HEPATOPROTECTIVE EFFECT OF MORINGA OLEIFERA LAM. LEAF ON BIOCHEMICAL PARAMETERS AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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Summary

Moringa Oleifera Lam (Called Shajna in Bengali) is a plant which has a long history of uses in liver disorders in this subcontinent. This study was done to evaluate the hepatoprotective effects of ethanol extract of leaf of this plant on paracetamol induced hepatotoxicity in albino rats. The total experiments were designed as follows I) Experiment- (Part I):- Induction of hepatotoxicity by single administration of paracetamol at a dose of 2 gm/kg body weight. 2) Experiment- (Part II). Effects of ethanol extract of Moringa oleifera Lam. on paracetamol induced hepatotoxicity in albino rats. The biochemical reports suggested that acute administration of paracetamol produced significant hepatotoxicity in experimental rats which was evidenced by significant increase in serum bilirubin, serum alanine aminotransferase (ALT) serum aspartate aminotransferase (AST) serum alkaline phosphatese (ALP) level, decrease in serum albumin and serum total protein level. When rats were treated with extracts of M. oleifera leaf in paracetamol induced hepatotoxic albino rats, the results showed marked reduction in serum bilirubin, serum ALT, serum AST and serum ALP level and increase of serum albumin level but not sigificant increase of total protein as compared with paracetamol treated group. The results of the present study revealed that ethanol extract of Moringa oleifera Lam. leaf has got protective effects against paracetamol induced hepatotoxicity. Further study is needed to find out the curative effect and to isolate the active principle and to explore out the exact hepatoprotective mechanism of this leaf extract.

Key words

Moringa oleifera lam; Hepatoprotective; Hepatotoxicity; Paracetamol.

Introduction

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxify toxic substances and synthesize useful material. It's typical position and functions make it the most essential organ but also prone to number of diseases [1]. Liver diseases are a serious health problem. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders. However, we do not have satisfactory remedy for serious liver diseases, most of the herbal drugs speed up the natural healing process of liver. So the search for effective hepatoprotective drug continues. Liver disease has become a global concern worldwide [2]. Uses of herbal drugs in the treatment of liver diseases has a long tradition, especially in Eastern medicine and can be traced back as far as 2100 B.C. in ancient China (Xia Dynasty) and India (Vedic period), but evidence for efficacy is sparse [3]. Now it is necessary to search for drugs of plant origin scientifically for the treatment of liver disease in order to replace currently used drugs of doubtful efficacy and safety. Shajna, scientific name is Moringa oleifera Lam. belongs to family Moringaceae may be source of drug for the protection of hepatotoxicity. This plant is available in our country and widely used by the traditional healers in the treatment of jaundice but scientific basis of such use is not specifically known. So in the present study, an attempt has been made to validate the traditional use of this plant as hepatoprotective against experimentally produced hepatotoxicity.

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Materials and methods

Animals:

Total 30 Albino rats (Rattus norvegicus) of age 3 to 6 months and body weight of 140-250 gm were used in this study, which were collected from Bangladesh Council of Scientific and Industrial Research (BCSIR).

Drugs, plants and chemicals:

i) Paracetamol (Acetaminophen) Powder (100 gm): Obtained from Glaxo Pharmaceuticals Bangladesh Limited.

ii) *Moringa oleifera* Lam. plant: Identified in the Industrial Botany division of BCSIR laboratory, Chittagong. Ethanol extract prepared in the laboratory of BCSIR.

iii) 95% Ethanol: Purchased from Chittagong Scientific Store, Chittagong.

iv) Propylene Glycol: Purchased from Chittagong Scientific Store, Chittagong.

Preparation of solution:

Solution of Paracetamol: 500 mg of Paracetamol powder (Active ingredient) was dissolved in 2 ml propylene glycol, so as to obtain a concentration of 250mg/ml.

Preparation of plant extract: 500 mg of plant (leaf) extract was diluted in 5ml of distilled water, so as to obtain a concentration of 100mg/ml.

Preparation of Ethanol extract of *M. oleifera leaf:*

The plant of *Moringa oleifera* Lam. (Shajna) was identified in the Industrial Botany Division of BCSIR laboratory, Chittagong. Freshly collected leaves of *M. oleifera* was washed separately, 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 plant collected in a container and concentrated under reduced pressure below 50°C through rotator vacuum evaporator. The concentrated extract was collected in a petridish and allowed to air dry for complete evaporation of ethanol. The concentrated extract then dried using freeze dryer. Finally an approximately 50 gm (10% w/w) dark green sticky mass was obtained and was kept in a refrigerator at 4ºC. During study 100 mg/ml concentrated extract was prepared by using distilled water.

Study design

For convenient purpose of study, the experiment was divided into 2 parts: Part - I & Part - II .

Experiments

Part I: Induction of hepatotoxicity by Paracetamol

18 rats were randomly selected for this part of experiment and divided into following three groups as Group-A1, Group-B1 and Group C1. Each group contained 6 rats.

