

HEPATOPROTECTIVE ACTIVITY OF DIFFERENT EXTRACTS OF GRAINS OF ELEUSINE CORACANA

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Summary

Herbal drugs are traditionally used in various parts of the world to cure different diseases. The present study has been conducted to evaluate the protective role of different extracts of grains of *Eleusine coracana* (Poaceae) against carbon tetrachloride (CCL₄) induced hepatotoxicity in rats. *E. coracana* exhibited significant hepatoprotective activity by reducing CCL₄ induced change in biochemical parameters that was evident by enzymatic examination. The extracts at an oral dose of 500 mg/kg exhibited a significant protective effect by lowering the serum levels of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP) and total protein. The activity of extract was comparable to the standard drug, Silymarin (100 mg/kg, p.o.). Histopathological observations also revealed that treatment with *Eleusine coracana* extracts protected the animal from CCL₄ induced liver damage. The results indicate that the different extracts of *Eleusine coracana* grains possess hepatoprotective activity on CCL₄ induced hepatic injury in rats.

Keywords: *Eleusine coracana*, Carbon tetrachloride, Hepatoprotective

Introduction

Liver is the largest organ in the body, which plays a vital role in regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction. The liver is expected not only to perform physiological functions but also to protect against hazards of harmful drugs and chemicals. (1) In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise. Hepatotoxicity is one of the very common ailments resulting in to a serious debilities ranging from severe metabolic disorders to even mortality. Hepatotoxicity in most cases is due to free radicals (2). In spite of this free radicals generated by hepatotoxins like CCl₄ may overpower the protective mechanism of the liver and cause hepatic damage. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. Several anti-inflammatory, digestive, anti-necrotic, neuroprotective, and hepatoprotective drugs have recently been shown to have anti-oxidant and/or anti-radical scavenging mechanisms as part of their activity (3). Herbal drugs are frequently considered to be less toxic and free from side effects than synthetic drugs. *Eleusine coracana* L. (Poaceae) commonly known as finger millet or ragi cultivated in India and many of the African countries (4). It is a popular millet of India, consumed without dehulling either raw or after germination or fermentation. The tiny millet grain has a dark brown seed coat, rich in polyphenols like phenolic acids and its derivatives, flavonoids and tannins. It is also rich in phytic acid, an antinutrient that binds minerals. All these compounds have been reported to have radical scavenging activity and can therefore serve as antioxidants (5). *E.coracana* has been claimed in traditional literature to be valuable against a wide variety of diseases. Indian Materia Medica describes the use of grains of *E.coracana* in the treatment of a number of ailments, including diuretic, depurative and tonic and is useful in vitiated conditions of pitta and kapha, burning sensation, hyperdipsia, strangury, renal and vesical calculi, leprosy, skin diseases, diuretic and general debility(6,7). The plant is known to possess diuretic, antilithiatic, antidiabetic, antioxidant and antimicrobial activities (8). A perusal of literature revealed that its hepatoprotective effect remain to be studied. Here in, we report the hepatoprotective effect of different extracts of grains of *E.coracana* against carbon Tetra Chloride (CCl₄) induced liver damage in rats.

Materials and Methods

Preparation of plant extract

The grains of *E. coracana* were dried in shade and were ground to get a coarse powder and subjected to soxhlet successive extraction using n-hexane, ethyl acetate, butanol and ethanol respectively. The extracts obtained were evaporated in rotary evaporator to get a powdery mass. The yield of different extracts was calculated. The powder extracts obtained were then subjected to phytochemical analysis to detect the chemical constituents present in each extracts.

Phytochemical screening

The crude extracts of grains of *E.coracana* were subjected to preliminary phytochemical screening for their presence of carbohydrates, flavonoids, glycosides, phenols and saponins, (9,10,11).

