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Original Article

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF 'KARIRA'-A VALUABLE AYURVEDIC PLANT

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ABSTRACT

Objective: 'Karira' is a common Sanskrit name of an important Ayurvedic medicinal plant, *Capparis decidua* Edgew. (family, Capparidaceae). It is a bushy shrub or small tree distributed throughout the dry areas of India and other parts of the world. As per traditional literature, various parts of the plant are widely used in the treatment of biliousness, jaundice, andas a liver tonic. Therefore the present study was aimed at the evaluation of hepatoprotective activity of Karira stem.

Methods: For the study, three types of extracts of *C. decidua* stem were prepared, viz. aqueous extract, alcoholic extract and 50% hydroalcoholic extract. The stem extracts were evaluated for their protective effects against carbon tetrachloride-induced acute hepatotoxicity in the rats in *in vivo* conditions.

Results: The results suggested that among all the three extracts, specifically the alcoholic extract at the dose of 300 mg/kg exhibited highest hepatoprotective activity, which was comparable to the standard drug, silymarin (50 mg/kg). The hepatoprotective effects were further confirmed by detailed antioxidant study, which revealed significant free radical scavenging activities and protection against lipid peroxidation offered by the extracts, along with preservation of the integrity of liver cells as evident from the histopathological study of the liver tissues.

Conclusion: The present investigation supports the traditional use of Karira plant in liver-related disorders.

Keywords: Karira, *Capparis decidua*, Capparidaceae, Hepatoprotective, Carbon tetrachloride, Antioxidant

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INTRODUCTION

Liver diseases, especially hepatitis, jaundice and cirrhosis have become one of the major concerns for humans of all ages due to the high rate of morbidity and mortality. In India also, Liver disorder is a common problem, where every year about more than 18,000 people are reported to die due to liver cirrhosis alone [1]. Liver diseases usually damage liver cell structures, preventing its proper physiological functioning, and decrease toxin neutralization and excretion of system and also cell repair. The dysfunction of liver can be caused by a variety of factors, which include congenital defects or abnormalities of the liver present at birth, metabolic disorders, autoimmune disorders, viral or bacterial infections, excessive alcohol consumption, decreased blood supply to liver, nutritional deficiencies, trauma or injury to hepatocytes, certain medications that are toxic to the liver or poisoning by toxins known as hepatotoxins [2]. The accumulation of membrane cytolytic bile acids damages liver cell membranes as they insert into lipid membranes. This damage is associated with the release of membrane enzymes such as alkaline phosphatase and gamma-glutamyl transpeptidase, as well as the induction of apoptosis. On the other hand, the accumulation of toxins due to the failure of the glutathione detoxification system accelerates oxidative stress, the death of hepatocytes and the progression of liver disease [3].

The drugs which are available in modern medicine only bring symptomatic relief and in most of the cases have no influence on the disease process; further their use is associated with the risks of relapses and dangers of untoward effects. Herbal medicines, used in Indian systems of medicine, are however claimed to be effective and safe in such ailments. The most successful liver-protective natural product is silymarin, a flavonolignan obtained from the 'Milk Thistle' [4]. Surprisingly, we do not have readily available satisfactory herbal formulations to treat severe liver disorders and screening of plants which are traditionally used in liver disorders may address this problem.

'Karira' is a popular sanskrit name for the plant *Capparis decidua* Edgew. belonging to Capparidaceae family. It is a bushy shrub or small tree that grows wildly in dry open wastelands and semi-arid zones of Deccan Peninsula, Western Rajasthan, Punjab, Sind, Central India, Gujarat, Arabia, Socotra, Egypt and tropical Africa [5]. Whole plant as well as individual plant parts are used in traditional medicine not only in India, but in other countries too [6]. In Ayurveda, the plant is used for treatment of jaundice and biliousness [5-8]. It is also considered to be useful as liver tonic and in the management of 'vata' [8, 9]. In Unani medicine also, the plant is used as liver tonic and prescribed for treating biliousness and improving the appetite. In Sudan, it is widely used in jaundice [5, 10].

The present study was undertaken to investigate the hepatoprotective potential of Karira stems, thereby generating the pharmacological data, whichwould support its traditional and folklore use in liver-related disorders.

