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AN EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *BACOPA MONNIERI (L.)* AGAINST PARACETAMOL-INDUCED HEPATOTOXICITY IN SWISS ALBINO MICE

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ABSTRACT
Bacopa monnieri L., locally known as 'Brahmi', is available widespread throughout Bangladesh. This plant contains significant phytochemical constituents and is well known for its numerous pharmacological properties, particularly, memory enhancing, anti-inflammatory, analgesic, antipyretic, sedative and anti-epileptic. A number of observations have been made on its hepatoprotective activities against chemical-induced liver toxicity in recent years. The present study was designed to evaluate the hepatoprotective activities of aqueous and ethanolic extracts of the aerial parts of *Bacopa monnieri L.* in paracetamol-induced hepatotoxic mice model. The animals were allocated into 7 groups and subsequent treatment was provided for 7 days. The toxic control group was given 400 mg/kg b.w./day (p.o.) of Paracetamol while 140 mg/kg b.w./day (p.o.) of Silymarin was administered to the normal control group. The treatment groups were given 200 & 400 mg/kg b.w./day (p.o.) of aqueous and ethanolic extracts of the plant respectively. The hepatoprotective effect of different plant extracts were assessed by determining serum levels of liver enzymes (ALT & AST) and bilirubin. Our study revealed that treatment with 400 mg/kg (b.w. p.o.) of aqueous and ethanolic extracts of the plant significantly reduced the levels of serum ALT, AST and bilirubin. In addition, the histopathological data of liver from a different animal group also supported the assessment of hepatoprotective activities of the plant based on biochemical parameters which confirms the restoration of the normal functional propensity of hepatocytes. To conclude, the present investigation suggests that high doses of different extracts of *Bacopa monnieri L.* offer potential protection against paracetamol-induced hepatotoxicity.

KEY WORDS: *Bacopa monnieri (L.)*, hepatotoxicity, hepatoprotective, aqueous extract, ethanolic extract, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin.

1. INTRODUCTION
The liver is the key organ that regulates all the biochemical, synthetic and excretory functions in our body. It is responsible for excretion of endogenous and exogenous toxic materials from the body. The liver remains continuously exposed to many undesirable chemical components like xenobiotics, environmental pollutants, chemotherapeutic agents as well as other drugs.[1] As a result, hepatic disorder is one of the most common and significant maladies resulting in serious impairment in various metabolic functions that may eventually lead to fatal condition. A number of liver injury tests including serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), albumin, bilirubin, leucine aminopeptidase etc. are carried out to assess the severity and outcome of certain liver diseases.[2,3] Paracetamol (acetaminophen) is widely used analgesic and antipyretic drug which is considered safe at its usual dose. However, an excessive dose of Paracetamol causes acute liver and kidney damage which can be fatal.[1] The drug is metabolized in the liver and when present in an excessive amount it induces oxidative stress as part of the drug is activated by an active liver enzyme (cytochrome P450) which produces toxic metabolites (N-acetyl-P-benzoquinone imine) that are responsible for hepatic necrosis and lipid peroxidation.[4-6] Thus, Paracetamol has been used in several earlier studies to induce liver damage in animal models.[7-9]

At present, management of liver disease has become very challenging for the health service community.[10] Multiple drugs are usually being prescribed for the treatment of chronic hepatic disorders which lead to side effects like gastrointestinal disturbances, allergic skin rashes and nausea.[11] In order to avoid the adverse effects caused by long term use of conventional

medicine, there is a growing demand of herbal medications all over the world.[12] Many plants play a significant role in the management of various liver diseases[8] by regenerating liver cells and hence protect against liver damage.[13] There are several phytochemical constituents such as flavonoids, terpenoids, phenols and steroids that have gained noteworthy attention in recent years due to their protective roles against conditions like oxidative stress, hepatotoxicity etc.[14-16] Sylimarin is marketed as one of the standard hepatoprotective herbal formulations in many countries including Bangladesh.[8]

Among many medicinal plants, *Bacopa monnieri L.* (Family – Scrophulariaceae), which is commonly known as Brahmi, is widely used in Indian Ayurvedic preparations for almost 3000 years[17] due to its various pharmacological activities including anticonvulsive, memory enhancer[18, 19], analgesic, cardiotonic, sedative, spasmodic, anti-cancerous activity, anti-anxiety agent, relieves and prevents stress[20], anti ulcerogenic activity[21-23], digestive aid as well as improve respiratory function in case of bronchoconstriction. Besides, experimental studies on different extracts of Bacopa monnieri L. have shown neuroprotective[24], antioxidant[20, 25] and hepatoprotective[26, 27] activities.