Animals

Studies were carried out using Wistar albino rats (150–200 g) of either sex procured from National Toxicology Centre, Pune, Maharashtra. The animals were grouped and housed in polyethylene cages with not more than six animals per cage and maintained under standard conditions with 12 hr natural light and dark cycle and were kept at room temperature (25±2°C). They were fed with standard pellet diet and water *ad libitum*. All the experimental process and protocols used in this study were approved by the Institutional Animal Ethical Committee (IAEC).

Acute toxicity study

Acute oral toxicity studies were performed according to OECD guideline 423 using Swiss albino mice. The animals were divided into 5 groups of three animals each. First group served as normal control. *E. coracana* extracts was administered orally to different groups at the dose level of 300, 500, 2000, 5000 mg/kg (p.o.) body weight. After dosing, the animals were observed for 2 hours and then intermittently for further 4 hours and finally recording mortality up to 24 hours till 14 days (12).

Carbon Tetra Chloride (CCl₄) induced hepatotoxicity

The animals were divided randomly into seven groups of six animals each. The hepatoprotective activity of the plant extracts was tested using CCl₄ model (13, 14).

- Group I:** Control group, received single dose vehicle once a day orally for 7 days.
- Group II:** Disease control, treated with vehicle daily for 7 days followed by CCl₄ (1.0 ml/kg i.p.) on 7th day with olive oil (1:1)
- Group III:** Standard control, treated with Silymarin (100 mg/kg p.o.) daily for 7 days followed by CCl₄ on 7th day.
- Group IV:** Treated with *E.coracana* n-hexane extract (500 mg/kg p.o.) daily for 7 days followed by CCl₄ on 7th day.
- Group V:** Treated with *E.coracana* ethyl acetate extract (500 mg/kg p.o.) daily for 7 days followed by CCl₄ on 7th day.
- Group VI:** Treated with *E.coracana* butanol extract (500 mg/kg p.o.) daily for 7 days followed by CCl₄ on 7th day.
- Group VII:** Treated with *E.coracana* ethanol extract (500 mg/kg p.o.) daily for 7 days followed by CCl₄ on 7th day.

After 36 hours of CCl₄ treatment, the animals were dissected under ether anesthesia. Blood from each animal was withdrawn by cardiac puncture and collected in previously labeled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 2000 rpm for 15 minutes. The separated serum were used for the estimation of various biochemical parameters namely SGPT, SGOT (15), ALP (16), and total protein (17).

Histopathological studies

A portion of the liver was cut into two to three pieces and fixed in 10% formalin. The paraffin sections were prepared and stained with haematoxylin and eosin. The thin sections of liver were made into permanent slides and examined under high resolution microscope with photographic facility and photomicrographs were taken (18).

Statistical analysis

The data were expressed as mean \pm SEM. Results were analyzed statistically by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test using Graph pad PRISM software and p- values $<$ was considered significant.

Result and Discussion

Preliminary phytochemical studies revealed the presence of carbohydrates, proteins, polyphenolics (flavonoids and tannins), steroids, saponins and triterpenes while alkaloids were found to be absent.

In acute toxicity studies it was found that animals were safe up to a maximum dose was 5000 mg/kg body weight. There were no changes in normal behaviour pattern and no signs and symptoms of toxicities and mortality were observed. On the basis of this study dose selected for the study was 500 mg/kg.

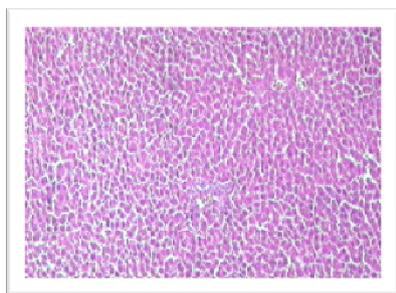
TABLE 1: EFFECT OF DIFFERENT EXTRACTS OF *E.CORACANA* GRAINS ON CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS.

