International Journal of Biological and Pharmaceutical Sciences Archive

ISSN: 0799-6616 (Online)

Journal homepage: https://ijbpsa.com/

(RESEARCH ARTICLE)

Check for updates

Investigation of *In-vivo* antioxidant and hepatoprotective potential of ethanolic extracts of *Bombax insigne* (Sw.) K. Schum on *Swiss albino* Rat

Md. Ataur Rahman, Runa Masuma *, Arpon Krishna Bala, Md. Al Foyjul Islam and Md. Shohel Hossain

Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

International Journal of Biological and Pharmaceutical Sciences Archive, 2023, 05(01), 087–096

Publication history: Received on 09 February 2023; revised on 20 March 2023; accepted on 23 March 2023

Article DOI: https://doi.org/10.53771/ijbpsa.2023.5.1.0025

Abstract

Medicinal plants are frequently used in traditional medicine and about 80% peoples of developing countries use this traditional therapy to treat their ailments. *Bombax insigne* (Sw.). K. Shcum is a large deciduous tree, locally known as Bon or Pahari shimul or Tula gachh. The present study was designed to investigate the antioxidant and hepatoprotective potential of ethanolic extract of *B. insigne* fresh bark. *In-vivo* antioxidant activity tests were performed by determining the lipid peroxidation and catalase concentration of the homogenized liver of *Swiss albino* rats receiving two doses (1.5 and 3 gm/kg body weight) of the plant extract with 750 mg/kg body weight of paracetamol. For liver function tests (LFT), total 35 rats were taken and divided into five groups containing seven animals in each. Group I received distilled water; group II received only paracetamol. Group III received paracetamol with silymarin. Group IV and V both were treated with paracetamol along with two doses of *B. insigne* extract. To evaluate the hepatoprotective potential, seven liver test markers such as total protein, albumin, total bilirubin, alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT) of rats were considered. Test's result for antioxidant activity demonstrated that the plant extract could inhibit the level of lipid peroxidation (LPO) induced by paracetamol. Enhancement of the level of the antioxidant enzyme catalase (CAT) was also observed. Paracetamol-induced hepatotoxicity in rats, as judged by the raised total protein, albumin, total bilirubin, serum enzymes, ALT, AST, ALP, GGT, were significantly prevented by the treatment with the extracts at different doses, demonstrating the hepatoprotective action of *B. insigne*. Histopathological observation also confirmed the hepatoprotective potential of *B. insigne*. Livers those were challenged with paracetamol exhibits the dishevelment of normal hepatic cells with centrilobular necrosis, inflammatory encroachment of lymphocytes and fatty changes were significantly protected by plant extract. The results of the present study suggested that *B. insigne* has antioxidant and hepatoprotective potential.

Keywords: Plant Extract; Paracetamol; Silymarin; Antioxidant; Hepatoprotective; Histopathology

1. Introduction

Liver disease after heart, stroke, chest disease and cancer, is the fifth common causes of death and now a days, it is a serious problem in the world [1]. Liver diseases linked with morbidity and mortality are viral hepatitis (hepatitis B and C), alcoholic liver disease (ALD), non-alcoholic fatty liver disease (FLD), liver cirrhosis and hepatocellular cancer [2]. Numerous liver diseases are manifested due to the presence of an oxidative stress component like reactive oxygen and reactive nitrogen radicals, superoxide, hydrogen peroxide, singlet oxygen, and hydroxyl that can be formed as a by-product of mitochondrial electron transport [3].

Free radical injury is rapidly emerging as a final common pathway for tissue damage. where Free radicals can establish chain reactions and causes oxidative damage. Oxidative damage significantly shows pathological role in human and manifest cancer, emphysema, cirrhosis, arteriosclerosis, and arthritis [4]. Excessive generation of ROS, which is induced by

* Corresponding author: Runa Masuma, Email: masuma@juniv.edu

various stimuli, when exceeds the antioxidant capacity of body, leads to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer [5]. Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase or antioxidant compounds such as ascorbic acid, tocopherols, and glutathione [6]. Antioxidants inhibit or delay the oxidation process by blocking the initiation or propagation of oxidizing chain reactions [7], by blocking harmful action of the free radicals or through scavenging the free radicals [8, 9,10]. Catalase enzyme, main regulator of hydrogen peroxide metabolism act as natural antioxidant [11,12].

Medicinal plants are frequently used in traditional medicine to treat different human diseases in different parts of the world [13], and primary source of medicine still now in rural areas of the developing countries [14]. Plants have formed the basis of sophisticated traditional medicine practices that have been used for thousands of years by people in China, India, and many other countries [15]. Plant secondary metabolites play an important role in health care for about 80% of the world's population [16]. About 80% peoples of developing countries use this traditional therapy to treat their ailments where plants are the major sources of traditional medicine [17-19]. Bangladesh has a rich and prestigious heritage of herbal medicines and more than 500 species of medicinal plants are estimated as growing in Bangladesh within which about 250 species of them are used for the preparation of traditional medicines and the majority of these plants have not yet undergone chemical, pharmacological and toxicological studies to investigate their bioactive compounds [20]. Examples like Crocin (isolated from stigmas of *C. sativus*), kaempferol (isolated from corms and petals of *C. sativus*) and Podophyllotoxin (isolated from rhizome of *P. hexandrum*) demonstrated strong antioxidant activity [21].

