EFFECTS OF CHENOPODIUM ALBUM LINN. PLANT EXTRACTS ON THE HISTOLOGY OF PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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Summary

This study was designed to evaluate the Hepatoprotective effect of extract of Chenopodium album Linn plant on the Histology of the liver of albino rats. Thirty (30) adult albino rats were divided into five (5) groups. Group 1 was the Control group that received distilled water for 7 days, group 2 was the vehicle control group that received single dose of 2 ml of propylene glycol and group 3 received single dose of Paracetamol (2gm/kg) Group 4 recieved only extract of Chenopodium album Linn plant for 7 days and Group 5 was pre-treated with 500 mg/kg of the ethanolic plant extract of Chenopodium album for 7 days and before inducing the liver damage on the 7th day with 2gm/kg of Paracetamol. On the 9th day rats were sacrificed and the liver was fixed immediately in formalin. The liver tissues was processed and stained in Haematoxylin and Eosin (H&E). The histological observations showed that the plant extract of Chenopodium album Linn prevent liver damage.

Key words: Hepatotoxicity; Hepatoprotective; Paracetamol; Chenopodium album Linn.

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Introduction

Over the past 100 years, the development and mass production of chemically synthesized drugs have revolutionized health care in most parts of the world. However, large sections of the population in developing countries still rely on traditional practitioners and herbal medicines for their primary care. In Africa up to 90% and in India 70% of the population depend on traditional medicine to help meet their health care needs [1]. Management of liver disease is still a challenge to the modern medicine. In the absence of reliable liver-protective drugs in allopathic medical practices, herbs play a vital role in the management of liver disorders. Many indigenous plants are used for the treatment of liver disorders [2]. Many species of Chenopodium are being used traditionally in indigenous system of medicine for the treatment of numerous ailments. C. album improves appetite, act as an antihelminthic, laxative, diuretic and tonic. It is also useful in abdominal pain and eye disease. It is useful as a pot herb in piles [3]. Paracetamol is a suitable analgesic and antipyretic. Paracetamol is effective in mild to moderate pain such as headache, myalgia, postpartum pain. Single dose of Paracetamol (2gm/kg body wt.) produce hepatotoxicity in rats [4]. In the present study, C. Album plants were evaluated for their hepatoprotective action in an animal model of hepatotoxicity induced by Paracetamol.

Materials and methods

This is an experimental cross sectional and comparative study which was carried out in the Department of Pharmacology and Therapeutics, Chittagong Medical College, Chittagong, in collaboration with Bangladesh Council of Scientific and Industrial Research (BCSIR) Centre, Chittagong, during the period from January 2013 to July 2013.

Animals: Albino rats of body weight 180–220 gm, bred in the BCSIR, Chittagong, were used in the study. The animals were fed on pellet diet and water ad libitum.

Drugs and chemicals

- Paracetamol (Acetaminophen) Powder (100gm): Obtained from Glaxo Pharmaceuticals Bangladesh Limited.
- Chenopodium album Linn plant: Identified in the Industrial Botany division of BCSIR laboratory, Chittagong. Ethanol extract prepared in the laboratory of BCSIR.
- 95% Ethanol: Purchased from Chittagong Scientific Store, Chittagong.
- Propylene Glycol: Purchased from Chittagong Scientific Store, Chittagong.
- 37% Formaldehyde: Purchased from Chittagong Scientific Store, Chittagong.

Preparation of ethanol extract of C album plant
The fresh plant of Chenopodium album Linn (Bathua Shak) collected from the Riazuddin market of Chittagong, was identified in the industrial botany division of BCSIR laboratory, Chittagong. Freshly collected whole plant of C. album was washed, cut into small pieces, air dried at room temperature for about 10 days and ground into powder form. Then the powder was macerated in 95% ethanol for 5-7 days at room temperature with occasional stirring. The ethanol extract of the plant collected in a separate container and concentrated under reduced pressure below 50°C through rotatory vacuum evaporator. The concentrated extract was collected in a petridish and allowed to air dry for complete evaporation of ethanol and then dried using freeze dryer. Finally an approximately 50gm (10% w/w) dark green sticky mass was obtained and was kept in a refrigerator at 4°C. During study 100 mg/ml concentrated extract of plant was prepared by using distilled water.

