Evaluation of liver protective activity of *Moringa oleifera* bark extract in paracetamol induced hepatotoxicity in rats.

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Abstract

Background: *Moringa oleifera* has been used in folk medicine to alleviate several diseases. In the present study, ethanolic extract of *Moringa oleifera* bark has been investigated to study its potential on paracetamol induced hepatotoxicity on model rats.

Methods: Rats (150–200 gm) were divided into 5 groups containing 6 animals each. Acute hepatotoxicity was induced by paracetamol (600 mg/kg body weight) administered once daily for one week whereas the extract of investigated plant was given orally throughout the whole experiment at 250 and 500mg/kg body weight. Silymarin (100mg/kg body weight) was given orally as standard hepatoprotective drug. The level of hepatic injury recovery was determined by

the estimation of liver enzymes like SGPT, SGOT, ALP, Bilirubin, Total protein,globulin and Albumin.

Results: Treatment with MO extract as well as standard hepatoprotective agent silymarin ameliorated plasma levels of hepatic enzymes. Body weight was improved significantly by MO extracts($p < 0.01$) , whereas liver weight was recovered insignificantly. SGPT , SGOT and ALP levels were improved very highly significantly ($p<0.001$) and highly significantly ($p<0.01$) at MO 250mg dose. While at the dose of 500 mg/kg ameliorated SGPT Level very highly significantly($p<0.001$) , SGOT Level highly significantly ($p<0.01$) but insignificant to ALP level.

Conclusion: The biochemical parameters provide evidence that the ethanolic extract of of *Moringa oleifera* bark has shown hepatoprotective activity.

Key words: *Moringa oleifera*, hepatoprotective activity, paracetamol induced hepatotoxicity, liver enzymes.

Introduction

Liver is the major organ which plays a key roles in processing of critical biochemical and physiological phenomena including metabolism and Detoxification of endogen and exogenous compounds, such as drugs and xenobiotics, homeostasis, growth, energy and nutrient supply [1, 2]. Hepatic injury could be occurred by hepatotoxic agents including drugs, alcohol and viral infections [3]. Liver diseases like jaundice, cirrhosis and fatty liver have been public health concern across the world. Prevalence of chronic liver disease worldwide is 18.5% and cirrhosis is 4.5 to 9.5% with a mortality of 2 million people each year [4]. In terms of medication, conventional or synthetic drugs are limited. Moreover they can have serious side effects [5]. Due

to the fact, a huge number of medicinal plants have been trialed to figure out regenerative and hepatoprotective activity [3].Approximately 160 phytochemical constituents originated from 101 plants have been reported to be potentially hepatoprotective [6]. At this present era, medicinal herbs have been a vital source of treatment of liver disease for instance , hepatitis, cirrhosis, and loss of appetite [7].Bangladesh, a country of great biodiversity of medicinal plants having a long history of use of traditional medicine along with phytotherapeutic potential mainly from native source. As a result , research in medicinal plants have been a huge area of discovering of promising lead compounds in Bangladesh[8,9].*Moringa oleifera* ,a medicinal plant of Bangladesh, belongs to the family moringaceae, is a small or middle sized tree, usually grows 10 -12 m in height .The plant is indigenous and abundantly seen in India, Pakistan, Bangladesh and Afghanistan. *M. oleifera* produce drumstick-like fruits. The flowers are white and quite small. It has teardrop shaped round and small leaves. Fruits and leaves are edible which are generally eaten as green vegetable [10, 11]. A good number of phytochmicals has been isolated and reported from various parts of *M. oleifera* which are 4-[(4'-O- acetylalpha-Lrhamnosyloxy) benzyl isothiocyanate, Niaziminin A, and Niaziminin B, 4-(alpha-1-rhamnopyranosyloxy)-benzylglucosinolate, quercetin-3-O-glucoside and quercetin-3-O-(6''-Malonyl-glucoside),Niazimicin (pyrrolemarumine400-O-a-L-rhamnopyranoside) and 40-hydroxyphenylethanamide(marumoside A and B) , Isothiocyanate, nitrites, thiocarbamates,O-(1heptenyloxy) propyl undecanoate, O-ethyl-4-(alpha-L-rhamnosyloxy) benzyl carbamate, methyl- p-hydroxybenzoate, beta- sitosterol, 4-hydroxyl mellein, vanillin, octacosonoic acid, Methionine, cysteine, D-glucose, kaemopherol, kaempferitin and ascorbic acid, protein, D-mannose , Moringine, moringinine, spirachin, 1,3-dibenzyl urea, alpha- phellandrene, p-cymene, Deoxy-niazimicine[11,12,13].A great number of pharmacological activity has also been reported