Bombax insigne is large deciduous trees named as silk cotton tree belong to the family Malvaceae, grey to greyish-brown in color, habitat in mixed deciduous and dry dipterocarp, ocky areas in evergreen, semi-evergreen and moist deciduous forests. Phytochemical analysis showed the presence of alkaloids, flavonoids, phenolics, cardiac sterols, triterpenoids, saponins, tannin and carbohydrates in the leaves of *B. insigne* [22,23]**.** Pharmacological studies found that it possessed antioxidant properties [24]. The bark of *Bombax insigne* along with *Pteris pellucida* and *Basella alba* are crushed together and taken for the treatment of spermatorrhoea in some places of India, Myanmar and Bangladesh [25].

For ensuring the medicinal and pharmacological properties of a medicinal plant, phytochemical screening of plant parts and various pharmacological studies are exceedingly needed. Liver function tests (LFT) are a helpful screening tool, which is an effective modality to detect hepatic dysfunction. The work described in this article was dedicated to investigate the *In-vivo* antioxidant and hepatoprotective properties of *Bombax insigne* bark extract as well as histopathological observation of experimental animals through this extract. This may be creating a good source of information related to liver disease for further investigative study.

2. Material and methods

2.1. Plant Collection, Identification and Plant Material Preparation

The bark of *B. insigne* plant was collected from Pablakhali reserve forest, Rangamati, Bangladesh and identified by Department of Botany, Jahangirnagar University as well as authenticated by the taxonomist of the National Herbarium of Bangladesh (voucher specimen accession number DACB No 46867)), Mirpur, Dhaka. The bark was washed, sun-dried and then, dried in a hot air oven (Size 1, Gallenkamp) at reduced temperature (not more than 50 ℃) and was grounded into coarse powders.

2.2. Plant Extraction

The powdered plant materials (1700 gm of bark part) were used for extraction by Soxhlet apparatus at elevated temperature (65 ℃) using ethanol. After extraction, the plant material was dried and used again for the next extraction. Extraction was considered to be complete when the plant materials become exhausted of their constituents that were confirmed from cycles of colorless liquid siphoning in the Soxhlet apparatus. Final extracts of bark were filtered carefully through a fresh cotton bed. The filtrates obtained were dried at the temperature of 40 ± 2℃ in a water bath to have a gummy concentrate of the crude extracts. The crude extract was stored in a cold and dry place and used in Scrutinizing antioxidant activity and evaluating of hepatoprotective properties.

2.3. Experimental Animals

For this experiment, *Swiss albino* rats of either sex, 7-8 weeks of age, weighing between (110-150 g) were collected from the animal research laboratory in the Department of Pharmacy, Jahangirnagar University, Dhaka, Bangladesh. Animals were maintained under standard environmental conditions (temperature: 25± 2℃, relative humidity: 55-65% and 12-hour light/12-hour dark cycle) and had free access to feed and water ad libitum. The animals were acclimatized to

laboratory condition for two weeks prior to the experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

2.4. Acute Toxicity Test and Selection of Suitable Dose for the Study

The acute toxicity study was conducted to find out LD_{50} of the test samples. Test animals were divided into seven groups comprises four animals in each and the test samples were administered orally to the test animals at seven different doses. These doses were administered repeatedly three times at one-hour interval and mortality was observed for every 1 hour for the next 6 hours. Finally, the test animals were observed after 24 hours for the death of certain populations. No mortality was found till 5000 mg/kg p.o [26].

2.5. Experimental Design

For evaluating the *In-vivo* antioxidant activity and hepatoprotective potential of *Bombax insigne* bark extract, a total of 35 test animals were assembled into five groups and each group contains seven animals (Table 1). There was 24 hours' free access for all the rats to normal diet spontaneously. By considering all the parameters that were done in the acute toxicity test, a suitable dose was selected for hepatoprotective activity determination through *Bombax insigne* bark extract.

Table 1 Group distribution of studied animals and the name of substances to be administered with their specific doses and schedule

Animal Groups	Abbreviation	No. of Rats	Treatments	Dose (per kg body weight)	Duration	Route of Administration
Group I (Normal)	Norm	7	Distilled Water	20 ml	15 days	Per Oral
Group II (Control)	Con	7	Paracetamol	750 mg		
Group III (Standard)	Std	7	Paracetamol + Silymarin	750 mg + 100 mg		
Group IV (Single Dose)	Bom 1.5 gm	7	Paracetamol + *B. insigne*	750 mg + 1.5 gm		
Group V (Double Dose)	Bom 3 gm	7	Paracetamol + *B. insigne*	750 mg + 3 gm		

2.6. Animal Maintenance, Sacrifice and Blood Collection

The rats were housed in plastic cages (bedding was hardwood chips and in metabolic cages and received a natural 12 h day- night cycle. The rats were provided with a standard laboratory pellet diet and water ad libitum. During the test period, body weight changes evaluated daily. At the end of the experimental period, the rats in each group were sacrificed by deep anaesthetization with ketamine hydrochloride (100 mg/kg body weight) injection followed by dissection. Blood samples (~4 mL) were collected by heparinized syringe (size: 5 mL) from inferior vena cava. After that, the liver was immediately removed and placed on an ice bag for calculating the weight. Then the organs weight ratio was calculated.