MATERIALS AND METHODS

Plant material

The stems of Karira (*C. Decidua*) plant were collected from Gujarat University, Ahmedabad during the flowering season and authenticated by a taxonomist at the Department of Botany, Gujarat University, Ahmedabad. After collection, the stems were cleaned, dried at room temperature, powdered to 60# and then used for the present study.

Selection of animals

Healthy untreated Albino rats of Wistar strain of either sex weighing 200 to 250 g were selected. All the animals were housed under standard conditions of 12 h light and dark cycle, at an ambient temperature 22±2 °C and relative humidity 60±5%. Animals had free access to standard pellet diet (commercial rat cubes from Pranav Agro Industries Ltd., Baroda, India) and water *ad libitum*. The protocol of

the experiment was approved by the institutional animal ethical committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Preparation of karira stem extracts

Three extracts, viz. aqueous extract, alcoholic extract and hydroalcoholic extract of the stem were prepared for the activity. Aqueous extract was prepared by refluxing 100 g of the air-dried powdered stem on water bath with 500 ml of distilled water for 2 h. After filtration, the resulting solution was evaporated under reduced pressure to yield a solid extract (Extract-A). Alcoholic extract was prepared by exhaustive extraction of 100 g of the powdered stem with 500 ml of absolute alcohol for 5 h, using a Soxhlet apparatus. After filtration, the solvent was evaporated under reduced pressure to yield a semi-solid extract (Extract-B). Hydroalcoholic extract was prepared by refluxing 100 g of the powdered stem on water bath with 50% of hydroalcoholic solution for 2 h. After filtration, the resulting solution was evaporated under reduced pressure to yield a semi-solid extract (Extract-C). ExtractsA, B and C were suspended in distilled water using acacia (1%) as suspending agent prior to administration, as per the dose, expressed as mg of extract per kg body weight of rat.

Preparation of standard drug

The standard drug Silymarin (Sigma, Mumbai) was suspended in distilled water before use, as per the dose, using acacia (1%) as suspending agent.

Experimental design for evaluation of hepatoprotective activity

All the three extracts were evaluated for their hepatoprotectiveactivity using carbon tetrachloride (CCl_4)-induced hepatotoxicitymodel [11, 12]. The dose of test extracts was decided based on the observations of acute toxicity study. The animals of either sex were divided into six groups of six animals each (n=6). The treatment was given as per the specifications shown in table 1.

Table 1: Grouping specification and treatment protocol for CCl_4-induced hepatotoxicity model

Group	Group name	Specification
I	Normal Control	Received only vehicle (distilled water), per orally (p. o.)
II	Toxic Control	Received only CCl_4 (1 ml/kg, i. p.) as 1:1 dilution in olive oil, every alternate day for 7 d i.e. on day 2, 4 and 6 (3 applications)
III	Standard drug treated	Received CCl_4 (1 ml/kg, i. p.) and Silymarin (50 mg/kg, p. o.), once a day for 7 d
IV	Extract-A treated	Received CCl_4 (1 ml/kg, i. p.) and Extract-A (300 mg/kg, p. o.), once a day for 7 d
V	Extract-B treated	Received CCl_4 (1 ml/kg, i. p.) and Extract-B (300 mg/kg, p. o.), once a day for 7 d
VI	Extract-C treated	Received CCl_4 (1 ml/kg, i. p.) and Extract-C (300 mg/kg, p. o.), once a day for 7 d

Liver function tests

After completion of experimental period, the rats were fasted overnight and blood samples were collected by puncturing the retro-orbital plexus under light ether anesthesia. Serum was separated and liver function tests were performed by using respective diagnostic kits from Accucare Diagnostic Ltd. Biochemical estimations of serum glutamic pyruvic transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase(ALP), gamma-glutamyl transferase (GGT), total and direct bilirubin, total cholesterol, total protein and albumin were done [13-19].

Pentobarbitone-induced sleeping time study

On 6th day of the study, all the groups were given pentobarbitone sodium (40 mg/kg body weight, i. p.) 2 h after CCl_4 treatment. The duration between loss of the righting reflex and its recovery was recorded [20].

Assessment of oxidative stress

For determining the effects on oxidative stress, liver tissue homogenate was prepared. For that, animals were sacrificed by deep dose of ether and livers were dissected out, rinsed with ice-cold distilled water, followed by sucrose solution (0.25 M). They were again rinsed with distilled water and one gram of liver tissue was homogenized in 10 ml ice-cold tris-hydrochloride buffer [21]. The prepared homogenates were centrifuged and used for the theex-vivo antioxidant activity, in which tissue protein, malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were estimated [21-25].

