

**CURATIVE POTENTIAL OF KASHNI (*CICHORIUM INTYBUS* LINN.)
EXTRACT AGAINST CARBON TETRACHLORIDE INDUCED
HEPATOCELLULAR DAMAGE IN RATS**

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Summary

Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. Kashni or *Cichorium intybus* Linn. is one such herb which is used in Indian medicine as tonic. It is said to be useful in fevers, vomiting, diarrhoea and enlargement of spleen. It has been reported to be useful in jaundice, liver enlargement, gout and rheumatism. Its seeds have been reported to be carminative, agglutinating and cholagogue. In the present study, extract of Kashni was studied for its curative activity against CCl₄ (2.0ml/kg; sc) induced chronic hepatocellular damage in albino rats. 50% ethanol extract of whole plant (25 and 50 mg/100g/day) of the herb was administered for 14 days. Curative potential was assessed using various biochemical parameters like serum alanine transaminase, aspartate transaminase and alkaline phosphatase. Liver tissue was subjected to histopathological studies. Rats treated with CCl₄ showed significant rise in serum levels of AST, ALT & alkaline phosphatase (SAP), reflecting liver damage. The herb was found to significantly decrease the raised serum enzyme levels, indicating the recovery of hepatocellular injury at the two doses of the extract. The results were confirmed by histopathological studies, which revealed less fatty changes and marked regenerative activity in the livers after administration of the extracts. It is suggested that ethanol extract of Kashni possesses significant antihepatotoxic potential and curative activity against carbon tetrachloride induced hepatocellular damage in rats.

Key words: Carbon tetrachloride, Hepatocellular injury, Kashni, *Cichorium intybus*, Curative activity.

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Introduction

In the absence of reliable hepatoprotective drugs in allopathic medical practice, herbal medicines are significant source of pharmaceutical drugs. These are more widely used these days than a few decades ago. This is because modern pharmaceuticals are out of reach of a large proportion of human population and because of reports of adverse side effects of many modern synthetic drugs.

Kashni (*Cichorium intybus* Linn.) belongs to the family Asteraceae (Compositae). It is commonly known as Chicory and is distributed in Europe, the Mediterranean region and Northern Asia. In India, it is found wild in Punjab, Andhra Pradesh and Kashmir. It is an erect perennial herb, 30-90cm in height with a fleshy taproot up to 75 cm in length.

The main constituents of Kashni reported to be present in root are inulin, reducing sugars and sucrose (1). Sesquiterpene lactones such as cichorides A, B and C, guanine type sesquiterpene lactones such as 8-deoxy, lactucopicrin; crepidiaside B and 11 & beta, 13-dihydrolactucin, two known germacrene type sesquiterpene glycoside-Picriside B and Sonchuside A- and Eudesmane type sesquiterpene glycoside Sonchuside C stand reported (2).

In the traditional system of medicine various uses have been attributed to Kashni. Bruised fresh leaves are applied externally for healing eye inflammations and boiled in broth for strengthening the digestion (3). Extracts of different varieties have been reported to show some promise to treat diseases characterized by tachycardia, arrhythmia and fibrillation (4). Both alcoholic and aqueous extracts potentiated pentobarbitone and ethanol induced hypnosis in mice, exhibited analgesia and potentiated morphine analgesia in rats (5). Root extract has shown anti-inflammatory and hepatoprotective activities (6)(7) (8).

The present study was undertaken to study the curative potential of ethanol extract of Kashni against CCl₄ induced chronic liver damage in albino rats.

Materials and Methods

Plant Material

Whole plant of Kashni was purchased from the local market. It was properly identified and authenticated by a plant taxonomist of the Department of Botany, University of Kashmir, Srinagar, India. The voucher specimen was deposited at the Department of Taxonomy, Kashmir University, J& K India.

Plant material and extracts

The whole plant of Kashni was freed from extraneous matter and dried in a well-ventilated room, the outside temperature being in the range of 18-32°C. It was then coarsely powdered and 50% ethanol extract was prepared as described in I.P, 1985 (9). The combined filtrate obtained was distilled under vacuum; the temperature of distillation being in the range of 33-44°C and distillate was evaporated to dryness in air to obtain a solid mass.

Animals

Albino rats of Wistar strain, weighing between 140-170g, were obtained from Central Animal House RRL Jammu after proper approval. They were housed under uniform animal husbandry conditions in polypropylene cages, fed with proper diet and water ad libitum. They were exposed to 12h. light-dark cycle, 18-32°C temperature and the relative humidity was in the range of 61-76%. All procedures were performed after the experimental protocol was approved by institutional animal ethical committee (IAEC) as per the guidance of the committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) at the Department of Pharmaceutical Sciences, University of Kashmir.