For hematological analysis, the injected blood samples (~1 mL) were directly transferred into EDTA containing tube and stored at -20°C. In the meantime, for biochemical analysis, the blood samples were collected in dry test tubes and were allowed to coagulate at ambient temperature for 30 min before centrifugation at 2,000 rpm for 10 min to separate the serum. The serum was stored at -20°C for further biochemical analysis.

2.7. Tissue Collection and Preparation of Liver Homogenate

The different tissues of rats were properly perfused with ice-cold phosphate buffer saline (PBS) through heart and then excised immediately. The liver sample was further perfused to remove remaining blood. The liver samples were blotted, minced and homogenized with ice-cold phosphate buffer (25mM, pH 7.4) to make approximately 10% (w/v) tissue

homogenate using polytron homogenizer. The homogenates were directly used to estimate lipid peroxidase enzymes and antioxidant catalase enzyme levels in liver tissue.

2.8. *In-vivo* Antioxidant Activity Evaluation by Lipid Peroxidation (LPO) and Catalase Concentration Estimation (CAT)

The extent of lipid peroxidation and catalase enzyme concentration are very pressing parameter for *In-vivo* antioxidant evaluation. After sacrificing the animals, liver tissues were collected, washed with immediately prepared normal saline and soaked in filter paper. One gram of the liver tissue was homogenized in 10 ml of 0.15M Tris buffer P^H 7 to7.4 and centrifuged at 3000 rpm at 4°C for 30 minutes. Supernatant was collected and biochemical assays were performed for determining absolute extent of lipid peroxidation and concentration of antioxidant enzyme catalase. These two antioxidant tests were performed by using the procedure used in the article [27].

2.9. Hepatoprotective Property Evaluation (*In-vivo*) through Liver Function Test

Seven mostly pertinent tests parameters were selected for ensuring the liver function test. These are Alanine transaminase (ALT), Alkaline Phosphatase (ALP), Aspartate Aminotransferase (AST), Gamma-Glutamyl Transferase (GGT), Albumin, Total Bilirubin and Total Protein.

2.10. Histopathological Observation

After sacrificing the rats by cervical dislocation, liver tissue was collected and washed in normal saline. The isolated livers were presented in formalin (13% v/v) (E Merck, Germany) and histopathological evaluation of the organs were made. The microscopic view of the tissues was photographed and compared with that of the controls.

2.11. Statistical Analysis

Data from the experiments were analyzed using the IBM Statistical Package for Social Science (SPSS) Statistics 22 for Windows and Microsoft Excel, Office 2013. Values were presented as Mean ± SEM (Standard Error of the Mean). Unpaired "t" tests One-way ANOVA followed by Dennett's multiple comparison test was done as the test of significance. $p<0.05$ was considered as the minimal level of statistical significance. $p<0.05$, $p<0.01$, $p<0.001$ are represented by single (*), double (**), triplet (***) asterisk(s).

3. Results

3.1. *In-vivo* Antioxidant Activity Evaluation

Ethanolic extract of bark of *B. insigne* showed sufficient reduction of lipid peroxidation and increased the production and release of catalase enzyme (Figure -1). In this study, there was the normal extent of lipid peroxidation in the animals of the normal control group but a significant increase of lipid peroxidation in paracetamol treated diseased control group whereas the standard group significantly reduced lipid peroxidation compared with the diseased control group. *B. insigne* at both doses showed significant reduction of lipid peroxidation. By comparing with the diseased control group, it was clear that the double dose of *B. insigne* bark had more capability to reduced lipid peroxidation revealed by significant reduction of lipoxygenase (LPO) than the single dose though the result of LPO for single dose was also significant (Figure 1).

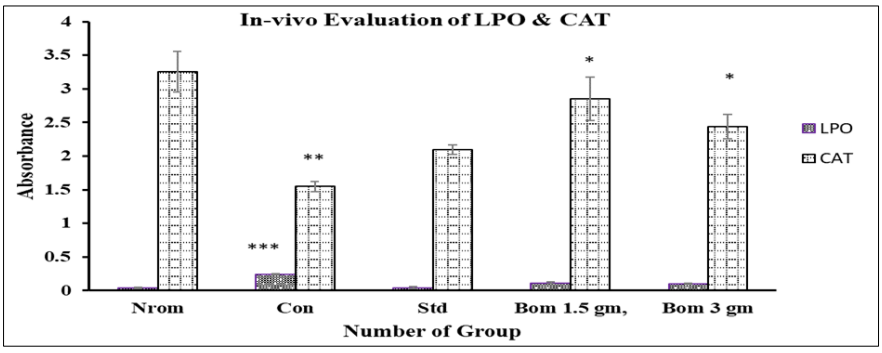

Figure 1 Changes of extent of lipid peroxidation and antioxidant enzyme Catalase concentration in liver tissue

In antioxidant activity evaluation, the diseased control group showed less quantity of catalase enzyme than the normal control group and it revealed the sign of toxicity. The standard group helped to restore the normal range of catalase concentration in the experimental animals. *B. insigne* single dose showed higher restoration capability than the double dose but both the single and double dose showed sufficient renovation capability compared to disease control group. No significant higher concentration was found in case of both doses of plant extract (Figure 1).