The plant is distributed in the warmer and wetlands regions of the world.[28] It is native to India, Bangladesh, Burma and many other countries in Asia.[29] The entire plant is used in traditional medicine[30] due to the presence of significant bioactive chemical constituents.[8, 28] Despite the widespread availability of *Bacopa monnieri (L.)* in Bangladesh, there are very few documentation regarding the pharmacological activities of the plant extract according to Bangladeshi studies. However, in a previous study the aerial part of different extracts of the plant showed the presence of flavonoid, phenols, alkaloids, terpenoids, tannins and steroids.[23] Further, one of the earlier studies exhibited the existence of considerable flavonoid and phenolic content in the crude extracts of Bangladeshi variety of Bacopa monnieri which indicated potent antioxidant properties of the plant.[29] Flavonoids are a large group of polyphenolic compounds that are responsible for detoxification of free radicals and hence prevents cellular necrosis.[31]

With regards to the above facts, our present investigation involves evaluation of the hepatoprotective activity of ethanolic and aqueous extracts of *Bacopa monnieri (L.)* by using Paracetamol-induced hepatotoxic model of Swiss Albino mice. The present investigation was based on the biochemical parameters and histopathology of the liver of experimental animals where Silymarin was used as standard hepatoprotective herbal therapeutic.

2. METHODS AND MATERIALS

2.1 Drugs and chemicals

Capsules of Silybin 140 (containing 140 mg of Silymarin as active) manufactured by Square Pharmaceuticals Ltd. and Ketamine Hydrochloride injection (Pentyl, 10ml vial) manufactured by ACI Ltd were purchased from the local drug store. Paracetamol (API) was obtained from the pharmaceutical industry Eskayef Bangladesh Ltd. Ethanol, tween 80 and all other reagent materials were purchased from Merck, Germany and were of analytical grade. Enzyme assay kits were obtained from Merck (Darmstadt, Germany).

2.2 Collection and extraction of plant material

Fresh plants of Bacopa monnieri L. was collected from Jahangirnagar University, Dhaka, Bangladesh on January, 2018. The identification of the plant material was confirmed from Bangladesh National Herbarium, Dhaka and the accession number is Bacopa monnieri - 46009. The aerial parts of the plant were selected, washed thoroughly and dried for the present experiment. The plant material was ground into coarse powder followed by preparation of aqueous and ethanol (98%) extracts according to the previously published protocol.[23] The drying, milling and extraction processes of the crude plant were carried out in the Pharmacognosy & Phytochemistry Laboratory of Department of Pharmacy, Primeasia University. The phytochemical screening was done using standard protocols.[32-34]

2.3 Experimental animals

The experimental work was carried out by using 42 Swiss Albino (male) mice of 4 weeks old weighing 30 – 35 gm. All animals were procured from the Pharmacy Department of Jahangirnagar University, Dhaka, Bangladesh. Animals were allocated into seven groups where each group contained six mice and housed in poly propylene cages. The animal house of Pharmacy Department of Primeasia University, Dhaka, Bangladesh was utilized for the present investigation where standard laboratory conditions like temperature (at 24° ± 2°C) and relative humidity (55 ± 5%) with light/dark cycle (12/12 hours) were maintained. The mice were allowed to acclimatize to laboratory condition for 14 days before the commencement of the experiment and were allowed free access of standard pellet diet and water ad libitum throughout the experimental period. Animal care and experimental work were conducted based on principles and guidelines approved by the Guide for the Care and Use of Laboratory Animals (NIH publication No: 85-23, revised in 1985). The hepatoprotective study procedures were approved by the Biomedical Research Center, University of Dhaka, Bangladesh.