Carbon tetrachloride-induced hepatocellular damage in rats

Rats of either sex were divided into four groups of eight animals each. First group served as vehicle control and received only 1% gum acacia suspension. Group II received CCl₄, 2.0 ml /kg body weight, subcutaneously, on alternate days for 14 days along with liquid paraffin (1:1). Groups III and IV received 50% ethanol extract of Kashni at the doses of 25 and 50 mg/100g/day respectively, for 14 days along with CCl₄. Kashni extract was administered as suspension, orally for 14 days. Weights of rats were recorded on every alternate date.

After 14 days of drug treatment and administration, the rats were fasted overnight; blood samples were collected from the retino-bulbar venous plexus. Serum was separated and subjected to AST (10), ALT (10) and alkaline Phosphatase estimations (11).

Livers of the rats were excised out, washed under tap water, dried between the folds of the filter paper; weighed and then preserved in 10% formalin for histopathological studies.

Statistical Analysis

Values are expressed as mean ± SE from the number of replications described in the test. Total variation present in a set of data has been estimated by ANOVA. The t-value was also calculated for two-sided test. $p < 0.05^*$, $p < 0.01^{**}$ were considered significant and $p < 0.001^{***}$ as highly significant.

Results

Administration of CCl₄ produced severe liver damage in rats, as evidenced by elevated levels of AST, ALT and SAP. Kashni extract when administered along with CCl₄ produced a fall in the AST, ALT and SAP levels; the fall was significant ($*p < 0.05$) at the dose of 25 mg/100g of Kashni while highly significant ($***p < 0.001$) fall in the enzyme levels was observed at the dose of 50 mg/100g of the extract (Table).

Table

Effect of ethanol extract of Kasni (*Cichorium intybus*) on Serum Enzyme levels, against Carbon Tetrachloride (CCl₄) -induced hepatocellular damage in Rats.

Group / SERUM ENZYMES	I Control (1% gum acacia)	II Only CCl ₄ (2 ml/kg)	III CCl ₄ (2 ml/kg) + <i>Cichorium intybus</i> (25mg/100g/day)	IV CCl ₄ (2 ml/kg) + <i>Cichorium intybus</i> (50mg/100g/day)
AST LEVELS (IU / L)	289.90 ± 18.94	501.42± 55.30 (n=6)	391.57± 52.18**	353.12 ± 28.34***
ALT LEVELS (IU / L)	158.35 ± 22.68	292.58± 22.51** (n=5)	228.80± 52.93*	163.16± 34.01***
SAP LEVELS (mM units)	704.91± 39.35	1651.25± 41.68*** (n=5)	1036.57± 14.94**	944.15± 43.38***
Groups compared	II Vs I	II Vs I	III Vs II	IV Vs II