3.2. *In-vivo* Hepatoprotective Property Evaluation

3.2.1. Assessment of Body Weight Variation

In body-weight variation assessment study, the mean body weight of the experimental animal was continuously increased in the normal group but in case of diseased control group there was no significant change in mean body weight. The growth of body weight of standard group was also normal. Comparing the average body weight of *B. insigne* single dose and double dose group with the diseased control, it revealed that *B. insigne* bark has weight restoration capability but it was insignificant and not enough (Table 2).

Table 2 The Evaluation of Body Weight Variation in the Experimental Animals

Body Weight Variation					
No. of Days	Group				
	Norm	Con	Std	Bom 1.5 gm	Bom 3 gm
IBW	127.5± 6.323	122.5± 7.736	126.17± 7.467	121.17± 7.049	124± 6.077
Day - 1	133.17± 5.952	124.67± 7.796	130.17± 7.016	123.67± 6.606	107± 19.732
Day - 3	140.83± 5.753	127±8.033	133.5±8.621	128.83± 6.740	120.83± 4.902
Day - 5	147±6.578	129.83±7.956	135.83±8.304	126.17± 8.076	121.83± 5.056
Day - 7	152.167±6.565	128.333±7.242	137.833± 8.463	125.667± 8.007	123.5± 4.552*
Day - 9	157.83±6.877	129.5±7.784	138.5± 8.053	122.33± 8.241**	123.33± 4.499*
Day – 11	162.67± 7.205	129.5± 6.602	137.5± 7.464	124.33± 8.543**	124.67± 4.991**
Day – 13	166.17± 6.858	133.17± 6.047	142± 8.238	128.33 ±8.441*	130.17± 5.582**
FBW	164.83± 7.054	132± 5.961	142.67± 8.413**	124.83± 9.293**	126.33± 5.463

*= p<0.05, **= p<0.01 and ***= p<0.001; IBW = Initial Body Weight, FBW = Final Body Weight, Norm = Normal Group, Con = Control Group, Bom 1.5 gm = *Bombax insigne* single dose, Bom 3 gm = *Bombax insigne* double dose.

3.3. The Evaluation of biochemical markers for assessment of Liver functions

To determine the condition of the liver, Total protein, Albumin, Total bilirubin of blood and four enzymes Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP) and Gamma-glutamyl transpeptidase (GGT) concentration were evaluated. The normal control group received distilled water had a sufficient level of total protein, albumin and a very low level of total bilirubin concentration where all were within the normal range but in case of paracetamol treated diseased control group, all the values increased and exceed the normal range (Figure 2). Comparing with the diseased control group, the standard group retained significant body proteins concentration which indicated the normal range and reduced bilirubin concentration and restored the animals at a healthy state. The single and double dose of ethanolic extract of bark *B. insigne* showed a sufficient reduction of T. protein, albumin and T. bilirubin level in paracetamol induced toxic liver compared to diseased control group and give similar protection as well as standard group (Figure 2).

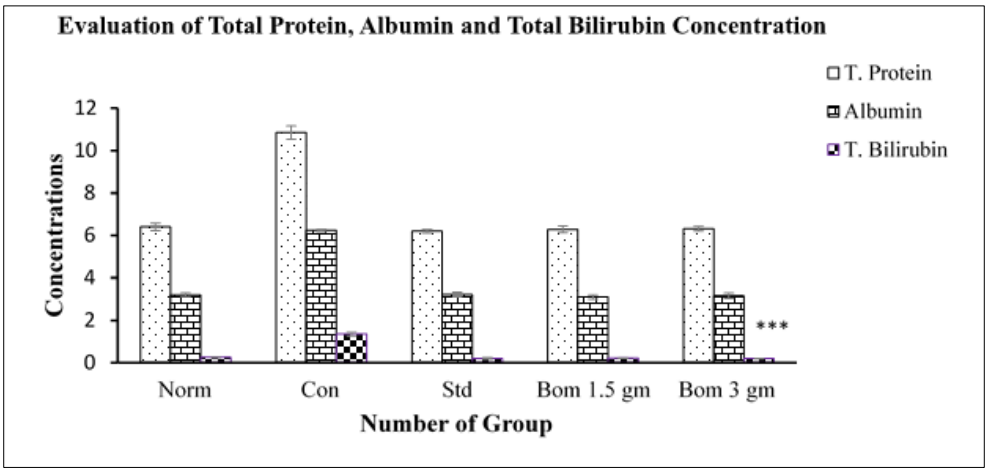

Figure 2 Evaluation of Total protein, Albumin and Total Bilirubin of the Experimental Animal (T. protein = Total protein, T. Bilirubin = Total Bilirubin)