Histopathology

Histopathological study of livers was carried out by microtomy to study the effect of test extracts of *C. decidua* stem on degenerative changes induced by carbon tetrachloride [26].

Statistical analysis

The results are expressed as mean±Standard Error of Mean (SEM). The data were statistically analyzed by using one-way Analysis of Variance (ANOVA) followed by Tukey's test, Dunnett's multiple comparison tests and linear regression analysis with GraphPad Prism 6 statistical software. Data were considered statistically significant at p value<0.05 [27].

RESULTS AND DISCUSSION

Liver function tests

Administration of CCl_4 (1 ml/kg, 1:1 solution in olive oil, i. p.) for three alternate days resulted into significant hepatotoxicity in rats, indicated by the elevated serum levels of SGPT, SGOT, ALP, GGT, total bilirubin, direct bilirubin and total cholesterol. Pretreatment of rats with the test extracts (at the dose of 300 mg/kg) exhibited a marked decrease in the elevated levels of all the markers, wherein Extract-B showed the most significant protection.

Administration of CCl_4 showed a significant lowering in total protein and albumin levels in rats. Extract-B elevated the decreased serum levels of protein and albumin both significantly ($p<0.01$), though the effect was lesser than the standard silymarin (fig. 1).

Pentobarbitone-induced sleeping time study

In the present study, CCl_4-induced severe hepatic damage, which was evident from CCl_4-induced prolongation of pentobarbitone hypnosis. CCl_4 induced a highly significant ($p<0.001$) rise in pentobarbitone-induced sleeping time as compared to the normal animals. Extract-B very significantly ($p<0.001$) reduced the time. The lowering in time by the Extract-B was also found to be comparable with silymarin (fig. 2).

Assessment of oxidative stress

In order to evaluate the effect of pretreatment with different extracts on CCl_4-induced lipid peroxidation, the levels of malondialdehyde were monitored. MDA serves as an indicator of oxidative damage and is considered as one of the principal products of lipid peroxidation. Results showed that MDA production in the liver in the toxic control group increased severalfold, as compared to the normal group. Consistent with the results of various enzymes at serum levels, pretreatment with silymarin and Extract-B significantly ($p<0.001$) decreased hepatic lipid peroxidation.

CCl_4 significantly decreased the antioxidant defence mechanism as evident from the reduced activity of superoxide dismutase and catalase levels in liver homogenates. Treatment with Extract-B increased the levels of SOD and catalase significantly ($p<0.01$ and $p<0.001$, respectively). The effect of Extract-B on catalase activity was comparable to silymarin.

CCl_4 administration caused a significant ($p<0.001$) reduction in GSH concentration and tissue protein in liver, as compared to normal animals. In animals pretreated with Extract-B, the liver GSH and protein contents were significantly found higher than that of the toxicant group (fig. 3).

Histopathology study

In the histopathological examination, liver of normal healthy rats showed normal hepatic histology, with normal central vein and portal triad structure along with hepatic plates lined by 2 to 3 cells line, sinusoids, few endothelial cells and kupffer cells.

Liver tissue of CCl_4-intoxicated rats showed extensive hepatocytes degeneration, foci of lymphocytic infiltration in periportal area, apoptic bodies and zonal necrosis with central vein dilatation.

Test Extract-B treated rat liver tissue showed histopathology nearly similar to standard silymarin-treated group, where areas of regeneration were clearly seen with mild inflammation and lymphocyte infiltration in the necrotic area. While slight recovery from the liver damage was observed in the Extract-C treated animals, Extract-A did not show significant improvement in the damaged liver tissues. So, as per the histopathological study also, Extract-B proved to have a significant hepatoprotective effect.