2.4 Acute toxicity study

Acute oral toxicity test was carried out based on OECD Guideline 423 (OECD 423).[35] Five mice were taken to which initially 2000 mg/kg and later on 5000 mg/kg doses of both aqueous and ethanol extracts of *Bacopa monnieri L.* were administered. The limit test was thus conducted as per OECD Guideline 423 (OECD 423) which involved observation of the mortality rate at different time intervals including 4, 6, 24 and 48 hours. Changes in behavior and body weight were also

observed. Further, grooming, hyperactivity, sedation, loss of righting reflex, convulsion parameters were measured.

2.5 Experimental design

The animals were divided into 7 groups (n = 6). Animals of all seven groups were subjected to biochemical tests and histopathological analysis. The experimental period involved 7 days and the hepatoprotective study of *Bacopa monnieri L*. extracts was conducted according to a previously published protocol.[8] 0.2% tween 80 (*v/v*) has been used a vehicle for all extracts and standard drug (Silymarin). Hepatotoxic mice model was developed by administering 400 mg/kg body weight of Paracetamol in 0.2% tween 80 (*v/v*) via oral route for once a day and the treatment was continued for 7 days.[8]

The following treatment pattern was carried out in the present study:

Group 1 – Normal control; the animals orally received distilled water alone for 7 days.

Group 2 – Pathological control, the animals orally received Paracetamol (400 mg/kg body weight/day) for 7 days (p.o.).

Group 3 – Standard control, the animals orally received Silymarin (140 mg/kg body weight/day) and Paracetamol (400 mg/kg body weight/day) for 7 days (p.o.).

Group 4 – The animals orally received aqueous extract of Bacopa monnieri L. (200 mg/kg body weight/day) and Paracetamol (400 mg/kg body weight/day) for 7 days (p.o.).

Group 5 – The animals orally received aqueous extract of Bacopa monnieri L. (400 mg/kg body weight/day) and Paracetamol (400 mg/kg body weight/day) for 7 days (p.o.).

Group 6 – The animals orally received ethanol extract of Bacopa monnieri L. (200 mg/kg body weight/day) and Paracetamol (400 mg/kg body weight/day) for 7 days (p.o.).

Group 7 – The animals orally received ethanol extract of Bacopa monnieri L. (400 mg/kg body weight/day) and Paracetamol (400 mg/kg body weight/day) for 7 days (p.o.).

2.6 Sample collection

Animals were allowed to fast 24 hours after the last treatment. After 7 day treatment, animals of all groups were subjected to euthanasia and sacrificed on the 8th day by cervical decapitation. The surgical protocol was maintained based on a previously established method where animals underwent general anesthesia with inhalation of ethylic ether and applying Ketamine hydrochloride (Pentyl) (100mg/kg of body weight).[36,37] Blood sample of each group was collected by retro orbital puncture into sterilized dry centrifuge tubes which was allowed to clot for 30 min at 37°C and then centrifuged at 2500 rpm for 10 minutes.[9]

2.7 Assessment of hepatotoxicity

The serum of each animal group obtained after centrifugation was subjected to a number of biochemical tests to determine serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin. Serum level of total bilirubin was measured based on previously described method by Kaplan.[38] Serum concentrations of alanine amino transferase and aspartate amino transferase were determined according to the earlier protocol by using enzyme assay kits.[39]

2.8 Histopathology study

After cervical dislocation, the liver of each group of animal was dissected out. The liver samples were washed with normal saline and fixed with 10% neutral buffered formalin at 4°C for 24 hours. Later, the liver tissues were washed in xylene and embedded in paraffin under hot air oven (560 C^0 for 24 hours) followed by preparing sections of 5 micron thickness using a microtome. These sections underwent alcohol-xylene series and then stained with haematoxylin and eosin (H&E).[40] The samples were then examined under the light microscope (LeicaMicrosystems, Germany). All procedures were conducted in Exim Bank Hospital, Department of histopathology, Dhaka, Bangladesh.

2.9 Statistical analysis

The results of biochemical tests were expressed as mean ± SD for all 7 experimental groups (n = 6). The data were statistically analyzed by one way ANOVA followed by Dunnett's test using GraphPad Instat3 (version 3.10). The statistically significant difference between control and the treatment groups was determined at 95% significant level (p<0.05). All data were presented in table and bar diagram.

