EFFECTS OF MORINGA OLEIFERA LAM. LEAF EXTRACTS ON THE HISTOLOGY OF PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

Maliha Ata[1] Selim Mohammmed Jahangir[2] Mahbubul Islam[3] Saiful Islam[4]

Summary

This study was designed to evaluate the Hepatoprotective effect of extract of Moringa oleifera Lam. leafon 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 Moringa oleifera Lam. leaffor 7 days and Group 5 was pre-treated with 500 mg/kg of the ethanolic plant extract of Moringa oleifera 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 Moringa oleifera Lam. prevent liver damage.

Key words

Moringa oleifera Linn; Paracetamol; Hepatotoxicity.

Introduction

In recent decades, there are many claims on the use of medicinal plants in the treatment of many diseases [1-2]. Despite the traditional claims, it is very important to have scientific knowledge regarding their therapeutic uses through detailed scientific investigation [3-4].

1. Assistant Professor of Pharmacology & Therapeutics Southern Medical College, Chittagong.
2. Professor of Pharmacology & Therapeutics Chittagong Medical College, Chittagong.
3. Associate Professor of Pharmacology & Therapeutics Chittagong Medical College, Chittagong.
4. Assistant Professor of Pathology Rangamati Medical College, Rangamati.

***Correspondence:** Dr. Maliha Ata
Email: dr.maliha59@gmail.com
Cell: 01910 837966

The use of medicinal herbs for treating ailments has been documented in the history of all civilizations [5-6]. From world health organization records,plant based remedies are now being used by 80% of the world population as their primary form of health care [7-8]. Consequently, herbal remedies are being investigated for their efficacy in their treatment of many conditions including hepatic injuries caused by Carbon tetra chloride or Paracetamol [9-10].

Many species of Moringaceae are being used traditionally in indigenous system of medicine for the treatment of numerous ailments. Shajna, scientific name is Moringa oleifera Lam belongs to family Moringaceae may be source of drugs for the protection of hepatotoxicity. It is believe to have variety usages which include combating malnutrition, anticancer and is being promoted as a panacea [11-14]. In many cases, published in-vitro (Cultured cells) and in-vivo (Animal) trials do provide a degree of mechanistic support for some of the claims that have sprung from the traditional medicine lore. For example, numerous studies now point to the elevation of a variety of detoxication and antioxidant enzymes and biomarkers as a result of treatment with Moringa or with phytochemicals isolated from Moringa [15-20]. In the present study, M.oleifera were evaluated for their hepatoprotective action on the histology of liver 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 g, 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.
- Moringa oleifera Lam. leaf: 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 Moringa oleifera Lam. (Shajna) collected from the plantation area of BCSIR, Chittagong, was identified in the industrial botany division of BCSIR laboratory, Chittagong. Freshly collected leaf of M.oleifera 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 leaf 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 [21].

Group 4 : Received Moringa oleifera leaf extract (500mg/kg) by stomach tube for 7 days.

Group 5 : Received Moringa oleifera leaf extract (500mg/kg) for 7 days and on the 7th 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 [22]. 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 [23].

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 (M.oleifera 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 (M.oleifera + Paracetamol), 5 rat showed grade A1 (score 1) necrosis and 1 rats showed grade A3 (score 3) necrosis. The mean value of histological score of liver necrosis in group 3 (Paracetamol treated) and group 5 (M.oleifera + Paracetamol) were 7.67±0.62 and 1.33 ±0.33 respectively. The decreasing mean histological score in liver necrosis was significant ($p<0.01$) in group 5 (M.oleifera + 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		5		1								1.33 ±0.33 ***

1 : Control – Distilled Water; **2**: Vehicle Control; **3** : Paracetamol treated
4 : *M.oleifera only*; **5** : *M.oleifera* + Paracetamol