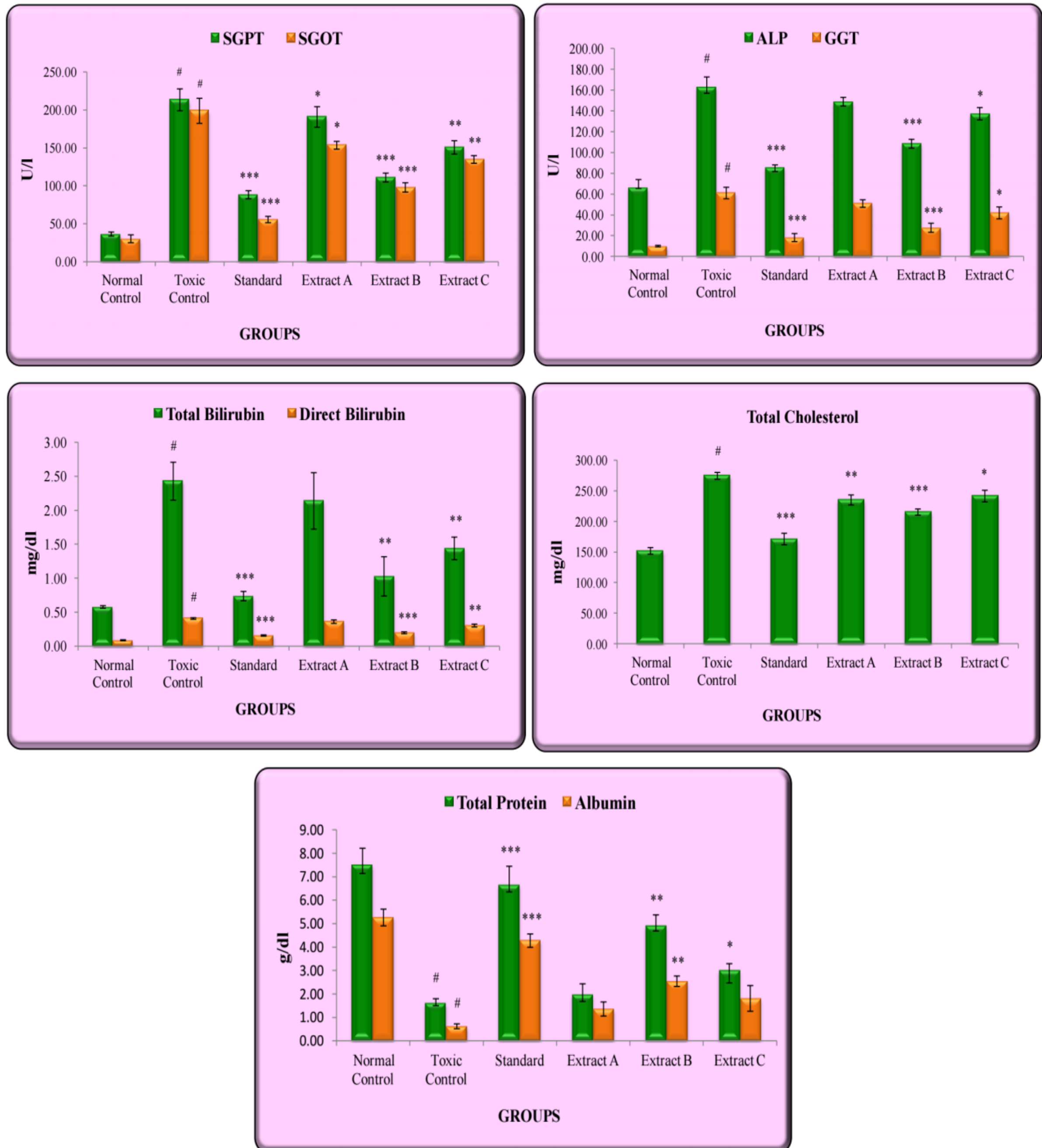

Fig. 1: Effect of test extracts on various markers of liver function test in CCl_4-induced hepatotoxicity in rats

Each bar represents mean±SEM of six observations. Comparisons are made between Group I vs II and Group II vs III, IV, V and VI; #significantly different from Normal control group, $p<0.001$, *significantly different from Toxic control group, $p<0.05$, **significantly different from Toxic control group, $p<0.01$, ***significantly different from Toxic control group, $p<0.001$.