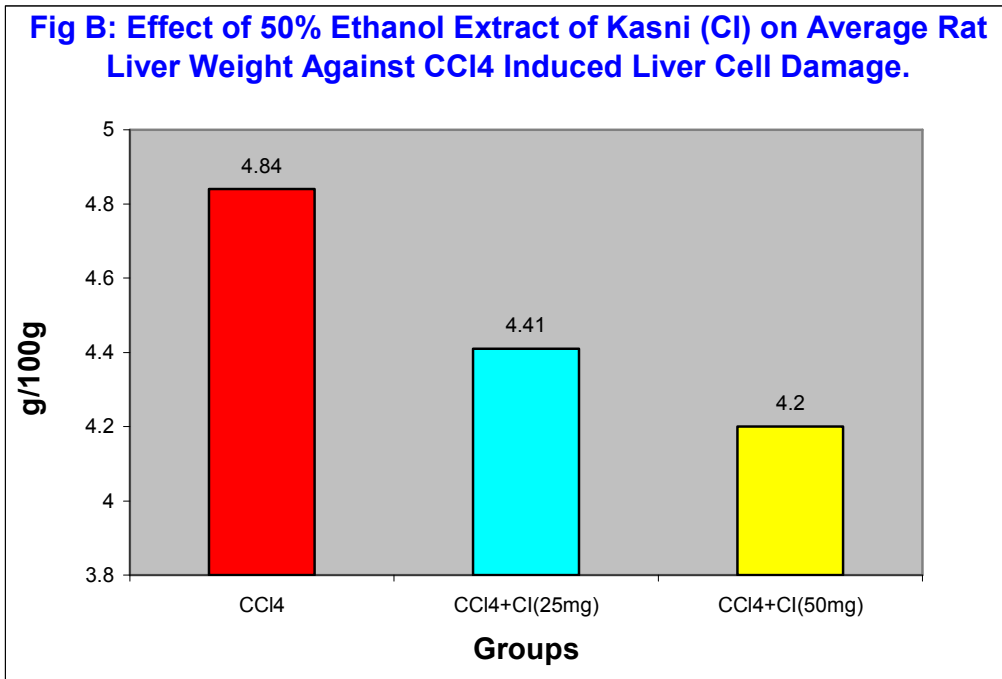
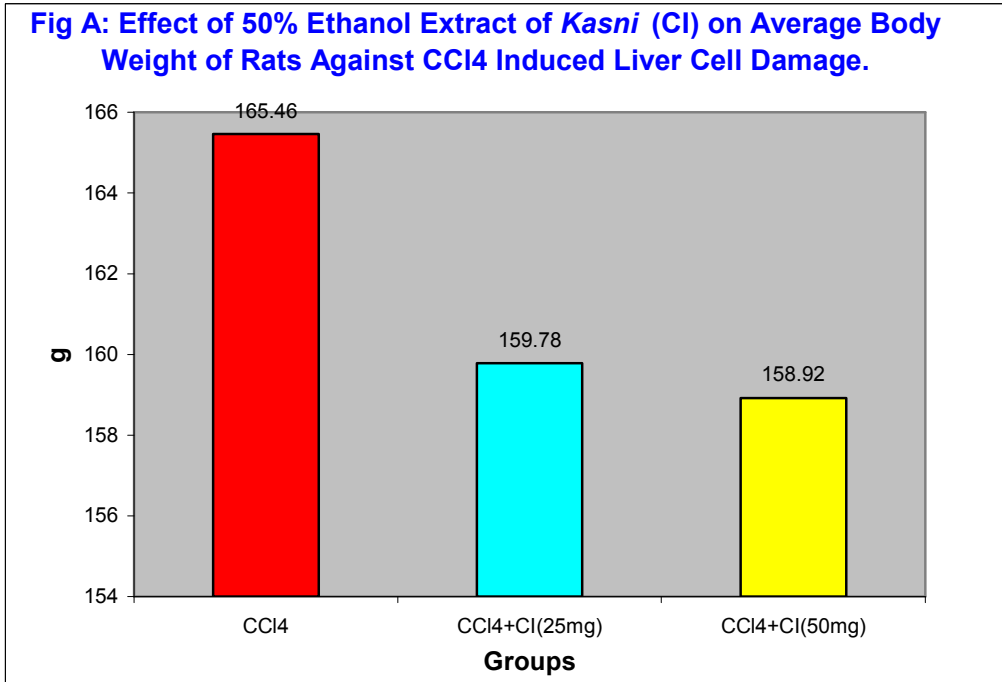
CCl₄ (2 ml/kg b.w) administered sc, twice a week for 14 days.

*p<0.05, **p<0.01, ***p<0.001.

Data shown are mean ± S.E.M; Number of animals in each group is given in parenthesis. Statistical significance calculated by ANOVA, using student 't' test. All comparisons made with Group I & II.

The average body weight decreased significantly (**p<0.01) in the rats that had received ethanol extract of Kashni at the doses of 25 and 50 mg/100g/day for 14 days as compared to the rats that had received only CCl₄ (Fig A).

A significant decrease (**p<0.01) in the average rat liver weight (Fig B) was observed in the rats treated with 25 & 50 mg/100g of Kashni compared to those rats that had received only CCl₄.



Histopathological examination of livers of rats that had received only CCl₄ for 14 days, revealed severe fatty changes in 90% livers (Fig 1). Centrilobular necrosis, focal fatty changes and centrivinous congestion were also observed in the livers of this group. At the dose of 25 mg/100g/day of Kashni extract, diffuse fatty changes were observed in 42% livers (Fig 2) while regenerative activity was observed in rest of the livers of this group. Marked regenerative activity was observed in 80% livers at the dose of 50 mg/kg/day of the extract (Fig 3) while fatty changes were observed in only 20% livers of this group.

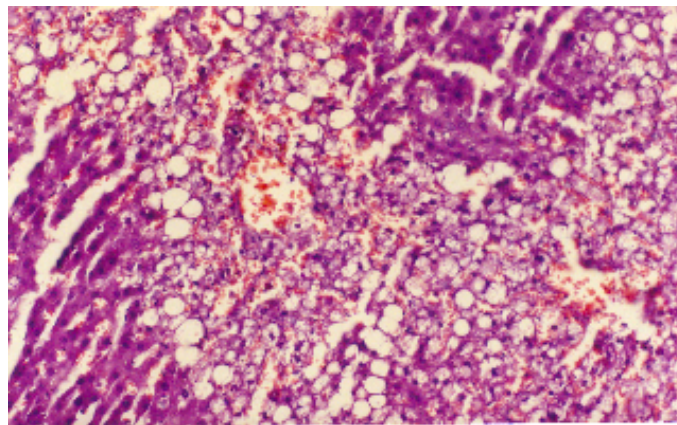


Fig 1: Carbon tetrachloride (2.0ml/kg body weight), s.c X 14 days. Severe fatty changes extending throughout the lobule with formation of fat cysts (H&E X 200).