3. RESULTS

The dried extracts of the different solvent systems were found to be 6.5gm and 8.3gm for 200gm of dried *Bacopa monnieri L.* in 500ml of water and ethanol respectively. Significant phytochemical constituents like alkaloids, flavonoids, phenols, tannins, steroids and terpenoids were found in both extracts.

3.1 Acute toxicity study

After the given dose of 2000 mg/kg and 5000 mg.kg, no changes in skin, fur, eyes and mucous membranes were found. Further, conditions like salivation, diarrhoea, lethargy, sleep, tremors, convulsions and coma were not observed. No noteworthy changes in body weight and behavior were observed. All animals remained alive with normal health conditions.

3.2 Biochemical parameters

Table 1: Effect of aqueous and ethanolic extracts of *Bacopa monnieri L.* on biochemical serum parameters in Paracetamol-induced hepatotoxicity on 8th day. The values are expressed in mean ± SEM for six animals in each group.

Group	Treatment	Liver Enzymes		Bilirubin (mg/dl)
		ALT (IU/L)	AST (IU/L)	
01	Normal	29.12±0.72	55.5±0.94	0.23±0.06
02	Hepatotoxic control (Paracetamol 400mg/kg b.w./day p.o.)	$217.75\pm1.03^{**a}$	$221.63\pm1.14^{**a}$	$1.38\pm0.10^{**a}$
03	Paracetamol + Silymerin (140 mg/kg b.w./day p.o.)	$36.38\pm1.84^{**b}$	$98.5\pm1.04^{**b}$	$0.3\pm0.02^{\eta b}$
04	Paracetamol + BMAE (200mg/kg b.w./day p.o.)	$68.63\pm0.85^{**b}$	$132.38\pm1.28^{**b}$	$1.04\pm0.06^{\eta b}$
05	Paracetamol + BMAE (400mg/kg b.w./day p.o.)	$39.00\pm1.47^{**b}$	$92.75\pm3.64^{**b}$	$0.35\pm0.09^{\eta b}$
06	Paracetamol + BMEE (200mg/kg b.w./day p.o.)	$41.63\pm1.43^{**b}$	$193.38\pm1.28^{\eta b}$	$0.41\pm0.08^{\eta b}$
07	Paracetamol + BMEE (400mg/kg b.w./day p.o.)	$24.88\pm1.64^{**b}$	$98.63\pm1.34^{**b}$	$0.25\pm0.07^{\eta b}$

Here, (**) indicates level of significance ($P< 0.01$) while (η) indicates statistically insignificant difference ($p>0.05$) from the respective group using ANOVA, followed by Dunnett's multiple comparison test ($^{**}p < 0.01$). ALT = Alanine transaminase, AST = Aspertate transaminase, BMAE = Aqueous extract of *Bacopa monnieri (L .)*, BMEE = Ethanolic extract of *Bacopa monnieri (L .)*. a = when compared with normal control, b = when compared with ulcer control.

In our present study, Paracetamol (400mg/kg b.w./day p.o.) treated group (Group 2) after 7 days developed considerable hepatotoxic condition by increasing serum levels of liver enzymes (ALT and AST) and bilirubin. It was found that the hepatotoxic control group exhibited significant difference in serum ALT (217.75±1.03 IU/L), AST (221.63±1.14 IU/L) and bilirubin (1.38±0.10 IU/L) levels when compared with normal group (ALT = 29.12±0.72 IU/L, AST = 55.5±0.94 IU/L and bilirubin = 0.23±0.06 mg/dl). Treatment with standard drug, Silymerin (140 mg/kg b.w./day p.o.), indicated improvement in terms of lowering serum levels of liver enzymes by showing significant difference (ALT = 36.38±1.84 IU/L and AST = 98.5±1.04 IU/L) in comparison with hepatotoxic group (Group 2) while no significant change was found in serum bilirubin level (0.3±0.02 mg/dl) when compared with the same group (Group 2).

