. The liver section of the animals treated with alcoholic extract of *Embllica officinalis* showed almost normal architecture as compared to CCl₄ treated group.

Antioxidant activity or the inhibition of the generation of free radicals is important against CCl₄ induced liver lesion.⁶

Flavonoids are well known for their antioxidant and hepatoprotective activities.⁷ Quercetin a bioflavonoid prevent in *Embllica officinalis* has been reported to prevent cytotoxicity in isolated hepatocytes by CCl₄.⁸

The tannoids of *Embllica officinalis* have been reported to enhance reactive oxygen species (ROS) scavenging activity in rat brain frontal cortex and straiatum enhancing the concentrations of the antioxidant enzymes SOD, catalase (CAT) and glutathione peroxidise (GPX) resulting in reduced lipid peroxidation.⁹

Phytochemicals screening revealed that *Embllica officinalis* contains active pharmacological constituents such as flavonoids, alkaloids, phytosterols and phenolic compounds. However, it has been already reported that such phytoconstituents like phenolic compounds, flavonoids, tannins are known to posses' hepatoprotective activity in various experimental models. Therefore, it has been suggesting that the hepatoprotective activity may be due to these active phytoconstituents present in the plant which is being also confirmed by the biochemical and histological parameters.¹⁰

Several other compounds like polyphenols, ellagic acid, gallic acid and tannins are present in very high amount along with vitamin C.¹¹

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Animal Ethics Committee (634/02/a/CPCSEA)

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