.Aqueous and alcoholic extracts of leaves and roots of *Moringa oleifera* has been reported to have a strong *in-vitro* anti-oxidant and radical scavenging activity[13] while Methanolic and ethanolic extract of leaves against pentylenetetrazole and maximal electroshock induced convulsions, demontrated significant anti-convulsant activity [14,15]. Potent anti-diabetic activity from different extracts of leaves , seed and pod, has also been reported[16,17, 18] an investigation against seed power also revealed its Anti-asthmatic activity[19,20]. Study on Ethanolic extracts of *Moringa oleifera* exhibited potent anti–tumor [21] and Anthelmintic activity [22, 23]. Another study of alcoholic extracts of leaves as well as seeds has showed effectiveness on isoniazid, rifampicin, and pyrizinamide induced liver damage[24]. However , our present study was concentrated on the bark extract of *Moringa oleifera* on paracetamol induced liver damage on Sprague Dawley rats . The objective of the present study was to obtain scientific data of the crude extract of MO on in vivo animal model to provide the support of biological activity of that extract against drug induced liver damage.

MATERIALS AND METHODS

Plant collection and Extraction

The plant *Moringa oleifera* (MO) bark was collected from Savar area, under Dhaka district , Bangladesh and was identified and authenticated by Md.Abdur Rahman, taxonomist , Dept. of Botany, Jahangirnagar University, Savar, Dhaka. The collected materials were thoroughly washed in water, cut into smaller parts and shed dried at room temperature for a week and pulverized in electric grinder to get extractable powder. Then powder was extracted in soxhlet apparatus with ethanol (96%). The extracts were filtered and collected in a container and then

dried with a rotary evaporator under reduced pressure to get viscous substance. Finally a solid mass, 25 gm, was obtained and preserved in a Petridis in the refrigerator.

Experimental animals

For the experiment, Sprague Dawley rats of either sex, weighing between 150-200g, were collected from the animal research lab in the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. Animals were maintained under standard environmental conditions (temperature: 27.0±1.0°, relative humidity: 55-65% and 12 h light/12 h dark cycle) and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee. [BBEC,JU/M2018(11)2]

Toxicity studies

Toxicity studies of the extracts were carried out in Swiss Albino mice of either sex weighing between 20 and 25 g. The extract was found to be safe till 5000 mg/kg p.o. Therefore, doses were selected as 250 mg/kg and 500 mg/kg b.w. [25].

Experimental design for the assessment of liver functions

Animal study was performed at Pharmacology Laboratory, Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342. The rats were housed in polypropylene cages at room temperature (27±2ºC). The rats were divided into five groups of 6 animals (n = 6) each [26].

Group I: received water (10 mL/kg p.o.) once daily for 7 days, and served as normal control

Group II: received water (10 mL/kg p.o.) once daily for 7 days and served as paracetamol control.

Group III: received standard drug silymarin (100mg/ kg p.o.). once daily for 7 days, serving as STD.

Group IV and V: received *Moringa oleifera* bark extract (250 and 500 mg/kg respectively) once daily for 7 days. In all groups except group-I paracetamol 600mg/kg bw p.o was administered once daily with respective treatment according to Tabassum N and Agrawal SS (2004) with slight modification with error and trial [27].

