ORIGINAL ARTICLE

Hepatoprotective effect of the pulp/seed of *Aegle Marmelos* correa ex Roxb against carbon tetrachloride induced liver damage in rats

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A number of herbal preparations are widely used in traditional system of medicine for the management of hepatic disorders. However, many of them have not been investigated for their described effects. *Aegle marmelos* Roxb is one such drug used in the treatment of hepatitis in folk medicine. Therefore, an attempt has been made to investigate for hepatoprotective effect of fruits of *Aegle marmelos* against carbon tetrachloride (CCl_4) induced hepatotoxicity in rats. Sixty Albino Wistar rats were divided into six equal groups of 10. Four groups received extracts of pulp/seeds of *Aegle marmelos* and intraperitoneal (i.p.) CCl_4 (0.2 ml/100 g) either before or after administration of pulp/seeds. Two groups were controls, one treated with CCl_4 and one with normal saline. Liver damage was assessed by plasma concentration of bilirubin and enzyme activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase. Treatment with aqueous extract of fruit pulp/seeds significantly reduced CCl_4-induced elevation in plasma enzyme and bilirubin concentration in rats. This study suggests that CCl_4-induced liver damage in rats can be ameliorated by treatment of extracts from fruits pulp/seeds.

Key words: *Aegle marmelos*, aminotransferases, carbon tetrachloride, hepatoprotective

INTRODUCTION

Liver is the key organ for detoxification and disposition of endogenous substances. It is continuously and widely exposed to xenobiotics, hepatotoxins, and chemotherapeutic agents that lead to impairment of its functions.[1] *Aegle marmelos* Roxb. (Rutaceae) is known as bael in English and found throughout India in dry hilly areas, gardens, and roadsides. Bael is generally cultivated near temples and dedicated to Lord Shiva, whose worship cannot be completed without the leaves of this tree.[2] Literature survey has revealed that fruits of *Aegle marmelos* are prescribed in the treatment of tuberculosis and hepatitis but no scientific study is reported regarding its hepatoprotective activity.[3,4] Therefore, the present study was conducted to investigate its hepatoprotective activity.

MATERIALS AND METHODS

Plant Material

Ripe fruits of bael were obtained from Badhani, Pathankot, District Gurdaspur, Punjab. The plant samples were identified and authenticated in the Herbarium: by Dr. N. N. Sharma, Sri Sai College of Pharmacy, Badhani, Pathankot, Gurdaspur, Punjab, India. The voucher specimen (SSCP-105) of the collected plant sample was deposited in the Pharmacognosy Museum, Sri Sai Institute of Pharmaceutical Education and Research, Badhani, Pathankot. The pulp was manually separated from the fruit and soaked in cold distilled water (1:3 ratios, weight to volume) and kept for 48 h at a temperature of 4°C. The seeds were rinsed clear of any pulp with water and dried at room temperature. The dried seeds were then grounded into a fine powder and immersed in cold distilled water (1:3 ratio, weight to volume) for 48 h at a temperature of 4°C.

Animals

Albino Wistar rats of either sex, weighing 180-200 g, were obtained from the Experimental Animal House, Sri Sai Institute of Pharmaceutical Education and Research, Badhani, Pathankot. All animals were given standard diet and water *ad libitum*. They were maintained at a relative humidity of 65 to 86%, a temperature of 23 to 25°C, and in a schedule of 12 h of light and 12 h of dark. Rats were weighed at the beginning and end of the study. Procedures involving animals and their care were conducted in conformity with Committee for the Purpose of Control and Supervision of Experiments on Animals (Regd. No.911/ac/95/CPCSEA).

Chemicals

CCl4 was obtained from Sigma-Aldrich. All other chemicals used were of analytical grade.

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Assessment of Hepatoprotective Activity

Sixty animals were randomly divided equally into six groups of 10 each.

Group 1 (controls): received normal saline orally (0.2 ml/100 g) for 16 consecutive days.

Group 2 (pretreatment experiment-pulp): allowed free access to aqueous extract of fruit pulp *ad libitum* for 28 consecutive days and treated with *i.p.* CCl_4 on Days 14, 15, and 16 of the treatment period.

Group 3 (post-treatment experiment-pulp): given aqueous extract of fruit pulp *ad libitum* for 14 consecutive days and treated with *i.p.* CCl_4 on Days 1, 2, and 3 of the treatment period.

Group 4 (pretreatment experiment-seeds): allowed aqueous extract of fruits seeds *ad libitum* for 28 consecutive days and treated with *i.p.* CCl_4 on Days 14, 15, and 16 of the treatment period.

Group 5 (post-treatment experiment-seeds): given aqueous extract of fruits seeds *ad libitum* for 14 consecutive days and treated with *i.p.* CCl_4 on Days 1, 2, and 3 of the treatment period.

Group 6 (CCl_4-treated control): injected *i.p.* with a fresh mixture of equal volumes of CCl_4 and olive oil for three consecutive days at the dose of 0.2 ml/100 g of body weight/day.

Twenty-four hours after the last treatment, the rats were euthanized by injecting *i.p.* sodium pentobarbitone (100 g/kg). Hepatoprotective activity was calculated.[5]

Hepatoprotective activity (%) = 1 – (PC - S)/(C - S) x 100 where PC, C, and S are the measurable variables in rats treated with bael fruits pulp/seeds with CCl_4, CCl_4, and saline-treated animals, respectively.