| Sr. No | Groups | SGPT | SGOT | ALP | Total Protein |
|--------|---|----------------------------------|---------------------------------|----------------------------------|--------------------------------|
| I | Control vehicle | 76.17 \pm 4.77 | 179.3 \pm 5.37 | 154.7 \pm 5.743 | 5.88 \pm 0.27 |
| II | CCl ₄ (1ml/kg) | 222 \pm 15.2 ^{***} | 336 \pm 31.41 ^{***} | 324.8 \pm 24.01 ^{***} | 3.9 \pm 0.35 ^{**} |
| III | Silymarin (100mg/kg) | 136.2 \pm 7.66 ^{**} | 213.3 \pm 14.24 ^{ns} | 185.8 \pm 12.77 ^{ns} | 5.48 \pm 0.36 ^{ns} |
| IV | n-haexane + CCl ₄ (500mg/kg) | 212.5 \pm 16.46 ^{***} | 305.3 \pm 19.46 ^{**} | 311.8 \pm 26.63 ^{***} | 3.95 \pm 0.26 ^{**} |
| V | E.acetate + CCl ₄ (500mg/kg) | 190.3 \pm 15.72 ^{***} | 285.5 \pm 28.98 [*] | 274.3 \pm 26.21 ^{**} | 4.35 \pm 0.34 [*] |
| VI | Butanol + CCl ₄ (500mg/kg) | 161 \pm 7.58 ^{***} | 240.5 \pm 22.01 ^{ns} | 218.7 \pm 27.26 ^{ns} | 4.683 \pm 0.32 ^{ns} |
| VII | Ethanol + CCl ₄ (500mg/kg) | 149 \pm 5.96 ^{***} | 224.5 \pm 25.87 ^{ns} | 208.7 \pm 13.64 ^{ns} | 5.217 \pm 0.53 ^{ns} |

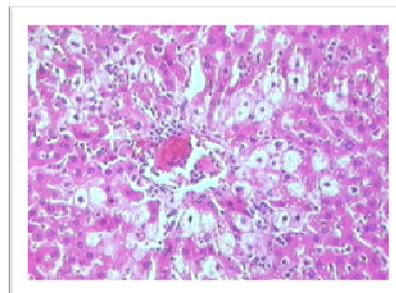
n=6; values are expressed as mean \pm SEM. Data found significant when one way ANOVA followed by Dunnett's multiple comparison test performed.

The activities of various biochemical enzymes in normal, CCl₄ control and treated groups were represented in Table 1. Results indicate that among these four extracts (n-hexane, ethyl acetate, butanol and ethanol) only butane and ethanolic extracts of *E.coracana* provide protection against the toxic effects of CCl₄ as compared to other two extracts. The activities of SGPT, SGOT and ALP were significantly increased with a significant decrease in total protein levels in CCl₄ control compared to normal control. The rapid elevation in the levels of serum aspartate transaminase indicates the extent of liver necrosis. Administration of the test extracts at a dose of 500 mg/kg, showed recovery against the toxic effect of CCl₄ on comparison with standard drug Silymarin (19).

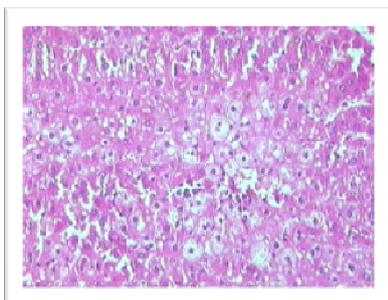
Histopathological examinations of liver sections of control group showed normal cellular architecture with distinct hepatic cells. CCl₄ treated liver showed an intense centrilobular necrosis and vacuolization, disarrangement and degeneration of normal hepatic cells, fatty changes. The Silymarin treated group showed almost normalization of fatty accumulation and necrosis. While among four different extracts especially butanolic and ethanolic extracts exhibited significant protection against CCl₄ intoxication which was evidenced by less centrilobular necrosis, less vacuole formation, less degeneration and regeneration of hepatic cells as compared to n-hexane and ethyl acetate extracts.