Rats were anesthetized using ketamine (500mg/kg, i.p.). After sacrifice, blood samples from each group of rats were collected and the serum was separated by centrifugation. Serum samples were subjected to liver function tests of enzymes such as glutamate pyruvate transaminase (GPT/ALT), glutamate-oxaloacetate tranaminase (GOT/AST) [28], alkaline phosphatase (ALP) [29], total bilirubin [30] and total protein by standard enzymatic colorimetric method.

Statistical Analysis

Statistical analysis for animal experiments was carried out using One way ANOVA following Bonferroni's post hoc test using SPSS 16.0 for windows. Data were presented as Mean ± SEM. The results obtained were compared with the vehicle treated paracetamol control group. *p* values <0.05, <0.01 and <0.001 were considered to be statistically significant, highly significant and very highly significant respectively.

Result and Discussion

Acetaminophen (AAP) is a frequently used analgesic that causes the formation of NAPQI and hepatic damage by GSH depletion. At high doses, more NAPQI will bind covalently to cellular macromolecules [31, 32, 33, 34]. Therefore, macromolecules like enzymes will leak from the damaged tissues into the bloodstream, [35] and a study of these enzyme activities in plasma has been found to be of great importance in the assessment of liver damage [36].

The increased levels of AST and ALT indicate cellular damage and loss of functional integrity of the hepatocytes [37]. The increase in ALP in liver disease reflects the pathological alteration in biliary flow [38].

The present study demonstrated that the MO extract decrease the SGPT very highly significantly ($p<0.001$), SGOT level highly significantly ($p<0.01$) at 500 mg/kg dose and also the ALP level significantly at 250 mg/kg ($p<0.01$)) (figure 3).

Reduction of the enhanced level of serum SGPT, SGOT, ALP and total bilirubin (figure 4) by MO extract seemed to offer protection and maintain the functional integrity of hepatic cells.

An abnormal increase in the levels of bilirubin in plasma indicates hepatobiliary disease and severe disturbance of hepatocellular function [39]. Prior oral administration of *Moringa oleifera* extract exhibited significant protection against AAP-induced hepatotoxicity. It decreased the levels of bilirubin (although it was statistically insignificant at both the doses) which is an indication of protection against hepatic damage caused by AAP .

Plasma proteins are mainly produced by the liver, the principle exception being immunoglobulins. Severe liver damage decreases the production of various proteins resulting in reduced serum levels of total protein, albumin, and/ or globulin [40, 41]. Decreased protein

production may render other abnormal test values. e.g. depletion of coagulation factors (all are globulins) may result in prolonged prothrombin or activated partial thromboplastin times [42].

The results indicate that protein level was slightly increased at 250 mg/kg and 500mg/kg dose which was insignificant. The serum albumin level was also insignificantly increased at both the doses although the globulin level was decreased (figure 5).

Through this study also the consumption of MO for 7 days was found to reduce the body weight as well as the liver weight of rats (figure 1 and 2).