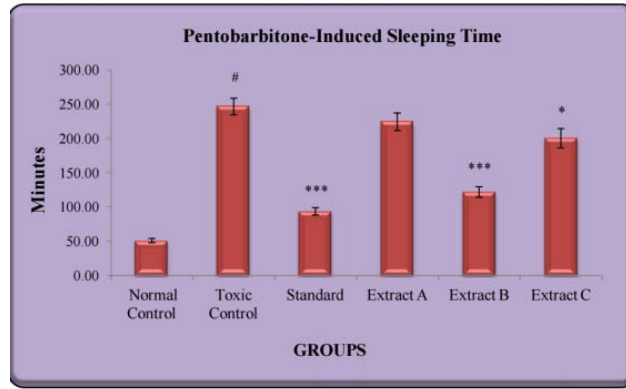

Fig. 2: Effect of test extracts on pentobarbitone-induced sleeping time in CCl_4-induced hepatotoxicity in rats

Each bar represents mean±SEM of six observations. Comparisons are made between Group I vs II and Group II vs III, IV, V and VI; #significantly different from Normal control group, $p<0.001$, *significantly different from Toxic control group, $p<0.05$, ***significantly different from Toxic control group, $p<0.001$.

Fig. 3: Effect of test extracts on liver oxidation markersin CCl_4-induced hepatotoxicity in rats

Each bar represents mean±SEM of six observations. Comparisons are made between Group I vs II and Group II vs III, IV, V and VI; #significantly different from Normal control group, $p<0.001$, *significantly different from Toxic control group, $p<0.05$, **significantly different from Toxic control group, $p<0.01$, ***significantly different from Toxic control group, $p<0.001$.

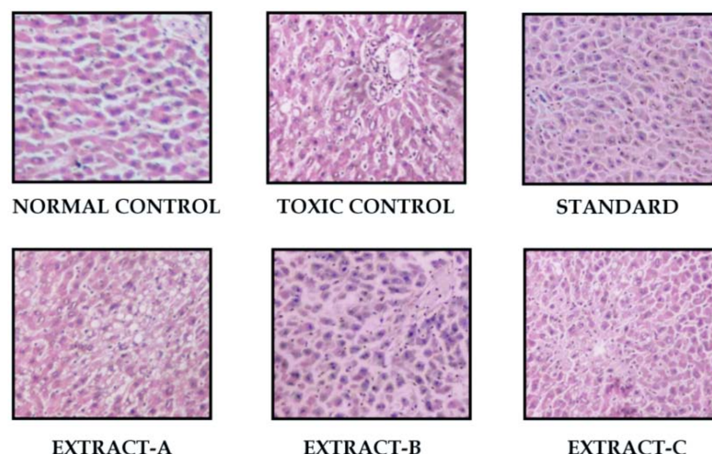

Fig. 4: Results of histopathology of rat liver tissues in CCl_4-induced hepatotoxicity

In a nutshell, CCl_4-induced damage to the liver significantly raised the serum levels of various enzymes in rats, particularly SGPT, SGOT and ALP, by releasing them into the bloodstream and increased MDA levels and decreased the levels of antioxidant enzymes, particularly SOD and catalase, in hepatic tissue. The inhibitory actions of the extracts on the rise of SGPT, SGOT and MDA levels in hepatic tissue observed in the present study indicated hepatoprotective activity of the extracts, which was comparable to the standard drug, silymarin (50 mg/kg). The effect on levels of antioxidant enzymes indicated the antioxidant potential of the extracts against the oxidative stress found in liver disorders. The mechanism by which the extracts provide protection could be the restoration of the integrity of cellular membrane, which was evident from a decrease in the number of necrotic cells in the histopathological studies. The results suggested that among all the three extracts, the aqueous (Extract-A) did not affect much of the parameters significantly, while the alcoholic extract (Extract-B) of *C. decidua* stem, at a dose of 300 mg/kg, is highly effective.

CONCLUSION

The present study incorporated the pharmacological evaluation of the hepatoprotective activity of three different extracts of the traditionally useful plant Karir (*C. decidua* stem), viz. aqueous extract (Extract-A), alcoholic extract (Extract-B) and 50% hydroalcoholic extract (Extract-C).

The activity was evaluated against carbon tetrachloride-induced hepatotoxicity in the rat liver. The results of the study demonstrated promising hepatoprotective activity of Karir stem and, thereby, providing a scientific base to the traditional claims of the therapeutic uses of this plant in hepatic disorders. The study also suggests that the dried alcoholic extract of Karir stem could be used as in formulation of a valuable hepatoprotective drug.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interest.

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