STUDY OF HEPATOPROTECTIVE ACTIVITY OF EMBLICA OFFICINALIS (AMLA) IN ALBINO RATS

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ABSTRACT

BACKGROUND

The study has been undertaken to evaluate the hepatoprotective activity of fruits extract of Emblica officinalis on carbon tetrachloride-induced hepatotoxicity on Albino rats.

MATERIALS AND METHODS

The present study was conducted in the Department of Pharmacology. The required amount of fresh fruits of Emblica officinalis were collected and prepared by percolation method using 90% ethyl alcohol. Oral toxicity test was performed as per OECD 423 guidelines. Liver damage was induced in Albino rats with carbon tetrachloride at the dose of 0.5 mL/kg/body weight subcutaneously. All the animals used for the experiment were kept under observation for daily food intake and body weight were measured after 7 days. The drugs were administered to the animals in the dose of 200 mg/kg/body weights by means of an intragastric feeding tube. The experiment was carried out for the period of 14 days.

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 and was compared with standard drug Silymarin. The statistical significance between groups was analysed using one way ANOVA followed by Dunnett's test. The significance was expressed by 'p' values as mentioned in the tables. P value of <0.05 was considered significant.

CONCLUSION

From this study, it can be seen that Emblica officinalis has significant hepatoprotective activity in rats with CCL4-induced liver injury.

KEYWORDS

Emblica Officinalis, Ethanol, Silymarin, Carbon Tetrachloride.

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BACKGROUND

The liver is one of the heaviest organs in the body and serves the principle function of maintaining the body's internal milieu. The liver is key to fulfilling this function as almost all absorption of foreign material into the body takes place in the gut and the portal blood draining nutrients into the systemic circulation. The liver is able to store and release a variety of substrates, vitamins and minerals and plays a crucial role in drug and bilirubin metabolism. The liver is also the largest reticuloendothelial organ in the body and its situation is important in removing infecting bacteria and bacterial products, which enter the body from the gut.[1] Drug-induced hepatotoxicity is a major cause of iatrogenic disease, accounting 1 in 600 to 1 in 3500 of all hospital admission and between 2-3% of all admission due to reactions.[2]

Emblica officinalis is found throughout the tropical and subtropical India, Ceylon, China and Malay Islands. It has been used as valuable ingredient of various medicines in India and abroad. Emblica officinalis (amla) is a small-to-medium sized tree, deciduous branchlets, feathery with distichous leaves, resembling a pinnate leaf. Dark greyish or light brownish, exfoliating in irregular plates, blaze pinkish brown to reddish green at the edge, 0.25-0.5 inch thick.[3] Apart from traditional uses, there are several reports on biological and pharmacological actions of amla based on modern scientific investigation especially as a novel therapy for acute pancreatitis,[4] antioxidant,[5] antitumour,[6] cytoprotective activity against chromium-induced oxidative injury in murine macrophages[7] and hepatoprotective[8] medicinal activity. Through pharmacological experiments on various plants used in traditional medicines are in order to establish their effectiveness and safety. Keeping in view above idea, the present study is undertaken to evaluate the hepatoprotective activity of Emblica officinalis (amla)

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fruit extract on carbon tetrachloride or CCL4-induced widely used in Indian medicine for treatment of various diseases.

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

Aim and Objectives

Hepatoprotective activity of fruit extract of Emblica officinalis (amla) as very few studies on Emblica officinalis (amla) have been conducted, therefore, the present study has been undertaken.

(i) To study the therapeutic activity of ethanolic fruit extract of Emblica officinalis (amla) on carbon tetrachloride-induced hepatotoxicity.

(ii) To compare the hepatoprotective activities of Emblica officinalis (amla) to a standard drug Silymarin on carbon tetrachloride-induced hepatotoxicity.

MATERIALS AND METHODS

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 powered drug was moised with 90% ethyl alcohol and allowed to remain for 6 hours in tightly covered container. Then, they were packed in a percolator and add enough menstruum 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 menstruum until the drugs is exhausted. Then, the residue obtained from percolation was put in a vacuum desiccators.[9]

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 and the study was approved by the Institutional Animal Ethical Committee (Registration No.-634/02/a/CPCSEA). 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 150-200 gm.

