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Hematopoietic and hepatoprotective activity of *Basella alba* L. fruit extract in experimental animal model

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Basella alba L. fruit extract (BAFE) has been used in rural eastern India by the traditional practitioners to treat anemia during pregnancy. In the present study, we have demostrated the hematopoietic and hepatoprotective activity of BAFE in animal model. Male Swiss albino rats were divided into: Gr. I, sham control; Gr. II, disease control (anemia or hepatotoxicity); Gr. III, standard drug treated; Gr. IV, BAFE treated (low dose); and Gr.V, BAFE treated (high dose). Anemia was induced in animals through blood loss at regular intervals and hematological, cytokine, antioxidant parameters and RBC morphology were done. Hepatotoxicity was induced in animals by CCl_4 (1 mL/kg/p.o.) and serum biochemistry, cytokine, antioxidant parameters and histology were done. There was a significant improvement of hematology (Hb, TC of RBC, TC of WBC, hematocrit, serum iron, TIBC), cytokine (IL 1β), antioxidant (SOD, catalase, LPO, GSH) and RBC morphology in BAFE treated animals when compared to Gr. II animals. Significant improvement in biochemical (AST, ALT, γGT, ACP, ALP and total bilirubin), cytokine (IL 1β, IL 4, IL 12, IL 17, cathepsin K and TNF α), antioxidant (SOD, catalase, LPO and GSH) and liver histology were observed in BAFE treated animals when compared to Gr. II animals. The present study confirmed the hematopoietic and hepatoprotective activity of BAFE in animal model and warrants further studies.

Keywords: Anemia, Antioxidants, Cytokines, Indian spinach, Iron deficiency, Malabar spinach

Basella alba L. is a fast growing, soft-stemmed edible green vegetable belonging to the family *Basellaceae*. Its stems are green or purplish in colour with fleshy, ovate or heart shaped leaves. Fruit is fleshy, stalkless, ovoid or spherical, 5-6 mm long, and becomes purple when mature (Fig. 1)[1,2]. The aerial part of *B. alba* is widely used in the tropical countries as a vegetable (Bengali name *Pui* and other common names: Indian spinach, Malabar spinach, Ceylon spinach, vine spinach, etc.). It has been used for the treatment of many diseases including dysentery, diarrhea, anemia, cancer, infection, ricket, cataract, etc.[2,3].

Traditional practitioners in rural Bengal use the ripened fruit of *B. alba* to treat anemia in pregnancy[4]. Bede community in Dhaka district of Bangladesh suggests that the leaf, stem and seed of *B. rubra* is useful to overcome physical weakness and anemia[5]. The leaves of *B. alba* have been found to be beneficial in treating hypertension and malaria[6]. Earlier studies showed the efficacy of *B. alba* leaf extract have antifungal, anti-inflammatory and analgesic

Fig. 1 — *Basella alba* plant and its fruit (inset image). (Picture from laboratory archive)

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Abbreviations: ACP, acid phosphatase; ALP, alkaline phosphatase; ALT, alanine amino transferase; AST, aspartate transaminase; BAFE, *Basella alba* fruit extract; γGT, γ-glutamyl transferase; GSH, reduced glutathione; Hb, Hemoglobin; IL, interleukin; LPO, Lipid peroxidation; SEM, Scanning electron microscope; SOD, Super oxide dismutase; RBC, Red Blood Cell; TC, Total count; TIBC, Total Iron Binding Capacity; TNF α, Tumor necrosis factor; WBC, Total count of White Blood Cell properties[7]. Many species of *B.* genus contain ascorbic acid, carbohydrates, proteins, flavonoids phenols and vitamins[8]. It ameliorates carbon tetrachloride-induced histopathological changes in the testes of experimental albino mice[9]. Methanolic extract of *B. alba* leaves protects against stress[10] and elevates the serum levels of testosterone and oestradiol, and increases the expression of aromatase mRNA in Leydig cells isolated from Sprague Dawley male rats[11]. It decreases blood cholesterol and protects against atherosclerosis and hypercholesterolemia in rabbit model[12,13]. The stem extract is used in wound healing[14]; and the whole plant extract protects against colorectal cancer by exerting its antioxidant and antiproliferative activity[15]. Leaf and seed extract of *B. alba* has been found to induce apoptosis in EAC cell line model[16,17]. It also has androgenic potential in experimental animal model[18]. *B. alba* leaves have hepatoprotective, antioxidant and anti-inflammatory activities[19,20]. Betacyanin, an active compound present in *B. alba* fruit have antioxidant and hematopoietic potential[21,22].