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

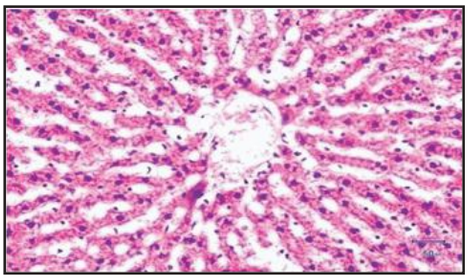

Figure 1 : 40x Photomicrograph of liver of group 1 rat (Control) showing normal architecture (H&E Stain)

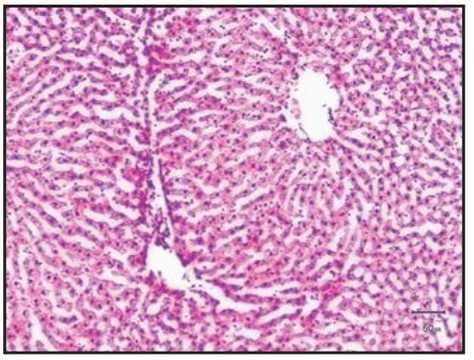

Figure 2 : 20x Photomicrograph of liver of group 2 rat (Vehicle control) showing normal architecture (H&E Stain)

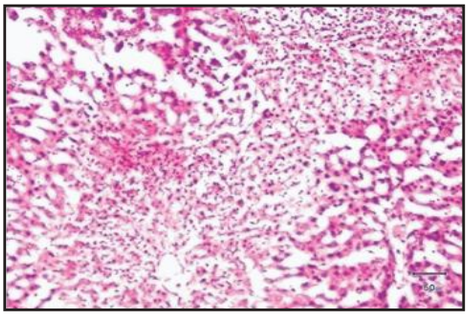

Figure 3 : 40x Photomicrograph of liver of group 3 rat (Paracetamol) showing severe necrosis (H&E Stain)

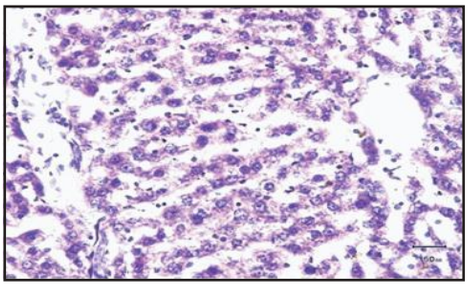

Figure 4 : 40x Photomicrograph of liver of group 5 rat (M.oleifera + Paracetamol) showing mild necrosis (H&E Stain)

Discussion

The liver can be injured by many chemicals and drugs. In the present study, Paracetamol was selected as a hepatotoxicant to induced liver damage. The primary objective of this study is to assess the hepatoprotective activity of M.oleifera against drug induced liver damage. 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 [24].

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). M.oleifera 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 can inferred that M. oleifera leaf extract has some protective effect on the liver as shown by the reduced damage in group 5.

The reduced necrosis of cells in the M.oleifera 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 [25]. Active chemical component such as flavonoid, glycoside, tannin, terpinoid are present in the ethanol extract of Moringa oleifera leaf [26]. Flavonoid, present in extract may have antioxidant property and hepatoprotective effect [27-28]. Ethanol is the most commonly used organic solvent by herbal medicine manufacturers because finished products can be safely used internally by consumers of herbal extracts [29]. Ethanol extract was found to be rich in phenolic and flavonoids and showed significant free radical scavenging activity [30].

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 [31].

As pretreatment with M.oleifera 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 [32].

Conclusion

Hepatoprotective activity of the ethanol extract of Moringa oleifera was studied. In this study, The extract of Moringa oleifera produced adequate hepatoprotective activity on albino rats.

Histopathological examination of the liver section of the rats treated with toxicant showed intense centrilobular necrosis and vacuolization. The rats treated with extracts along with toxicant showed sign of protection against these toxicants to considerable extent as evident from minimum of necrosis. Thus it is concluded that the extract exhibited significant dose dependent hepatoprotective activity.

Disclosure

All the authors declared no competing interest.

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