On the other hand, treatment with different doses (200 & 400mg/kg/b.w./day) of both aqueous and ethanolic extracts of *Bacopa monnieri L.* significantly lowered ($p<0.01$) serum ALT and AST levels when compared with Paracetamol-induced hepatotoxic group (Table 1). However, 200mg/kg b.w./day of ethanolic extracts of *Bacopa monnieri L.* caused no significant change ($p>0.05$) in serum AST level in comparison with hepatotoxic control group. Furthermore, all treatment groups (Group 4 - 7) receiving different doses of plant extracts (200 & 400mg/kg/b.w./day of aqueous and ethanolic extracts of *Bacopa monnieri L.*) indicated insignificant differences ($p>0.05$) in case of serum bilirubin levels as compared to the Paracetamol treated hepatotoxic group.

3.3 Evaluation of histopathology of liver

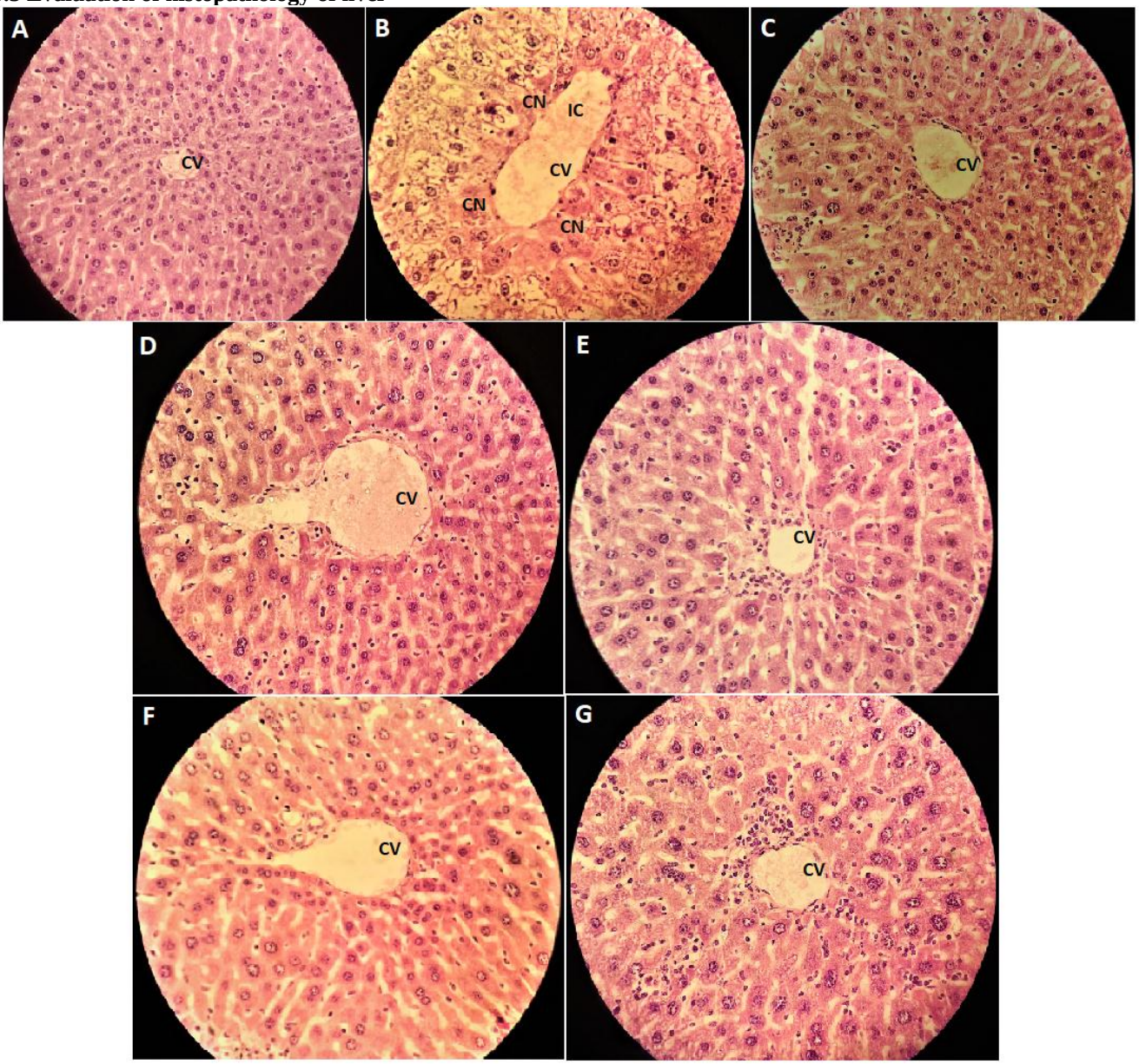

Figure 1: : Histopathological images of the liver of experimental animals; A: Group 1 = Normal control, **B: Group 2** = Hepatotoxic control (Paracetamol 400mg/kg b.w./day p.o.), **C: Group 3** = Hepatotoxic control + Silymerin (140 mg/kg b.w./day p.o.), **D: Group 4** = Hepatotoxic control + 200mg/kg aqueous extract of *Bacopa monnieri (L .)*, **E: Group 5** = Hepatotoxic control + 400mg/kg aqueous extract of *Bacopa monnieri (L .)*, **F: Group 6** = Hepatotoxic control + 200mg/kg ethanolic extract of *Bacopa monnieri (L .)*, **G: Group 7** = Hepatotoxic control + 400mg/kg ethanolic extract of *Bacopa monnieri (L .)*. Liver tissue of each group underwent H&E staining and microscopic assessment was done at 400X magnification, scale bar: 40µm. Here, **CV, CN and IC** indicate central vein, cell necrosis and inflammatory cell respectively.

The normal control group (Group 1) showed typical architecture of liver tissue with the central vein through which group of hepatocytes are tunneled (Fig. 1A). Paracetamol-induced hepatotoxic group (Group 2) caused pronounced necrosis of liver around the centrizonal or centrilobular region (area around the central vein) with infiltration of inflammatory cells within the enlarged central vein (Fig 1B). Morphological improvement of liver tissue was observed in case of treatment with Silymerin (140 mg/kg b.w./day p.o.) where normal hepatocytes were found around the central vein with reduced infiltration of inflammatory cells (Fig. 1C). It was found that low doses (200 mg/kg b.w./day) of both aqueous and ethanolic extracts of *Bacopa monnieri (L.)