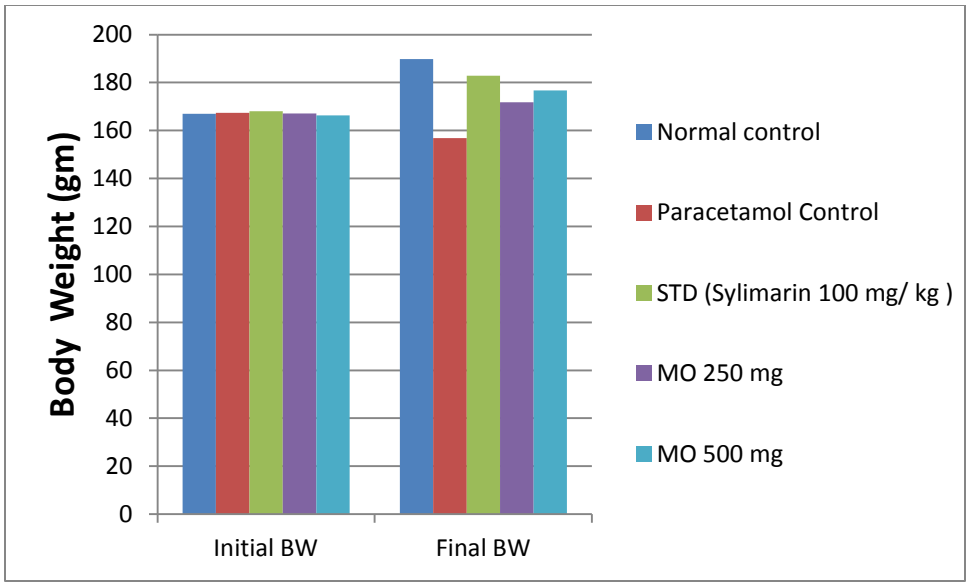

Figure 1. Effect of *Moringa oleifera* bark on the body weight in paracetamol induced hepatotoxicity in rats.

N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values were presented as Mean±SEM, n=6. *($p< 0.05$) = significant, ** ($p< 0.01$) = highly significant, *** ($p< 0.001$) = very highly significant as compared to paracetamol control.

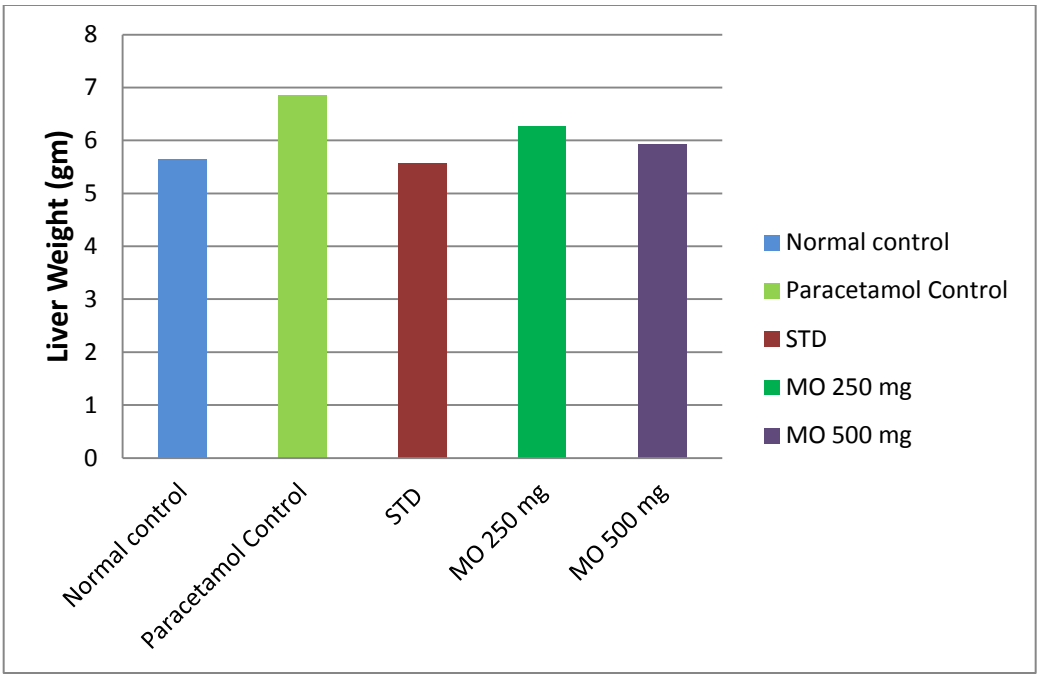

Fig. 2 : . Effect of *Moringa oleifera* bark on liver weight in paracetamol induced hepatotoxicity in rats.

N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values were presented as Mean±SEM, n=6. *($p< 0.05$) = significant, ** ($p< 0.01$) = highly significant, *** ($p< 0.001$) = very highly significant as compared to paracetamol control.