Experimental procedure
The animals were randomly divided into five groups. Each contained six animals.
Group 1 : Normal rats, received distilled water 2ml per oral through stomach tube for 7 days.

Group 2 : These rats served as vehicle control and received single dose of vehicle for Paracetamol i.e. propylene glycol 2ml orally by stomach tube.

Group 3 : Received single dose of Paracetamol solution in propylene glycol at a dose of 2gm/kg body weight in the strength of 250mg/ml of solution.

Group 4 : Received Chenopodium album plant extract (500mg/kg) by stomach tube for 7 days.

Group 5 : Received Chenopodium album plant extract (500mg/kg) for 7 days and on the 7^{th} day toxic dose of Paracetamol (2gm/kg) administered per oral through stomach tube.

All the rats were sacrificed on the 9th day that is, 48 hours after Paracetamol administration [5]. Livers of all rats were collected for histopathology.

Parameter: Histological parameters
An electrical binocular microscope was used to study the histological details of the tissue sections. Any abnormal changes in the hepatic architecture were carefully noted. Sections of each liver were examined for any evidence of necrosis. All sections of liver were examined under light microscope. Grading of liver necrosis was done in the following manner [6].

A : Piecemeal Necrosis
Grade A0 - Absent.
Grade A1 - Mild (Focal, few portal area).
Grade A2 - Mild/Moderate (Focal, most portal area).
Grade A3 - Moderate (Around 50% of the tracts or septa).
Grade A4 - Severe (Continuous around more than 50% of the tracts or septa).

B : Confluent Necrosis
Grade B0 - Absent.
Grade B1 - Focal confluent necrosis.
Grade B2 - Zone 3 necrosis in some areas.
Grade B3 - Zone 3 necrosis in most areas.
Grade B4 - Zone 3 necrosis plus occasional portal-central bridging.
Grade B5 - Zone 3 necrosis plus multiple portal-central bridging.
Grade B6 - Panacinar or multiacinar necrosis.

For the convenient of the study or statistical analysis, Grade A0/B0 is expressed as score 0, then grade A1= score 1, grade A2= score 2, grade A3= score 3, grade A4= score 4, grade B1= score 5, grade B2= score 6, grade B3= score 7, grade B4= score 8, grade B5= score 9 and grade B6= score 10. This arrangement is according to non progressive to progressive stage of liver necrosis.

Statistical Analysis

Mean score of hepatic necrosis of each group of rat is measured & then expressed as mean ± standard error of mean (Mean±SEM). Significance of difference between groups were assessed by Student's 't' test. Student's 't' test was done by using Statistical Package for the Social Sciences (SPSS) version 18. $P<0.05$ considered significant and $p<0.01/0.001$ considered highly significant.

Results

In histological examination of livers, no evidence of necrosis was observed in rats of group 1 (Control) group 2 (Vehicle control) and group 4 (C. Album only). Whereas all the rats of group 3 (Paracetamol treated) showed necrosis of varying grades. The mean values of histological score of liver necrosis in group 1, group 2 and group 3 were 0.0, 0.0 and 7.67±0.62 respectively. The increase in mean histological score of liver necrosis was highly significant ($p<0.001$) in group 3 in comparison with group 1 and group 2. Among the 6 rats of group 5 (C. album + Paracetamol) 1 rat showed grade A1 (Score 1) necrosis and 3 rats showed grade A4 (Score 4) necrosis, 1 rat showed grade B3 (Score 7) and 1 showed grade B5 (score 9) necrosis. The mean value of histological score of liver necrosis in group 3 (Paracetamol treated) and group 5 (C. album + Paracetamol) were 7.67±0.62 and 4.67±1.02 respectively. The decreasing mean histological score in liver necrosis was significant ($p<0.01$) in group 5 (C. album + Paracetamol) as compared to group 3 (Paracetamol treated). The results are shown in table 1.