* (Groups 4 & 6) produced less improved hepatocytes around much enlarged central vein (Fig. 1D & F). On the other hand, high doses (400 mg/kg b.w./day) of different extracts of *Bacopa monnieri (L.)* (Groups 5 & 7) showed better improvement of liver tissue morphology with less

necrosis within the centrizonal region and moderate inflammatory cell infiltration (Fig. 1E & G).

4. DISCUSSION

Hepatocytes are prone to toxicity as they conduct the detoxification process in our body.[41] Therefore, liver injury is the most common abdominal injury and it is frequently assessed by estimating serum levels of liver enzymes like alanine aminotransferase (ALT) and aspartate aminotransferase (AST) which represent 90% of total liver enzymes.[8] In the injured liver, these enzymes are found in high concentration in serum as they leak out of hepatocytes due to erosion of functional integrity of hepatocellular membrane.[42] Bilirubin is another fundamental biomarker for evaluating liver injury. Hyperbilirubinemia is a common observation made in patients suffering from numerous liver diseases. Damaged and dead hepatocytes resulting from extensive heme destruction in injured liver cause excessive release of bilirubin.[43]

There are many drugs and chemicals that are responsible for frequently causing liver damage and can lead to life threatening liver diseases like liver cirrhosis, hepatitis etc.[44] Paracetamol has been well recognized as an agent to produce liver toxicity in experimental animals. Studies have been conducted in previous years where paracetamol has been used to induce hepatic injury in rodents for evaluation of hepatoprotective activities of chemical or drug moieties.[7,45,46] In the present investigation, oral administration of paracetamol for 7 days potentially injured liver tissues of experimental mice by extensively increasing levels of liver enzymes (ALT and AST) and bilirubin in serum than the normal levels found in the normal control group. The microscopic analysis of liver tissue also exhibited extensive destruction of hepatocytes by paracetamol overdose causing enzymes to leak out into the circulation. Hyperbilirubinemia is seen to be is directly associated with the degree of histological injury of hepatocytes.[2,47]