All of the three enzymes without Alkaline Phosphatase showed normal concentration range in the animals of the normal control group. Alkaline Phosphatase showed high level beyond the desired range. At paracetamol treated diseased control group all enzymes level was out of the normal range which revealed the damaged condition of the liver (Figure 2). The standard group restored the normal range of all enzymes without Alkaline Phosphatase because this enzyme was sufficiently high in concentration from the starting point of the study. The single dose of ethanolic extract of *B. insigne* did not exhibit any good results and had not sufficient capacity to furbish up normal state. The double dose of ethanolic extract of *B. insigne* bark had the precise capability to restore in normal range better than the standard group (Figure 3). Double dose can protect liver toxicity induced by paracetamol and significantly reduced the level of ALT, AST and GGT.

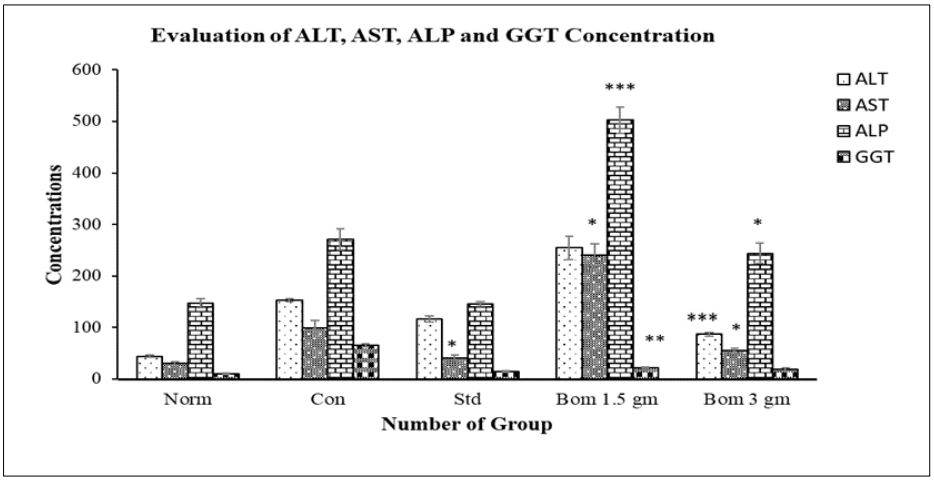

Figure 3 Evaluation of ALT, AST, ALP and GGT Concentration of the Experimental Animals (ALT = Alanine transaminase, AST = Aspartate Aminotransferase, ALP = Alkaline Phosphatase, GGT = Gamma-Glutamyl Transferase)

3.4. Results of Histopathological Observation

Histopathological study of livers of the normal control group showed a normal hepatic architecture. Hepatocytes are arranged in a plate of hepatocytes, with sinusoidal between the hepatocyte plate. Livers challenged with paracetamol showed disarrangement of normal hepatic cells with massive centrilobular necrosis, inflammatory infiltration of lymphocytes and fatty changes (Figure 4.1). The figures (Figure 4.2) represent the standard group which treated with Silymarin which also represents the finest protection. Moderate protection was observed in case of ethanolic extract of *B. insigne* of 1.5 gm/kg body weight group animals (Figure 4.3). The ethanolic extract of *B. insigne* of 3 gm/kg body weight treated rats exhibited significant protection against paracetamol intoxication as evident by the presence of normal hepatocyte and absence of necrosis with minimal inflammatory conditions (Figure 4.4).

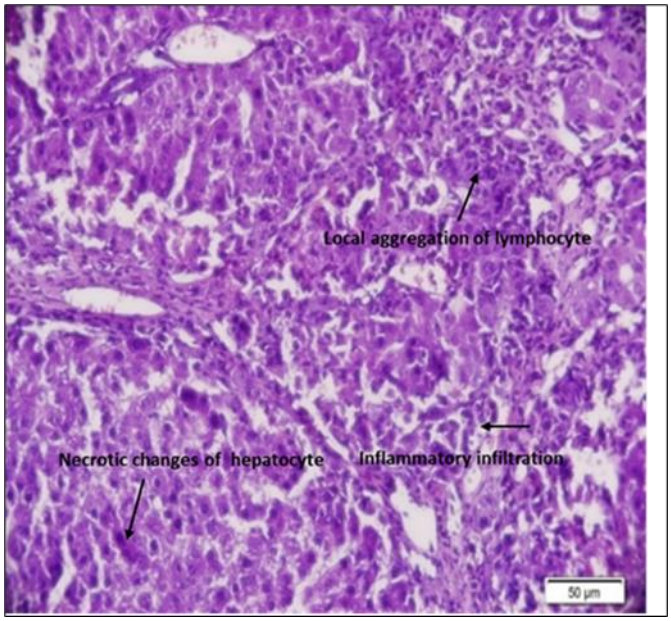

Figure 4.1 Disease control group (Paracetamol 750mg)