Acute toxicity test was done for the ethanolic extract of Emblica officinalis following OECD 425 guidelines.[10] An arbitrary dose 200 mg/kg was selected for the study as the extract was found safe even at doses more than 2000 hepatotoxicity in Albino rats. Emblica officinalis (amla) is mg/kg without any sign of toxicity or mortality. Silymarin tablets were collected and crushed into powder. The stock solution was prepared by dissolving 100 mg of Silymarin in 5 mL of normal saline and used as a standard drug in doses of 100 mg/kg body weight/day.

Carbon tetrachloride 0.5 mL/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 0.5 mL/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 5 mL/kg orally 14 days.

Group II- Received carbon tetrachloride 0.5 mL/kg/body weight with olive oil 1:1 volume subcutaneously for 7 days + normal saline from 8-14 days.

Group III- Received carbon tetrachloride 0.5 mL/kg/body weight with olive oil 1:1 volume subcutaneously 7 days and Emblica Officinalis Extract (EOE) 200 mg/kg/body weight orally for next 7 days.

Group IV- Received carbon tetrachloride 0.5 mL/kg/body weight with olive oil 1:1 volume subcutaneously for 7 days and Silymarin suspension 100 mg/kg/body weight orally for next 7 days.

All the animals used for the experiment were kept under observation for daily food intake and body weights were measured again after 7 days. The drugs were administered to the animals in the dose mg/kg/body weights by means of an intragastric feeding tube.

On the 15th day, six animals from groups I, II, III and IV were selected at a time for estimation of liver function. After collection of blood, the animals were selected to collect liver tissue for histopathological examination.

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 and was compared with standard drug Silymarin.

The statistical significance between groups was analysed using one way ANOVA followed by Dunnett's test. The significance was expressed by 'p' values as mentioned in the tables. P value of <0.05 was considered significant.

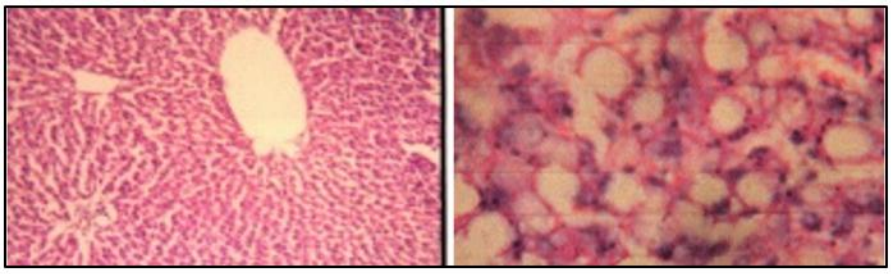

Figure 1 ***Figure 2***

Fig. 1) Normal; Fig. 2) CCL4 then Vehicle Treated Showing No Regenerative Areas

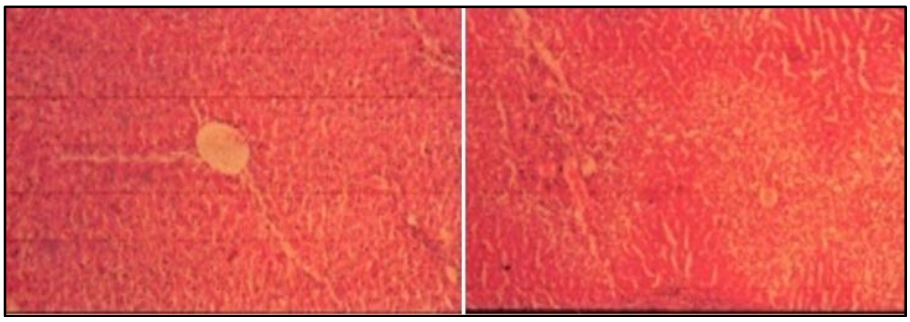

Figure 3. CCL4 then Emblica Officinalis Showing Normal Hepatocytes with Regenerative Areas

In acute oral toxicity tests, the LD50 of Emblica officinalis to be more than 2000 mg/kg.

