

Hepatoprotective effect of *Cichorium Intybus linn* (Kasni) Extracts against Carbon Tetrachloride induced Liver Damage.

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Abstract

Background: To assess the effect of aqueous and alcoholic extracts of *Cichorium intybus linn*, frequently used by Hakims and traditional healers, on liver, against the carbon tetrachloride (CCl₄) induced hepatic injury.

Methods: In this experimental intervention study, forty rats were subdivided into four sub groups, ten in each group. Group I was kept as standard control. For this study three groups (II, III, IV) were made in which aqueous and alcoholic extract was given prior to hepatic damage by CCl₄. Hepatoprotective effect was assessed by measuring serum levels of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP).

Results: Very significant hepatoprotective effect ($p < 0.001$) was observed by alcoholic extract while aqueous extract showed no significant effect against CCl₄ induced hepatic injury.

Conclusion: Alcoholic extract of *Cichorium intybus linn* exhibits a very significant hepatoprotective effect.

Introduction

Phytotherapy is the use of plants, plant extracts or pure chemicals isolated from natural products to treat a disease¹. It is estimated that out of 250,000 to 500,000 species of plants, only 1-2% of terrestrial plants have been reasonably well investigated. There is considerable potential to find new useful drugs from plants¹. Although today the synthetic drugs outnumber the natural ones but many drugs have their origin in natural source². A number of pharmacological products have been derived from the plants, for example morphine, digoxin, quinine, atropine, vinblastine and vincristine³. Modern medicine is now beginning to accept the use of standardized plant extracts. Garlic and ginseng have been extensively studied and found useful in cardiovascular disorders such as hyperlipidemia, hypertension, cardiac and cerebral insufficiency⁴. All herbal plants are not safe⁵. Two cases were reported who were taking germander to reduce their weight. Both patients developed hepatitis after 5-6 months and their serum aspartate aminotransferase (AST) and

serum alanine aminotransferase (ALT) were raised. With cessation of treatment hepatitis disappeared within eight weeks but when treatment was started by one of the patients with germander again, hepatitis recurred. Therefore it is significant to scientifically evaluate the effectiveness and safety of plants for human use. *Cichorium intybus linn* is now a domesticated plant cultivated for food, fodder and medicine⁶. It is used in treatment of jaundice, liver enlargement, gout and rheumatism. *Cichorium intybus linn* is an erect herb, 30-90 cm in height found in Punjab and Andhra Pradesh. Phytochemicals are distributed in whole plant⁶. Present work was carried out to evaluate the hepatoprotective (pre treatment) effect of Aqueous and alcoholic extracts of seeds of the *Cichorium intybus linn* against carbon tetrachloride induced hepatotoxicity in the albino rats of sprague dawley strain.

Material and Methods

The seeds of *Cichorium intybus linn* were procured in sufficient quantity from a dealer at the herbal market, made clear of dust by straining. Seeds were identified and authenticated by the taxonomy section of the department of Biological sciences, Quaid-i-Azam University, Islamabad. Seeds of *Cichorium intybus linn*, 500g were powdered and kept in dark tinted large sized glass jar at 4°C in refrigerator out of which 200g of powder was transferred in stopper flask along with 250ml of distilled water occasionally agitated for one day at room temperature. Mixture was filtered through a Whatman filter paper No.7. Filtrate of 150 ml was obtained and poured in petridishes and dried at 40°C under reduced pressure in Toyo vacuum drying oven, Seisakusho Co. Japan. Powder obtained from drying was 9g, which was stored in refrigerator and dissolved in 5ml of distilled water before use. Alcoholic extract was prepared by mixing 200g of powdered seed of *Cichorium intybus linn* with 250ml absolute ethanol in stopper flask for one day, filtered by Whatman filter paper No.7. Filtrate obtained was 120 ml, dried in Toyo vacuum

drying oven Seisakusho Co. Japan below 40°C under reduced pressure. Powder obtained was 12g, which was stored in refrigerator and dissolved in 5ml of distilled water before use.

Forty male albino rats of sprague dawley strain of body weight between 150-200 g were obtained from animal house of National Institute of Health, Islamabad and were kept in the wire cages at the animal house of Quaid -i- Azam university, Islamabad under standard conditions. Animals were divided in four groups with ten animals in each group.

Group I was standard control. Animals in this group were only given normal saline 10ml /kg mixed in olive oil 7.5 ml/kg orally with the help of gastric tube. Four doses were given at 12 hourly intervals. All animals were sacrificed on third day.

Group II animals served as sham control. They were given four doses of normal saline mixed with olive oil 1.5ml/kg at 12 hourly intervals. CCl₄ (1.5ml /kg) was administered one hour after last dose of normal saline.

Group III animals were given four doses of aqueous extract of the seeds of Cichorium intybus linn 500mg/kg orally with gastric tube at 12 hourly intervals, CCl₄ 1.5 ml/kg was given along with olive oil, one hour after last dose.