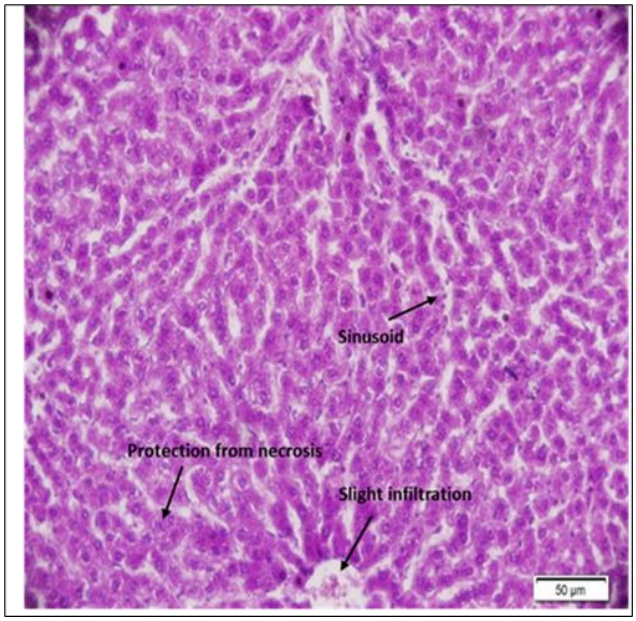

Figure 4.2 Standard group (Paracetamol 750mg + Silymarin 100 mg)

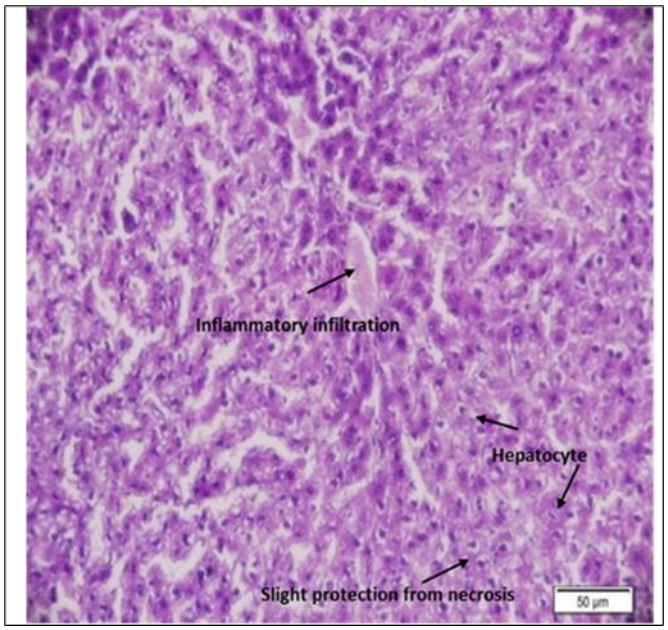

Figure 4.3 Treated group (Paracetamol 750mg + B. insigne 1.5 gm/kg)

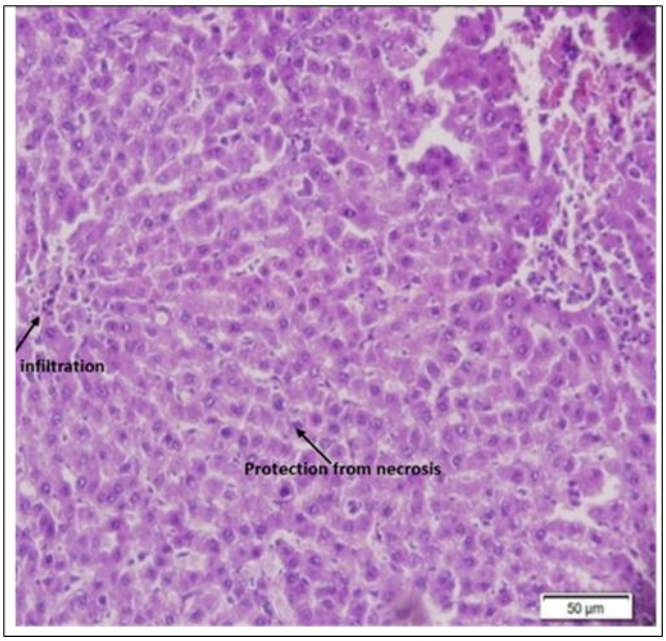

Figure 4.4 Treated group (Paracetamol 750mg + B. insigne 3 gm/kg)

4. Discussion

For ensuring *In-vivo* antioxidant activity, the extent of lipid peroxidation and the level of catalase enzyme were considered for this study. Considering the hepatotoxic characteristic, paracetamol was selected for *In-vivo* study to generate artificial liver toxicity. The increase in malondialdehyde (MDA) levels (end product of lipid peroxidation) in the liver suggest enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals. The elevations in the levels of end products of lipid peroxidation in the liver of rat treated with paracetamol in diseased control group indicated that paracetamol had a sufficient level of toxicity. By comparing with the diseased control and standard group, it was clear that the double dose of *B. insigne* bark had more capability to reduce lipid peroxidation than the single dose.