Table I : Distribution of different group of rats according to various grades of liver necrosis (With mean score in each group)

Study Groups	No. of Rats (n)	A_0/B_0 = 0	A_1 = 1	A_2 = 2	A_3 = 3	A_4 = 4	B_1 = 5	B_2 = 6	B_3 = 7	B_4 = 8	B_5 = 9	B_6 =10	Score (Mean ± SEM)
		No Necrosis	Piecemeal Necrosis					Confluent Necrosis					
1	6	6											0
2	6	6											0
3	6							1	3		1	1	7.67 ±0.62 ***
4	6	6											0
5	6		1			3			1	1			4.67 ±1.02 *

1 : Control – Distilled Water 2: Vehicle Control 3 : Paracetamol treated
4 : C. album only 5 : C. album + Paracetamol

* = Significant ($p < 0.05$)
*** = Highly Significant ($p < 0.01/0.001$)
NS = Not Significant ($p > 0.05$)

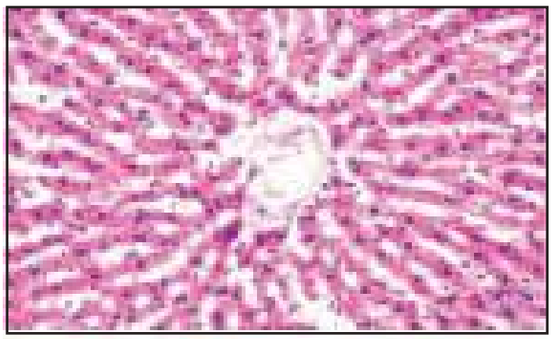

Fig 1 : 40 x Photomicrograph of liver of group 1 rat (Control) showing normal architecture (H&E Stain)

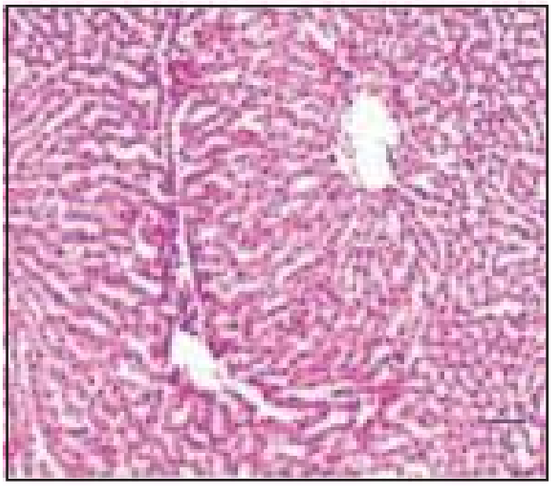

Fig 2 : 20 x Photomicrograph of liver of group 2 rat (Vehicle control) showing normal architecture (H&E Stain)

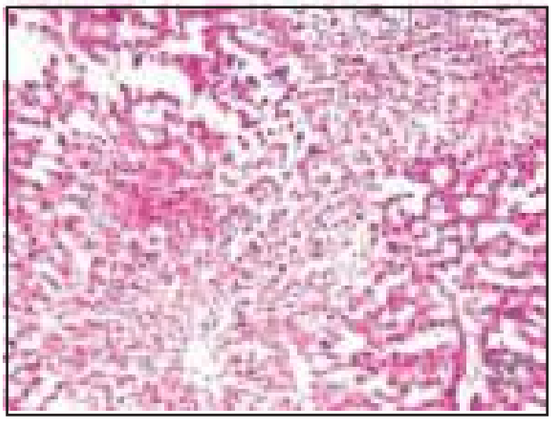

Fig 3 : 40 x Photomicrograph of liver of group 3 rat (Paracetamol) showing severe necrosis (H&E Stain)