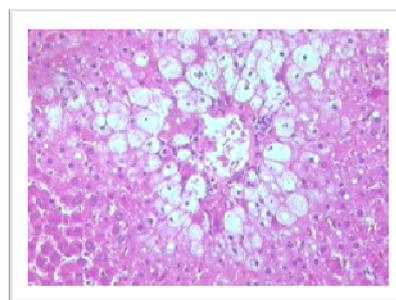
(A)



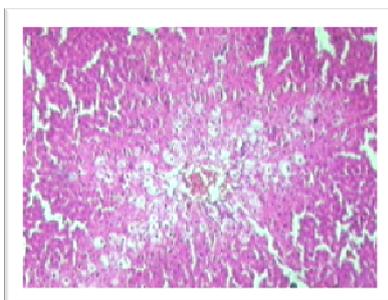
(B)



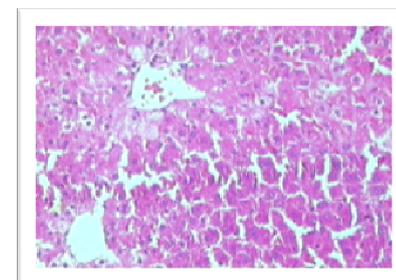
(C)



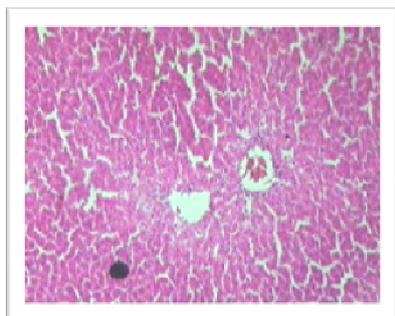
(D)



(E)



(F)



(G)

Fig. 1. The photomicrographs of liver section taken from rats. (A) control group; (B) CCl₄ + olive oil (1:1, 1 ml/kg, i.p.); (C) CCl₄ ± Silymarin (100 mg/kg, p.o.); (D) CCl₄ ± n-haexane extract of *E.coracana* (500 mg/kg, p.o.); (E) CCl₄ ± ethyl acetate extract of *E.coracana* (500 mg/kg, p.o.); (F) CCl₄ ± butanol extract of *E.coracana* (500 mg/kg, p.o.); (G) CCl₄ ± ethanol extract of *E.coracana* (500mg/kg, p.o.).

The phytochemical studies performed in the present study confirmed that the grains of *E.coracana* possess flavonoids, alkaloids, glycosides and tannins. After completion of oral acute toxicity as per OECD guideline 423, MTD found to be more than 5000 mg/kg and drug was found to be safe. Effective dose selected for study was 500 mg/kg.

For determining the hepatoprotective activity of the different extracts of the grains of *Eleusine coracana* in rats, carbon tetrachloride was used as hepatotoxin. This chemical has a well documented evidence of causing hepatic injuries and thus are preferred and are commonly used models for the screening of hepatoprotective agents.

Carbon tetrachloride induces hepatic damage due to its Cytochrome P-450 enzymatic system which catalyzes hepatic conversion into reactive trichloromethyl radical (-CCl₃) which upon reaction with oxygen radical gives trichloromethyl peroxy free radical (-OOCCL₃). This radical forms covalent bond with sulphhydryl group of several membrane molecules like glutathione which is considered as the initial step in the chain of events leading to lipid peroxidation and hepatic tissue destruction.

The comparative study in the test doses of four extracts especially butane and ethanolic extracts of *E.coracana* showed significant inhibition of the biological parameters, however is not equal to that of inhibition which was shown by the standard drug Silymarin towards the normal.

Conclusion

From the phytochemical tests, it was observed that the butane and ethanolic extract of the grains of *Eleusine coracana* contains glycosides, tannins and flavonoids which may be responsible for the antioxidant activity.

The in vivo study was evaluated using the biochemical estimations, histopathological study. The study clearly demonstrated that the grains of *Eleusine coracana* have got hepatoprotective activity that is comparable with Silymarin.

It can thus be concluded that the butanol and ethanolic extracts of grains of *Eleusine coracana* showed significant hepatoprotective activity as compared to n-hexane and ethyl acetate extracts.

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