Catalase (CAT) is an enzymatic antioxidant that showed highest activity in the red cells and the liver and decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals. It is beneficial if the catalase enzyme concentration level in the body is high because it is an antioxidant enzyme. In this study the normal control group showed a sufficiently high quantity of catalase enzyme, whereas the diseased control group showed less quantity and it revealed the sign of toxicity. The standard group helped to restore the normal range of catalase concentration and *B. insigne* single dose showed higher restoration capability than the double dose but both the single and double dose showed sufficient renovation capability.

The liver disease greatly impacts nutritional status [28]. Other potential malnutrition causes include digestion and absorption abnormalities due to chronic liver failure as well as metabolic abnormalities [29] . Moreover, weight loss during this period is difficult to assess because ascites, edema and risk factors for these conditions have not yet been well-assessed. The current study aimed to evaluate weight loss and its association with liver disease. In this study, Comparing the average body weight of *B. insigne* single dose and double dose group with the diseased control group, it revealed that's *B. insigne* has weight restoration capability but it was insignificant and not enough.

An albumin test measures how well the liver is making the proteins that the body needs. Low albumin levels can be a sign of liver damage. [30]. Bilirubin is a yellow fluid made in the body when red blood cells are broken down. If the liver is damaged, bilirubin can leak out of the liver into the blood and can show abnormally high serum bilirubin levels suggest underlying liver disease [31]. All values were normal in the normal group and abnormal in the diseased control group without the alkaline phosphatase. Compare and contrast with the diseased control group, the standard group contained significant body proteins within the normal range and restored the animals in a healthy state. The double dose of ethanolic extract of *B. insigne* showed significant beneficial effect than the single dose.

Enzymes are a good biochemical parameter to detect liver damage. Alanine Transaminase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (ALP) and Gamma-glutamyl transpeptidase (GGT) are enzymes mainly found in your liver. Consistently high level of these enzymes represents the damage of liver [30]. ALT, AST, ALP and GGT serum concentration was measured in each experimental animal. The single dose of ethanolic extract of *B. insigne* had not sufficient capacity to furbish up the normal state of the liver but the double dose had the precise capability to restore in normal range better than the standard group. The revitalization of serum markers by *B. insigne* suggests that they can condition the hepatocytes, to protect the membrane integrity against paracetamol-induced leakage of marker enzymes into the circulation. The above changes can be considered as an expression of the functional improvement of hepatocytes, which may be caused by an accelerated regeneration of parenchyma cells.

The histopathological observations in paracetamol-treated rats showed severe necrosis, neutrophil infiltration, and liver cell disarrangement. This could be due to the formation of highly reactive radicals because of oxidative threats caused by paracetamol. On the other hand, ethanolic extract of *B. insigne* bark 1.5 gm/kg and 3 gm/kg doses could remodel sufficiently the hepatocyte architecture that was changed by administration of toxic doses of paracetamol but exerts significant hepatoprotection against paracetamol-induced liver toxicity.

5. Conclusion

In the present investigation, it was observed that the animals treated with paracetamol resulted in significant hepatic damage as shown by the elevated levels of serum markers. These changes in the marker levels will reflect in hepatic structural integrity. The ethanolic extract of *B. insigne* bark reduced lipid peroxidation and increased the level of catalase enzyme and also attenuated the elevated levels of the serum markers at both doses. Finally, after analyzing all the experiments, it can be said that the ethanolic extract of *B. insigne* bark had sufficient antioxidant and hepatoprotective potential because antioxidant has sufficient capabilities to maintain normal liver function enzyme concentration within the body.

Compliance with ethical standards

Acknowledgments

The authors would like to express their gratitude to Md. Shafiq, laboratory assistant of Pharmacology and Toxicology Lab, Department of Pharmacy, Jahangirnagar University for his kind cooperation in conducting the Research work.

Disclosure of conflict of interest

The authors affirm that they have no known competing financial or interpersonal conflicts that would have appeared to have an impact on the research presented in this study.

Statement of ethical approval

Ethical approval had been taken from the Biosafety, Biosecurity and Ethical committee of Jahangirnagar University before performing the research work on *Swiss albino* mice. The reference no. of ethical clearance is BBEC, JU/M 2020 3(3).

References

[1] Williams R. Global Challenges in Liver Disease. perspectives in clinical herpetology. 2006 Sep, 521.

[2] Desai MA, Kotak KP, Patel SS. Investigation of epidemiology and etiology of liver diseases and characterization of its association with various factors. Asian Journal of Pharmaceutical and Clinical Research. 2015; 8(2): 346.

[3] Hodgson E, Levi PE. A Text Book of Modern Toxicology. 3'rd ed. Department of Environmental and Biochemical Toxicology North Carolina State University: A john Wiley & Sons, Inc., Publication. 2004; 263-267.

[4] Halliwell B, Gutteridge JMC. Oxygen toxicity, oxygen radicals transition metal ions and diseases. Biochemistry Journal. 1984; 2191-2194.

[5] Elmastas M, Gulcin I, Beydemir S, Kufrevioglu OI, Aboul-Enein HY. A study on the in-vitro antioxidant activity of Juniper (Juniperus communis L.) Fruit Extracts. Analytical Letter. 2006; 39(1), 47-65.

[6] Niki E, Shimaski H, Mino M. Antioxidantisim-Free Radical and Biological defense, Gakkai Syuppan Center, Tokyo, Japan. 1994; 3.