Group A1 (Control): The rats in this group served as control and received distilled water 2 ml per oral through stomach tube for 7 days.

Group C1 (Vehicle control): The rats of this group served as vehicle control and received single dose of propylene glycol (vehicle for Paracetamol) 2 ml orally by stomach tube.

Group B1 (Paracetamol treated): This group received single dose of Paracetamol solution in propylene glycol at a dose of 2 gm/kg body weight in the strength of 250mg/ml of solution. Animals were sacrificed 48 hours after the administration of Paracetamol [4]. Blood was collected by cardiac puncture [5].

Part II : Effects of pre treatment with *Moringa oleifera* leaf extract on Paracetamol induced hepatotoxicity in rats. 12 rats were randomly selected for this part of experiment and divided into following two groups as Group-A2, Group-B2. Each group contained 6 rats.

Group A2 (*Moringa oleifera* leaf extract): Rats of this group received *Moringa oleifera* leaf extract (500mg/kg) by stomach tube for 7 days.

Group B2 (*Moringa oleifera* leaf extract + Paracetamol): Rats of this group 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 [4]. Blood sample was collected from each rat in separate test tube and investigated for biochemical parameters.

Collection of blood and separation of serum

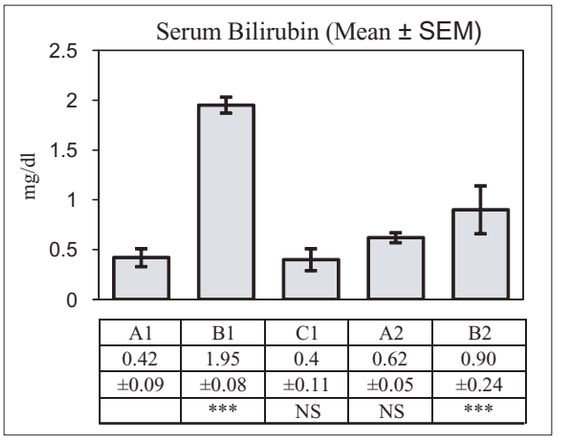
Blood was collected by cardiac puncture from each rat in separate test tubes. Test tubes were placed in inclined position, in the dark about 1 hour, at room temperature. The clot was loosened from the wall of the test tubes with a fine clean stick taking care to avoid haemolysis. Serum was then drained off carefully into the centrifuge tube and centrifuged at 3000 rpm for 3-5 minutes. The serum was then collected in the tips and was used for biochemical parameters.

Parameter studied: Biochemical parameters

a) Estimation of serum bilirubin - DCA method
b) Estimation of serum alanine amino transferase (ALT) - Kinetic method
c) Estimation of serum aspartate aminotransferase (AST) - Kinetic method
d) Estimation of serum alkaline phosphatase (ALP) – Kinetic method
e) Estimation of serum albumin Photometric colorimetric test for Albumin (BCG Method)
f) Estimation of serum total protein Photometric Colorimetric test for total proteins (Biuret Method)

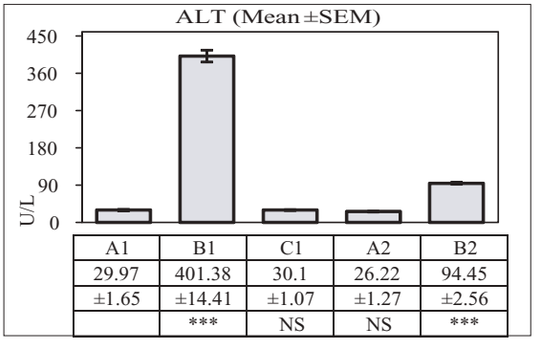
Statistical analysis

All the results have been expressed as mean ± standard error of mean (Mean±SEM). Significance of difference between groups were assessed by using Student's 't' test with $p<0.05$ was considered significant (*) and $p<0.01/0.001$ was considered highly significant (***). $p>=0.05$ was considered Not Significant (NS).



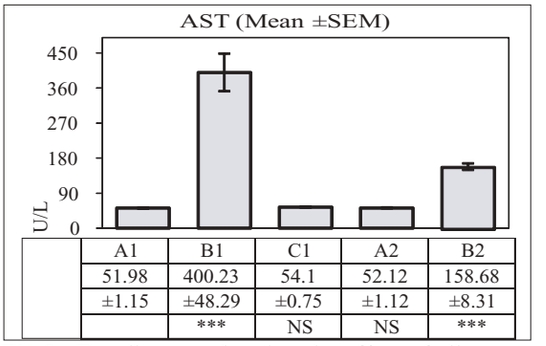
A1	B1	C1	A2	B2
0.42	1.95	0.4	0.62	0.90
±0.09	±0.08	±0.11	±0.05	±0.24
	***	NS	NS	***

Fig 1 : Bar diagram showing the effect of all group of rats on Serum Bilirubin level