In the present study, we evaluated the hematopoietic and hepatoprotective activity of *Basella alba* fruit extract (BAFE) in the animal model.

Materials and Methods

Materials

The following reagents and chemicals were used in the present experiment: 5,5'dithiobis (2-nitrobenzoic acid) (Sigma, USA), ACP assay kit (Spinreact, Spain), ALP assay kit (Spinreact, Spain), ALT assay kit (Spinreact, Spain), AST assay kit (Spinreact, Spain), dextrose (SRL, India), dextrose saline 5% (Pharma Corporation of India, India), disodium hydrogen phosphate (SRL, India), drabkin's reagent (Span Diagnostics Ltd., India), eosin (SRL, India), ethanol (Bengal Chemicals, India), ethylenediamine tetraacetic acid (SRL, India), formaldehyde (Merck, Germany), glacial acetic acid (SRL, India), hematoxylene (SRL, India), hydrogen peroxide (Merck, Germany), interleukin 12 ELISA kit (R&D, USA), interleukin 17α ELISA kit (R&D, USA), interleukin 1β ELISA kit (R&D, USA), interleukin 4 ELISA kit (R&D, USA), iron assay kit (Labkit, India), methelene blue (SRL, India), paraffin (Emerck, Germany), potassium dihydrogen phosphate (SRL, India), pyrogallol (SRL, India), sodium bicarbonate (SRL, India), sodium chloride (SRL, India), sodium chloride (SRL, India), sodium dihydrogen phosphate (SRL, India), thiobarbituric acid (SRL, India), total iron binding capacity assay kit (Labkit, India), tricarboxylic acid (SRL, India), trisaminomethane (SRL, India), trisodium isocitrate (Merck, Germany), xylene (Merck, India) and γ glutamyl transferase assay kit (Spinreact, Spain).

Basella alba fruit extract

Dried *B. alba* fruit (Fig. 1) was collected from M/s United Chemical and Allied Products, Kolkata, India during the month of September-November. The procured specimen was authenticated by Department of Botany, University of Calcutta. Dried whole fruit (40 g) was soaked in 200 mL of distilled water and was kept at 4-8°C for 24 h. It was filtered and the filtrate (cherry red coloured) was collected. The filtrate was centrifuged at 5000 rpm for 30 min and the supernatant was termed as *B. alba* fruit extract (BAFE). The dry weight of BAFE was measured. It was kept at 4-8°C for further use and expressed in terms of dry weight.

Animals and animal grouping

Swiss albino male mice (20 ± 2 g) of age 8-9 weeks were obtained from M/s Chakraborty Enterprise, Kolkata, India (CPCSEA Registration no. 1443/PO/b/11/CPCSEA). The animals were housed in appropriate cages in a well-ventilated room with controlled atmosphere (temperature: 25±1°C, humidity: 50±5% and 12:12 h light:dark cycle). All the animals were provided with chattu, bread, green vegetables, Bengal gram and water *ad libitum*. An acclimatization period (5 days) was allowed before the commencement of the experiment. All experimental protocols described in this study were approved by the institutional animal ethical committee (Reference number: IAEC/ Revised Proposal/ AG-01/ 2012; dt: 01.02.2013), Department of Physiology, University of Calcutta and the animals were maintained as per the guideline of the Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA), Government of India. Animals (n=6) were divided into 5 groups: Gr. I sham control; Gr. II, disease control (anemia or hepatotoxicity); Gr. III, standard drug treated; Gr. IV, BAFE treated (low dose); and Gr. V, BAFE treated (high dose).

Iron deficiency anemia model

Iron deficiency anemia (IDA) was induced in mice as stated by Jones *et al*[23]. Blood (300 µL) was taken out in three alternate days (0, 2nd and 4th day), and the lost volume was replenished by same amount of 5%

dextrose saline (p.o.). From 5th to 15th day the animals of Gr. III were treated with standard drug iron-sucrose (1.5 mg/kg/i.p.). Animals of Gr. IV & V received BAFE of 75 mg/kg/p.o., and 125 mg/kg/p.o., respectively. On 16th day, blood was collected from retro-orbital plexus and assays were done.