[7] Tutour BL. Antioxidative activities of algal extracts: Synergistic effect with vitamin E. Phytochemistry 1990; 29: 3759-3765.

[8] Geber M, Boutron-Ruault MC, Hercber S, Riboli E, Scalbert A, Siess MH. Food and cancer: state of the art about the protective effect of fruits and vegetables. Bulletin Cancer. 2002; 89: 293-312.

[9] Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. American Journal of Medicine. 2002; 113: 71S-88S.

[10] Serafini M, Bellocco R, Wolk A, Ekstrom AM. Total antioxidant potential of fruit and vegetables and risk of gastric cancer. Gastroenterology Journal. 2002; 123: 985-991.

[11] Gaetani GF, Ferraris AM, Rolfo M. Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocytes. Blood. 1996; 87:1595-1599.

[12] Mueller S, Riedel HD, Stremmel W. Direct evidence for catalase as the predominant H2O2 removing enzyme in erythrocytes. Blood. 1997; 90: 4973-4978.

[13] Palombo EA. Phytochemicals from Medicinal Plants used in the treatment of Diarrhea: Modes of action and effects on intestinal function. Phytotherapy Research. 2005; 20:717-724.

[14] Chitme HR, Chandra R, Kaushik S. Studies on anti-diarrheal activity of Calotropic gigantean R. BR. In experimental aminals. Journal of Pharmaceutical Science. 2003; 7:70-75.

[15] Sneader W. Drug Discovery: A History, Wiley, Chichester, UK. 2005.

[16] Wohlmuth H, Oliver C, Nathan PJ. A review of the status of Western herbal medicine in Australia. Journal of Herbal Pharmacotherapy 2002; 2: 33-46.

[17] Kim HS. Do not put too much value on conventional medicines. Journalof Ethnopharmacology. 2005; 100:37-39.

[18] Hossain SJ, El-Sayed M, Aoshima H. Antioxidative and anti-α-amylase activities of four wild plants consumed by pastoral nomads in Egypt. Oriental Pharmacy and Experimental Medicine 2009; 9(3):217-224.

[19] WHO 1978. WHO and UNICEF, Primary health care: Report of the international conference on health care, Amla-Ata, USSR, 1978; 6-12.

[20] Ghani A. Medicinal Plants of Bangladesh. 2nd Ed. The Asiatic Society of Bangladesh. Dhaka, Bangladesh, 2003; 31, 39-40, 418, 500-504, 589-580.

[21] Dar RA, Brahman PK, Khurana N, Wagay JA, Lone ZA, Ganaie MA, Pitre KS. Evaluation of antioxidant activity of crocin, podophyllotoxin and kaempferol by chemical, biochemical and electrochemical assays. Arabian Journal of Chemistry. 2017 (Feb), Vol 10(1):S1119-S1128.

[22] Pandya DJ, Desai TR, Nadpara NP, Mehta HA, Modi AM. Pharmacognostic study and establishment of quality parameters of leaves of Linn. Inter. Journal of Pharmacognosy and Phytochemistry Research 2010; 2(3): 1-5.

[23] Yemul NB. Floral Pigments from: An Endemic Tree Species of East-Asia. International Journal of Scientific Research in Science and Technology. 2018; 4(5): 387.

[24] Mohapatra P, Sharma A, Dixit Y. A review on phyto and medicinal properties of Bombax and Phyllanthus species. International Journal of Engineering Applied Science and Technology 2020; 5(1): 522-530.

[25] Bangladesh Ethnobotany Online Database, 2019. "http://www.ebbd.info/bombax-insigne.html. Web. 02 March, 2019.

[26] Kim JW, Ishibashi H, Yamauchi R, Ichikawa N, Takao Y, Hirano M, Koga M, Arizono K. Acute toxicity of pharmaceutical and personal care products on freshwater crustacean (Thamnocephalus platyurus) and fish (Oryzias latipes). Journal of Toxicology Sciences. 2009; 34(2): 227-232, DOI https://doi.org/10.2131/jts.34.227

[27] Lalee A, Bhattacharaya B, Das M, Mitra D, Kaity S, Bera S, Samanta A. Hepatoprotective activity of ethanolic extract of Aerva sanguinolenta (Amaranthaceae) against paracetamol induced liver toxicity on Wistar Rats. NSHM Journal of Pharmacy and Healthcare Manage. 2012 Feb; 3: 57-65

[28] Ferreira AL. Production and nutritional value of shoot area of cassava, maniçoba, and pornunça. Revista Brasileira de. Saúde e Producao Animal. 2009; 10 (1):983-990.

[29] Henkel AS. Nutritional support in patients with chronic liver disease. Nature Clinical Practice Gastroenterology & Hepatology. 2006; 3(4):202–9. [: 1743–4378 (Print)].

[30] American Liver Foundation, 2016. Liver function Test. "https://liverfoundation.org/.Web. Wednesday, February 2019.

[31] Thapa BR, Walia A. Liver Function Tests and their Interpretation. Indian Journal of Pediatric 2007; 74: 663.