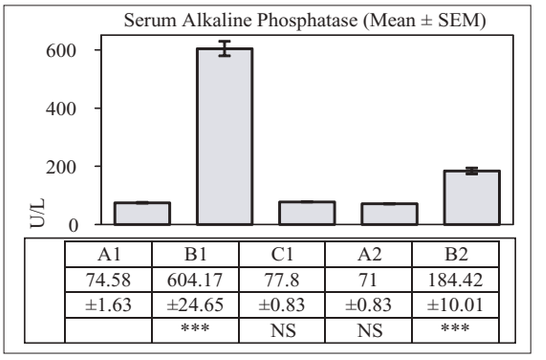
A1	B1	C1	A2	B2
29.97	401.38	30.1	26.22	94.45
±1.65	±14.41	±1.07	±1.27	±2.56
	***	NS	NS	***

Fig 2 : Bar diagram showing the effect of all group of rats on ALT level



A1	B1	C1	A2	B2
51.98	400.23	54.1	52.12	158.68
±1.15	±48.29	±0.75	±1.12	±8.31
	***	NS	NS	***

Fig 3 : Bar diagram showing the effect of all group of rats on AST leve



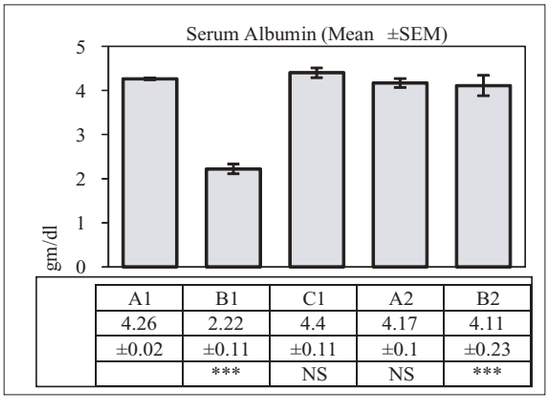
A1	B1	C1	A2	B2
74.58	604.17	77.8	71	184.42
±1.63	±24.65	±0.83	±0.83	±10.01
	***	NS	NS	***

Fig 4 : Bar diagram showing the effect of all group of rats on Serum ALP level

Results

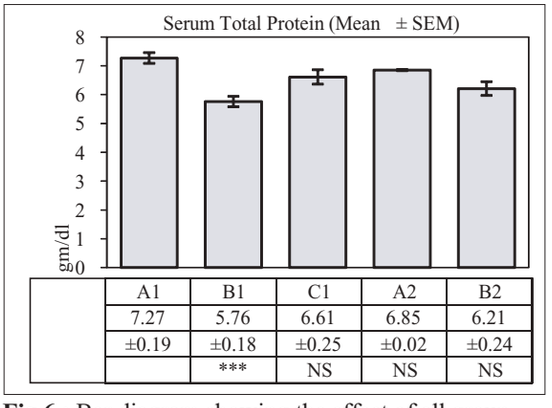
In this study, all the biochemical markers (Serum ALT, AST, Alkaline phosphatase & bilirubin) increased to highly significant level after Paracetamol administration as compared to the control group and the vehicle control group. These findings indicate that there are extensive liver damage after toxic dose of paracetamol administration. Serum albumin & total protein decreased highly significantly as compared to the control and the vehicle control group after Paracetamol administration. It was observed that when only ethanol extracts of *M. oleifera* was given

to one groups of rats, there was no significant changes in biochemical parameters as compared to the control group. These findings suggest that *M. oleifera* do not alter normal hepatic function. Pretreatment with *M. oleifera* in Paracetamol induced hepatotoxic rats showed reduction of serum bilirubin, ALT, AST and ALP level which were statistically highly significant as compared to the Paracetamol treated group. There was highly significant increase of serum albumin level as compared to the Paracetamol treated group but no significant change was observed in case of serum total protein.



	A1	B1	C1	A2	B2
	4.26	2.22	4.4	4.17	4.11
	±0.02	±0.11	±0.11	±0.1	±0.23
		***	NS	NS	***

Fig 5 : Bar diagram showing the effect of all group of rats on Serum Albumin level



	A1	B1	C1	A2	B2
	7.27	5.76	6.61	6.85	6.21
	±0.19	±0.18	±0.25	±0.02	±0.24
		***	NS	NS	NS

Fig 6 : Bar diagram showing the effect of all group of rats on Serum Total Protein level

Discussion

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 [6]. As pretreatment with M. oleifera produces lesser biochemical changes, it is easily understood that these 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 [7-8].

Conclusion

This study reveals that ethanol extract of Moringa oleifera Lam. leaf have protective effect against drug induced hepatotoxicity.

Further studies may be conducted on other species like rabbit, mouse, guinea pig etc. to find out both protective and curative effects of this plant against drug induced hepatotoxicity and to isolate the active principle that is responsible for these effects.

Disclosure

All the authors declared no competing interest.

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