Liver Function Test Results

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 CCL4 7 days and normal saline next 7days	25 ± 1.85[a]	56± 1.18[a]	24 ± 1.18[a]	4.6 ± 0.17[a]	0.7 ± 0.19[a]
III (CCL4 7 days and EO next 7 days)	15 ± 0.90[b]	45 ± 0.93[b]	12 ± 0.57[b]	6.7 ± 0.16[b]	0.9 ± 0.18[b]
IV (CCL4 7 days and sily next 7 days)	20 ± 1.66[c]	32 ± 1.15[c]	16 ± 0.79[c]	5.9 ± 0.21[c]	1.1 ± 0.12[c]
ANOVA	F10.39 df3,20 P<0.05	F387.59 df3,20 P<0.05	F67.40 df3,20 P<0.05	F26.25 df3,20 P<0.05	F29.08 df3,20 P<0.05

Table 1. Results Expressed in Mean ± SEM (n=6)

Values are expressed as SEM (n=6).

One way ANOVA followed by Dunnett's test.

[b]p<0.05, when compared with CCL4 group (III-II).

[c]p<0.05, when compared with CCL4 group (IV-II).

[b]p<0.05, when compared with group (III-IV).

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

DISCUSSION

The purpose of the study was to evaluate the hepatoprotective activity of the alcoholic extract of the fruit of Emblica officinalis to a standard drug Silymarin on carbon tetrachloride-induced hepatotoxicity in Albino rats. Bhattacharyya D et al (2003) reported that Himoliv (HV) is a multi-herbal formulation containing Picorrhizakurroa, Boerhavia diffusa, Tinospora cordifolia, Andrographis paniculata, Phyllanthus Emblica were earlier investigated for their protective effects against different models of experimental hepatotoxicity. They reported hepatoprotective activity against 1 mL/kg and paracetamol 1 mg/kg induced hepatic damage in the dose (0.5 mL/kg) and (1 mL/kg) body weight orally showed significant decrease level of SGOT, SGPT and alkaline phosphate. Gulati RK et al (1995) reported that Phyllanthus emblica (100 mg/100 gm) and quercetin (15 mg/100 gm) along with Country Made Liquor (CML) showed significant decrease in elevated levels of SGPT, cholesterol and total lipids in serum. In histopathology, hepatic architecture was maintained in CMML treated group, but there were moderate fatty changes with occasional fat cysts. In Phyllanthus emblica treated group, 60% rats showed protection with reduced fatty changes as compared to CML treated group. In 40% rats, mild fatty changes were seen with few fat cysts. In quercetin-treated animals, histopathology revealed mild changes as compared to CML-treated group in all the animals.[11] Carbon Tetrachloride (CCL4) is a synthetic compound also known as tetrachloromethane 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 P_{450} dependent, free radicals initiate the process of lipid peroxidation, which is generally caused of inhibition of enzyme activity.[12]

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, vacuolisation and macrovesicular fatty changes. The liver section of the animals treated with alcoholic extract of Emblica 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.[13]

Flavonoids are well known for their antioxidant and hepatoprotective activities.[14] Quercetin, a bioflavonoid prevent in Emblica officinalis has been reported to prevent cytotoxicity in isolated hepatocytes by CCL4.[15]

The tannoids of Emblica officinalis have been reported to enhance Reactive Oxygen Species (ROS) scavenging activity in rat brain frontal, cortical and striatal concentrations of the antioxidant enzymes SOD, Catalase (CAT) and Glutathione Peroxidise (GPX) resulting in reduced lipid peroxidation.

GC-MS chromatogram of leaves of methanolic extract of Emblica officinalis showed four major peaks indicating the presence of 1,2,3-benzenetrial (synonym- pyrogallol), 2-furancarboxyaldehyde, 5-(hydroxymethyl)-(synonym:5-hydroxymethylfurfural), 2-acetyl-5 methylfuran ((synonym-5-methyl-2-furylmethylketone), benzoic acid 3,4,5-trihydroxy- (synonym- gallic acid) were the major components in the extract. The phytochemicals that contribute to the medicinal property of the plant.[16]

Phytochemicals screening revealed that Emblica 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 possess hepatoprotective activity in various experimental models. Therefore, it has been suggested 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.[17]

In the present study, the hepatoprotective activity of Emblica officinalis against CCL4-induced liver injury in Albino rats was probably due to the antioxidant activity present in it.

CONCLUSION

From this study, it can be seen that Emblica officinalis has significant hepatoprotective activity in rats with CCL4-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. Few studies have been undertaken to fully evaluate the molecular and biochemical basis of its pharmacological action as a hepatoprotective. That is why Emblica officinalis, a commonly used natural herb, deserve further consideration in order to establish its potential as a safe economical hepatoprotective agent.

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