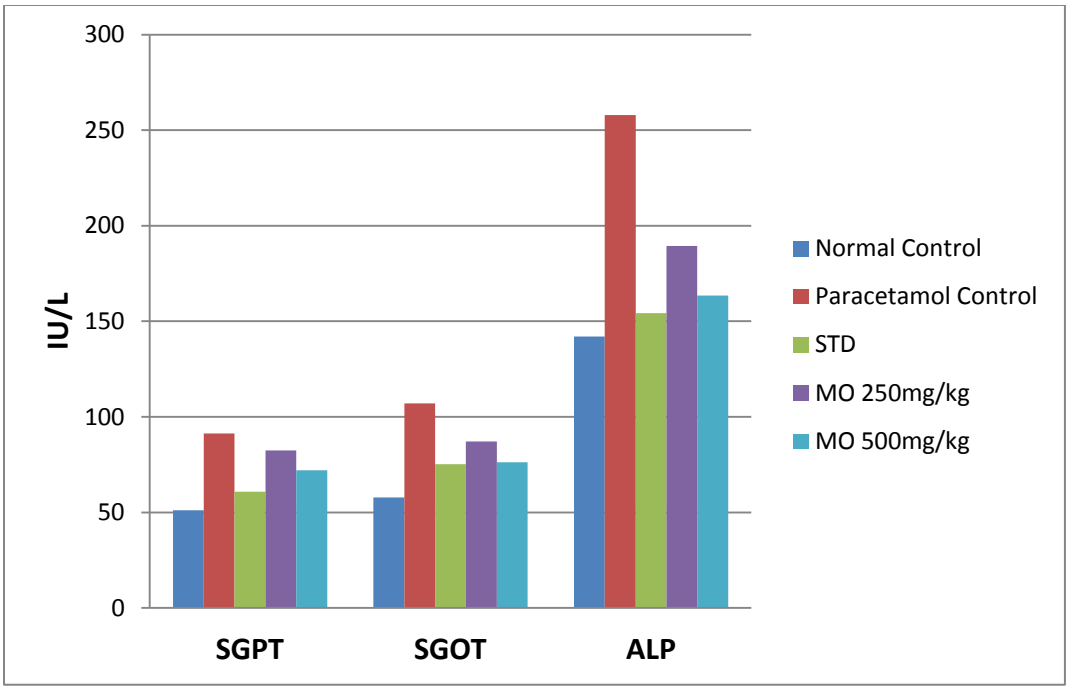

Figure 3. Effect of *Moringa oleifera* bark on SGPT, SGOT and ALP level in paracetamol-induced hepatotoxicity in rats.

N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values were presented as Mean±SEM, n=6. *($p< 0.05$) = significant, ** ($p< 0.01$) = highly significant, *** ($p< 0.001$) = very highly significant as compared to paracetamol control.