Group IV animals were given alcoholic extract of seeds of Cichorium intybus linn 500mg/kg orally at 12 hourly intervals by gastric tube, CCl₄ 1.5ml/kg was given one hour after last dose of alcoholic extract. All animals in each group were anaesthetized with injection ketamine 100mg/kg IM in thigh muscle, 24 hours after the last treatment and 3ml blood was extracted by cardiac puncture at maximum pulsating point, using 24G sterile disposable syringes. Blood was allowed to clot and serum was separated by centrifugation at 3000 rpm for 15 minutes. The supernatant layer of serum was sucked by micropipette and plunged in to cuvette and stored in refrigerator at 4°C. ALT, AST and ALP were estimated on the same day using the kit prepared by Boehringer Mannheim Co, Germany using preprogrammed 4010 spectrophotometer of the same company. Results were expressed as mean ±SEM and all statistical comparisons were made by means of student t- test. P value <0.05 was regarded as significant and p<0.001 highly significant. Statistical analyses were done using computer program statistica (1994).

Results

Serum ALT, AST and ALP measured results are presented in the Table I. ALT, AST, and ALP were

significantly higher in group II animals as compared to group I (p<0.001) and the levels of the enzymes in group IV receiving alcohol extract were very significantly less than group II animals (p<0.001) whereas the serum level of enzymes in group III receiving the aqueous extract were significantly less than group II animals (p<0.05)

Table 1: Levels of ALT, AST, and ALP (effect of the aqueous and alcoholic extracts of Cichorium intybus linn on CCl₄ induced hepatic damage)

Serum enzyme U/L	Group I	Group II (n=10)	Group III (n=10)	Group IV (n=10)
SALP	303± 6	483± 12**	406±19*	299±13 NS
SAST	113± 5	588± 16***	526 ± 18***	166± 9***
SALT	57± 4	475± 7***	443± 13***	71± 4*

Comparison between mean values of group I with group II, group III and group IV

NS: no significant increase compared to group 1 enzyme activity (p>0.05)

* Significant increase compared to group 1 enzyme activity (p<0.05)

** Very significant increase compared to group 1 enzyme activity (p<0.01)

***Highly significant increase compared to group 1 enzyme activity (p<0.001)

Discussion

In this study CCl₄ was used as the hepatotoxic agent as it is commonly used by research workers to study the hepato-protective action of plants 7,8,9. Carbon tetrachloride by homolytic cleavage is converted to trichloromethyl free radical (CCl₃·), which reacts with oxygen, and even more reactive specie trichloromethyl peroxy free radical (Cl₃ COO·) is formed which is cytochrome P450 oxygenase dependent¹⁰. Compounds such as Chlorophenothane, alcohol and phenobarbitone, which induce such enzymes, enhance hepatotoxic effects. Injury to hepatocytes is severe and rapid in onset (within 30 minutes) and degradation of hepatocytes leading to release of enzymes in plasma within 02 hours mitochondrial injury also occurs¹⁰. Due to cell damage by CCl₄ AST and ALT are released in circulation markedly where as ALP is raised only slightly¹¹. With CCl₄ only parenchymal cells are damaged and sinusoids remain intact. Damaged cells undergo autolysis and remaining cells proliferate repairing damage with in 5-6 days¹². Prevention of damage may be due to antioxidants such a Vit-E, glutathione etc. Extent of damage depends upon the net balance

between free radical formation and its termination¹². In group II animals ALT and AST were markedly increased compared to group I ($p < 0.001$) and ALP was less increased ($p < 0.01$). Slight increase in ALP activity is due to swelling of hepatocytes¹³.

In this study aqueous extract did not protect the liver against CCl_4 induced injury. ALT, AST and ALP in-group III animals were close to group II animals than to group I animals. Where as the alcoholic extract in group IV animals prevented the increase in ALT, AST and ALP levels which were close to group I animals than to the group II animals. Hepatoprotective action could be due to flavonoids or polyphenolic compounds, which are alcohol soluble and present in many plants. Flavonoids and polyphenolic compound have potent antioxidant activity, which protect the liver against free radical injury¹⁴. Ethanolic extract of *Cleome viscosae* linn has significant hepatoprotective activity¹⁵. Daily oral administration of methanolic extract of *Comiphora berryi* (Arn) Engle bark produces dose dependent reduction in serum levels of liver enzymes against CCl_4 induced hepatic injury¹⁶. Aqueous extract of *Osbeckia aspera* leaves can significantly decrease in vitro concentration of 1,1, diphenyl -2,2 Picylhydrazyl a free radical and inhibit xanthine oxidase activity suggesting the plant extract contains compound(s) with antioxidant activity¹⁷. In vitro *Cichorium intybus* linn and *Solanum nigrum* cause the inhibition of free radical mediated DNA damage¹⁸. Most of plants also act as inhibitors of microsomal drug metabolizing enzymes (MDME), thus providing hepato-protective activity¹⁹. An alkaloid protopine present in different plants such as *Fumaria indica*, *Corydalis tuber* and *Corydalis meifolia*, inhibits MDME thus providing protection against CCl_4 and paracetamol induced hepatotoxicity by preventing their conversion into their respective toxic metabolites²⁰. *Osbeckia aspera* and *Osbeckia octandra* have been used for treatment of liver disease and in vitro studies suggest they have antioxidant properties¹⁷. In this respect flavonoids and other poly phenolic compounds have received great attention²¹.

From the present study it can be proposed that alcoholic extract exhibits significant hepatoprotective action. Alcohol soluble compound(s) is (are) present in seeds of *Cichorium intybus* linn which is (are) active ingredients. There may be poly-phenolic compounds or flavonoids that have significant antioxidant activity, which protect the liver against the free radical injury by preventing lipid peroxidation of cell membrane.

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