Blood Sampling

Blood was collected in heparinized tubes from the inner canthus on the 29th day or the 16th day in the pre or post-treated groups, respectively. Plasma was separated by centrifugation at 900 rpm for 10 min at 4°C and used for determining the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and bilirubin concentration using the Hitachi autoanalyzer-902.

Statistical Analysis

Values reported are means ± SE (n = 10). Experimental results were statistically analyzed using the Student's t-test followed by ANOVA. P value less than 0.01 considered significant.

RESULTS

The CCl_4-treated animals exhibited a significant increase ($P < 0.01$) in plasma enzyme activity and bilirubin concentration compared with saline-treated control rats [Table 1]. A significant reduction was found in elevated AST, ALT, and ALP values in rats subjected to both pre- and post-treatments with the aqueous extracts of both bael fruit pulp and seeds. Liver enzyme values were higher in the four experimental groups than in the saline-treated controls, but the liver enzyme values were decreased to about half of those found in CCl_4-treated control animals for all liver function tests except bilirubin. Expressed in percentage of protection provided, both bael fruit pulp and seeds given pre- or post-treatment were hepatoprotective [Table 2].

Table 1: Effect of pre- and post-treatment with aqueous bael fruit pulp and seeds on CCl_4 induced liver damage in albino wistar rats

Treatment	Aspartate aminotransferase (U/L)	Alanine aminotransferase (U/L)	Alkaline phosphatase (U/L)	Bilirubin (µmol/L)
Control	108.6 ± 3.29	35.43 ± 1.33	156.2 ± 2.64	0.17 ± 0.03
Bael fruit pulp pre-treatment	134.6 ± 3.12	46.80 ± 1.78[a]	181.5 ± 1.98[a]	0.19 ± 0.01
Bael fruit pulp post-treatment	124.0 ± 4.27[a]	44.31 ± 0.76[a]	193.2 ± 4.82[a]	0.20 ± 0.01
Bael fruit seeds pre-treatment	163.1 ± 4.63[a]	59.60 ± 1.56[a]	180.9 ± 3.03[a]	0.21 ± 0.01
Bael fruit seeds post-treatment	158.7 ± 6.45[a]	56.90 ± 1.23[a]	171.8 ± 10.13	0.20 ± 0.00
Carbon tetrachloride	283.4 ± 2.99[a]	85.6 ±1.99[a]	253.6 ± 5.45[a]	3.30 ± 0.07[a]

Data are expressed as mean ± SE (n = 10), [a]Significantly different than control at $P < 0.01$.

Table 2: Hepatoprotective activity of bael fruit pulp and seeds in CCl_4 induced hepatotoxicity in albino wistar rats

Clinical chemistry liver function indicator	Aspartate aminotransferase	Alanine aminotransferase	Alkaline phosphatase	Bilirubin
Bael fruit pulp pre-treatment (% Protection)	83.06	76.60	75.16	99.23
Bael fruit pulp post-treatment (% Protection)	89.89	82.32	61.97	99.07
Bael fruit seeds pre-treatment (% Protection)	68.66	51.11	71.37	96.03
Bael fruit seeds post-treatment (% Protection)	71.23	54.99	82.54	99.03

% protection = 1 – (PC – S) / (C - S) x 100, where PC, C, and S are the measurable variables in rats treated with bael fruit pulp or seeds plus CCl_4, CCl_4, and saline treated animals, respectively

DISCUSSION

Liver cirrhosis induced by CCl_4 is perhaps the best-studied model of liver cirrhosis.[6] Several mechanisms underlying this toxicity have been suggested.[7] The reduction of CCl_4-induced elevated plasma activities of AST, ALT, ALP, and bilirubin level in animals pre- and post-treated with the aqueous extracts of bael fruits pulp or seeds shows their ability to restore the normal functional status of the poisoned liver and also to protect against subsequent CCl_4 hepatotoxicity. The mechanism by which the fruits pulp and seeds induces its hepatoprotective activity is not certain. The inactive metabolite (CCl_4), is transformed to a free radical through the microsomal cytochrome P-450-dependent enzyme, resulting in activation of CCl_4 toxicity. Hepatoprotective activity of any drug is the ability of its constituents to inhibit the aromatase activity of cytochrome P-450, thereby favoring liver regeneration. On that basis, it is suggested that flavonoids in *Aegle marmelos* could be a factor contributing to its hepatoprotective ability through inhibition of cytochrome P-450 aromatase.[8] In addition, hesperidin present in the fruits of bael reduces signs of hypovitaminosis C in experimental animals which in turn may also play a role in hepatoprotection. It is demonstrated that hepatic microsomal drug metabolism decreases in ascorbic acid deficiency and is augmented when high supplements of the vitamin are given to experimental animals.[9,10]

CONCLUSIONS

This study clearly demonstrates that aqueous extracts of bael fruits pulp and seeds are effective in the treatment and prevention of CCl_4-induced hepatic cytotoxicity. The data suggest that the daily oral consumption of an aqueous extract of the bael fruits and seeds as a part of the diet *ad libitum*, was prophylactic to CCl_4 poisoning, achieving about 80% protection with fruits pulp and 70% with seeds. A similar percentage protection was achieved when the aqueous extracts of the fruits pulp and seeds were used as a cure against CCl_4 poisoning after toxicity was induced. Of greater importance to the public is the effect of ingesting normal *ad libitum* of bael fruits, particularly because it is an inexpensive and effective prophylactic and/or treatment against liver cytotoxicity and a dynamic liver support.

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