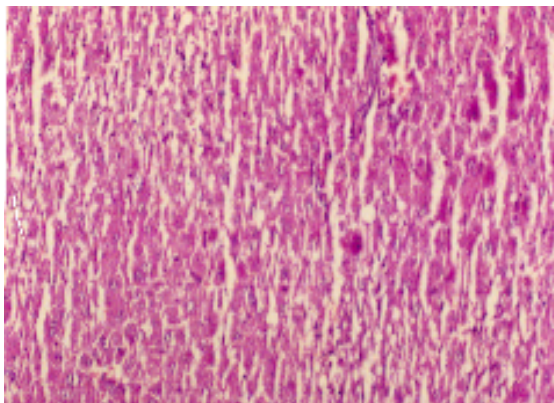


Fig 2: Carbon tetrachloride (2.0ml/kg bodyweight), s.c + Kasni (25mg/100g/day) X 14 days. Regenerative activity (H&E X 200).

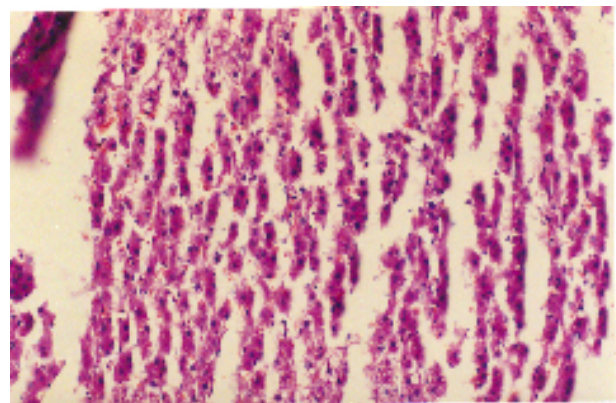


Fig 3: Carbon tetrachloride (2.0ml/kg body weight), s.c + Kasni (50 mg/100g/day) X 14 days. Marked regenerative activity, anisonucleosis. (H&E X 200).

Discussion

Liver holds a position of singular importance in the overall functions of the human body. Besides its secretory and excretory functions, it effectively controls numerous vital metabolic functions. The normal functioning of the liver can be disturbed by various infections, infiltrations or toxic agents such as alcohol, drugs and environmental factors (12).

CCl₄ intoxication in rodents is a commonly used model of both acute and chronic liver injury. Its administration causes hepatocyte injury that is characterized by centrilobular necrosis followed by hepatic fibrosis. Its metabolism occurs predominantly in the pericentral zone (zone 3) of the liver where its products cause hepatocyte injury, which can be of both short and long-term consequences to the liver (13)(14). So in the present study the curative effect of 50% ethanol extract of Kashni was investigated using CCl₄ as chronic hepatotoxic model.

CCl₄ was given subcutaneously, at the dose of 2.0ml/kg b.w (along with liquid paraffin 1:1), twice a week for 14 days, as it has been reported to produce centrilobular necrosis and fatty changes in the liver (15). The assessment of liver functions was done by performing biochemical estimations and by histopathological studies of liver sections. Kashni extract was observed to offer protection and maintain the structural integrity of hepatic cells against CCl₄ induced chronic liver damage. The protection offered was more significant and much better at the dose of 50 mg/ 100 g/day than with 25 mg/100 g dose. This was evidenced by significant fall (*p<0.05) in the AST, ALT and SAP levels at the dose of 25 mg/100 g while highly significant fall (**p<0.01) in these enzyme levels was observed at the dose of 50 mg/ 100g when given concurrently with CCl₄

Histopathological studies also revealed that the dose of 50mg/100g dose of Kashni extract afforded more protection as 80% livers were found with normal hepatic architecture at this dose while 58% livers were normal when administered 25mg of the extract.

The above results are in corroboration with our earlier study on this herb where antihepatotoxic activity was observed when the extract was given prophylactically for five days against paracetamol induced hepatocellular damage in mice (16). In an earlier study (17) (18) have also reported that total aqueous extracts of *Cichorium intybus* seeds reduced the levels of AST & ALT in Paracetamol-Carbon tetrachloride induced hepatotoxicity

Conclusion

It can be concluded that 50% ethanol extract of Kashni, when administered at the doses of 25 & 50 mg /100g, demonstrated antihepatotoxic activity / curative effect against CCl₄ induced hepatocellular damage in rats while optimal curative effect was seen at the dose of 50 mg/100g of Kashni extract. The Kashni (*Cichorium intybus*) extract thus possesses both prophylactic and curative activity against acute and chronic hepatotoxic models.

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