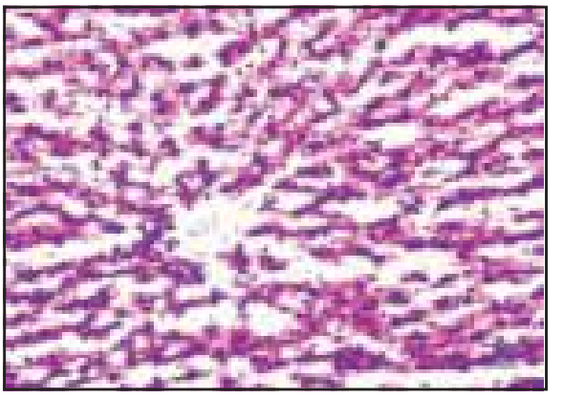

Fig 4 : 40 x Photomicrograph of liver of group 5 rat (C. album + Paracetamol) showing mild to moderate necrosis (H&E Stain)

Discussion

Liver is an organ involved in many metabolic functions and is prone to xenobiotic injury because of its central role in xenobiotic metabolism. Hepatotoxic drugs cause damage to the liver [7]. Paracetamol is a common antipyretic agent which is safe in therapeutic dosage but can produce fatal hepatic necrosis in human, rats, and mice with toxic doses. It is mainly metabolized in liver to excretable glucuronide and sulfate conjugates [8]. Paracetamol was used in this study to induce the liver damage [9].

Histopathological examination of liver sections of the control (Group 1), vehicle control (Group 2) showed regular cellular architecture with distinct hepatic cells, sinusoidal spaces, and a central vein. The hepatocytes are polygonal cells with well preserved cytoplasm, nucleus with prominent nuclei (Fig 1&2). Liver section of paracetamol-treated rats (Group 3) showed gross necrosis of the centrilobular hepatocytes characterized by lymphocytic infiltration and portal triads (Fig 3). C. Album treated animals (Group 5) show protection against liver damage by minimal necrosis in centrilobular and regeneration of hepatocytes (Fig 4). Based on the results obtained, we therefore inferred that C. Album plant extract has some protective effect on the liver as shown by the reduced damage in group 5.

The reduced necrosis of cells in the C. Album treated animals (Group 5) might be due to the presence of chemical constituents which have hepatoprotective properties. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes [10]. Flavonoid, tannin and phenolic compound present in the ethanol extract of plant of Chenopodium album. Flavonoid, present in extract may have antioxidant property and hepatoprotective effect [11,12]. Ethanol extract was found to be rich in phenolic and flavonoids and showed significant free radical scavenging activity [13].

Paracetamol toxicity results from formation of an intermediate reactive metabolite (NAPQI) which binds covalently to cellular proteins, causing cell death. In therapeutic doses, this toxic intermediate metabolite is detoxified in reactions requiring glutathione, but in overdose, glutathione reserves become exhausted and results in the generation of free radicals following the depletion of glutathione. Antidotes for Paracetamol act by replenishing hepatic glutathione [14].

Glutathione (GSH) is the main intracellular non protein sulfhydryl compound which plays an important role in the maintenance of cellular proteins and lipids in their functional states. NAPQI binds to GSH, forming a conjugate which results in conversion of GSH to an oxidized form of glutathione. When GSH levels are lowered, the toxic effects of oxidative insult exacerbated, resulting in increased membrane and cellular damage [15,16].

As pretreatment with C. album extracts produces minimal histological changes, it is easily understood that this extracts deserve credit for giving protection to liver. The proposed reasons behind the protection are possibly the GSH preservation or replenishment and antioxidant properties of those extracts [17].

Conclusion

Mean value of hepatic necrosis of C. Album pretreated group of rats decrease significantly as compared to that of Paracetamol treated group of rats, we therefore inferred that ethanolic plant extract of Chenopodium Album Linn plant has an appreciable ability to prevent damage to the liver.

The present study thus justifies the traditional use of Chenopodium Album Linn plant in the treatment of liver diseases and also points out that Chenopodium Album Linn plant warrants future detailed investigation as a promising hepatoprotective agents. However the exact mechanism & active compounds need to be clarified in future studies.

Disclosure

All the authors declared no competing interest.

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