Earlier studies on ethanolic and methanolic extracts of *Bacopa monnieri* showed hepatoprotective properties against CCl_4 induced liver injury in rodent models.[27,48,49] In our current work, we treated paracetamol-induced hepatotoxic mice model with 200 and 400 mg/kg b.w. of aqueous and ethanolic extracts of *Bacopa monnieri (L.)* for 7 days. Both extracts exhibited hepatoprotective activities at high doses (400 mg/kg b.w.) by markedly reducing serum ALT and AST levels. No significant difference was found in case of serum levels of bilirubin in either of the plant extracts when compared with the toxic level (Group 2). However, the mean (mean ± SEM) serum bilirubin level of mice treated with 400 mg/kg b.w./day of ethanolic extracts of *Bacopa monnieri (L.)* (0.25±0.07 mg/dl) was found to be much close to its level observed in normal group (0.23±0.06 mg/dl). A former study showed a considerable reduction of serum liver enzymes and bilirubin levels in paracetamol-induced hepatotoxic animals treated with 300 mg/kg b.w./day of ethanolic extracts of *Bacopa monnieri (L.)*.[50] Ethanolic extracts of this plant extensively prevented hepatic damage in many studies by significantly reducing serum levels of bilirubin and other biomarkers.[27,51-53] It has been reported that pretreatment with the extracts of *Bacopa monnieri (L.)* prevented the elevation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine.[54]

The microscopic analysis of hepatic tissue morphology was carried out via Hematoxylin and Eosin staining which revealed several alterations in tissue architecture of different animal groups. Overdose of paracetamol established extensive liver damage with enlarged central vein comprising of centrizonal necrosis along with cell inflammation, and infiltration of inflammatory cells. Treatment with a high dose of plant extracts (400 mg/kg b.w./day p.o.) demonstrated considerable improvement of the architecture of hepatocytes with reduced necrotic and degenerative changes (Fig. 1 E & G). This observation was similar to the histological results of mice administered with Silymerin (140 mg/kg b.w./day p.o.). However, treatment with low doses of different extracts of *Bacopa monnieri (L.)* (200 mg/kg b.w./day p.o.) showed partial recovery of hepatocytes with considerable necrosis around the moderately enlarged central vein and substantial infiltration of inflammatory cells (Fig. 1 D & F). This suggested that high dose of both aqueous and ethanolic extracts of the plant offered better protection against hepatic damage. In the present study, the biochemical parameters well corresponded with the histological data. Although the changes in serum bilirubin levels in treatment groups (Groups 4-7) evidenced no significant difference when compared to hepatotoxic control group (Group 2), the treatment with ethanolic extract of 400 mg/kg of *Bacopa monnieri (L.)* (0.25±0.07 mg/dl) displayed results much similar to that of normal group (0.23±0.06 mg/dl). This indicated that, considering all the parameters for evaluation of present study, 400 mg/kg of ethanolic extract of *Bacopa monnieri (L.)* revealed better hepatoprotective effect with maximum protection against liver damage. In the earlier studies, similar results were observed with 200 and 300 mg/kg b.w./day of ethanolic extracts of this plant where treatment was continued for a longer period of time.[52; 55] Nevertheless, from the previous findings, we speculate that due to considerable saponin content (bacoside-A) within the extracts of *Bacopa monnieri (L.)*, it possesses potential hepatoprotective properties.[27,56]

Although, in India, extensive research on hepatoprotective activities of *Bacopa monnieri (L.)* has been performed in the past years[49], minimal interest was observed in the studies of hepatoprotective properties of this plant in Bangladesh. However, a significant population of Bangladesh rely on natural products for the treatment of various ailments since the country possess a great diversity in plants.[57] Therefore, it can be suggested

that the plant may be utilized as a promising source of herbal remedy for various liver diseases within the Indian subcontinent.

5. CONCLUSION

In conclusion, the findings of our present study unveiled that due to presence of potential phytochemical constituents like flavonoids and saponins in the aqueous and ethanolic extracts of *Bacopa monnieri (L.)*, it protected the liver from paracetamol-induced hepatotoxicity. However, further analysis is required for isolation and characterization of these phytochemical compounds in order to obtain a clear understanding of these protective mechanisms by the plant extracts.

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