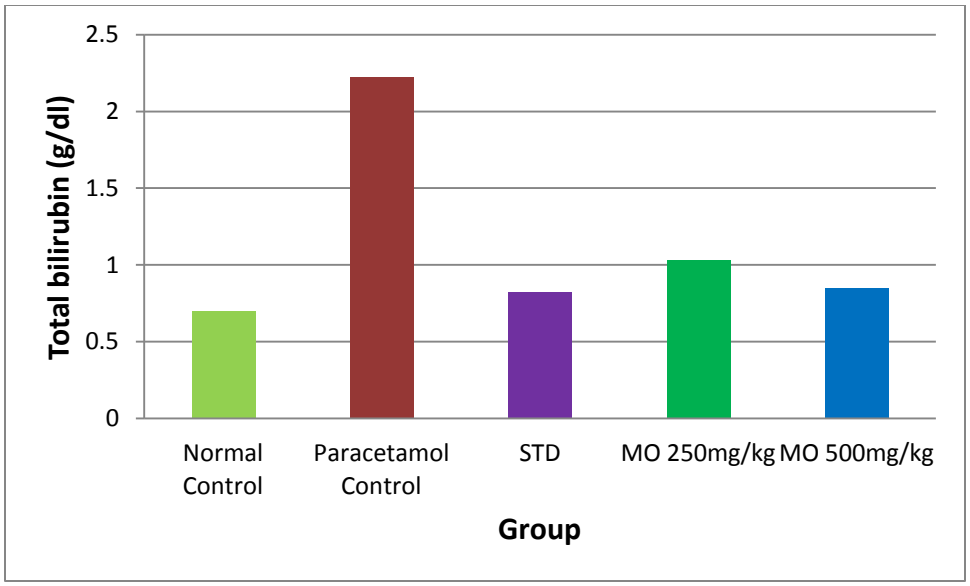

Figure 4 : Effect of *Moringa oleifera* bark on serum bilirubin level in paracetamol-induced hepatotoxicity in rats.

N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values were presented as Mean±SEM, n=6. *($p< 0.05$) = significant, ** ($p< 0.01$) = highly significant, *** ($p< 0.001$) = very highly significant as compared to paracetamol control.

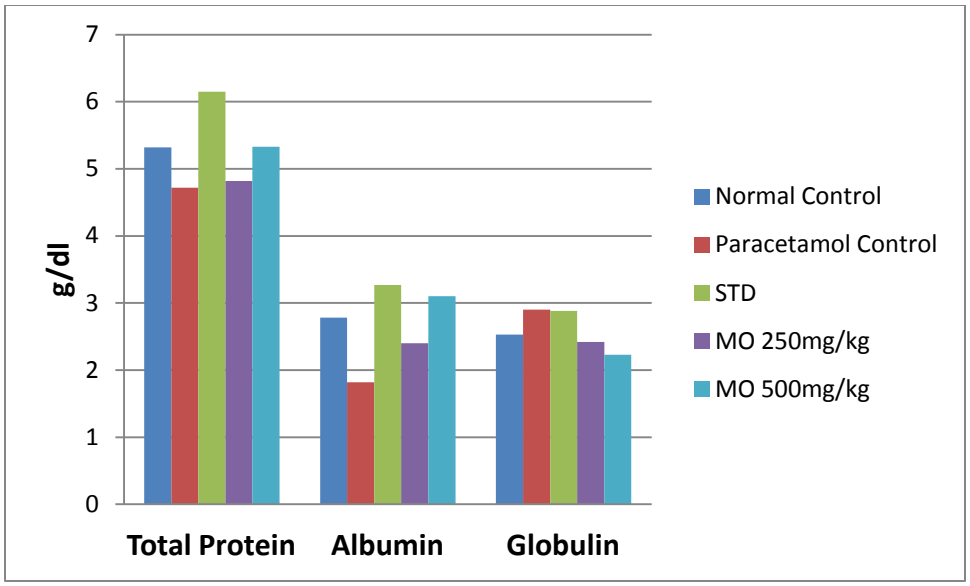

Figure 5: Effect of *Moringa oleifera* bark on serum total protein, albumin and globulin level in paracetamol-induced hepatotoxicity in rats.

N.B: Data were analyzed by one way ANOVA following Bonferroni post hoc test. Values were presented as Mean±SEM, n=6. *($p< 0.05$) = significant, ** ($p< 0.01$) = highly significant, *** ($p< 0.001$) = very highly significant as compared to paracetamol control.

Conclusion

The above study showed that, the treatment with *Moringa oleifera* bark extract was able to protect the changes induced by AAP. On the basis of the above results it can be concluded that *Moringa oleifera* has significant hepatoprotective value against paracetamol induced liver injury. It is thought that the possible reason of liver protective activity of *Moringa oleifera* could be due to the presence of phytochemicals in the ethanolic bark extract. As the crude extract contain a great number of phytocompounds , therefore, further studies are recommended with isolated pure compounds to identify the lead and determine the mechanism(s) involved.

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