Hepatotoxicity model (*in vivo*)

Hepatotoxicity was induced in mice using carbon tetrachloride (CCl_4) as previously described[24]. Animals of Gr. III were treated with standard drug (Silymerin, 10 mg/kg/p.o.) for day 0 to day 10. Animals of Gr. IV & V were treated with BAFE of 75 mg/kg/p.o., and 125 mg/kg/p.o., respectively for day 0 to day 10. On 11th day, CCl_4 was induced in animals of all the groups except the control group at a dose of 1 mL/kg/p.o. On 13th day, blood was collected from retro-orbital plexus of all animals and assays were done. Liver tissues were collected for tissue histology.

Hepatotoxicity model (*in vitro*)

In vitro hepatotoxicity was assessed in liver slice model as described earlier[25]. Livers were collected from adult fasted Swiss albino male mice and 100 mg of liver slices were taken in each of the groups (n=6): Gr. I (Sham control), Gr. II (paracetamol treated), Gr. III (silymerin treated), Gr. IV (BAFE treated, low dose) and Gr. V (BAFE treated, high dose). To avoid inter-experimental variations due to the circadian rhythm of the mice, isolation of the livers was performed between 9 and 11 A.M. Slices were prepared from the whole liver and randomly selected for incubation and sampling. As soon as the liver slices were made, they were incubated with phosphate buffered saline with 25 mM D-glucose. Slices were transferred to 4 mL glass bubbler kept at 37±1ºC in presence of oxygen (95% O_2 + 5% CO_2) containing 2 mL phosphate buffered saline. About 1.0 mM paracetamol was added in the glass bubbler of groups II-V liver slices. At the same time 50 µg of silymerin, 50 µg BAFE and 100 µg BAFE were added to glass bubbler of groups III-V liver slices. All the liver tissue slices were incubated for 2 h, after which the incubated supernatant fluid was collected, and the biochemical parameters (AST and ALT) were estimated using kits (Labkit, India) and spectrophotometer (Biorad, SmartSpec Plus).

Hematopoietic parameters

Blood hemoglobin from each group of animals was measured by cyano-hemoglobin method[26]. Total count of RBC was tested by using hemocytometer[26]. Hematocrit value was determined by Wintrobe tube method[26]. Serum iron concentration and total iron binding capacity (TIBC) were estimated using biochemical kit (Labkit, India). Scanning electron microscopy (SEM) was done to examine the size, shape and morphology of RBC in all the animal groups using JEOL JSM-7600F SEM.

Antioxidant parameters

Blood was collected from all animals, serum was separated and reduced glutathione (expressed as µM/mg protein), superoxide dismutase (IU/mg protein) and lipid peroxidation (MDA/mg protein) were estimated from the serum[27-29]. Serum protein was estimated by the method of Lowry *et al*.[30] to express the antioxidants in terms of enzyme activity/mg of protein. Heparinised blood was centrifuged for 10 min at 3000 rpm and 1% solution of packed cell volume was used as RBC lysate. From this lysate, catalase (µM/mg hemoglobin) was estimated according to Beers & Sizers[31].

Liver marker

Serum AST, ALT, γGT, ACP and ALP were measured using commercial kit (Spinreact, Spain) and UV-vis spectrophotometer (Biorad, USA).

Cytokine marker

Serum interleukins 1β, 17, 12 and 4, Cathepsin K and TNF α of animals was done by using ELISA kit (R & D Products, U.S.A.) and ELISA reader (Biotec, India).

Tissue histology

Liver tissues were collected, fixed in 10% buffered formalin for 24 h. The tissues were then dehydrated in graded (50-100%) ethanol followed by clearing in Xylene. Paraffin (56-58°C) embedding was done at 58±1°C for 4 h, followed by paraffin block preparation. Paraffin sections (5 µm) were cut using a rotary microtome (WESWOX Optik, MT 1090). Paraffin sections were deparaffinised with xylene, stained with hematoxylin- eosin, followed by mounting in DPX with a cover slip. The tissues were observed with a bright field microscope (Motic, Germany) and photographs were captured with Motic software (Motic Images Plus 2.0 software).

Statistical analysis

Statistical significance was evaluated by one way analysis of variance (ANOVA) and $P < 0.05$ was considered statistically significant. Values were expressed as mean ± standard error of mean (n=6).

Results

Effect of BAFE on iron deficient male albino mice

Hematopoietic markers

In Gr. II anemia control animals, there was a significant ($P < 0.05$) decrease in blood hemoglobin (36.6%), total count of RBC (45.9%) and hematocrit (29.7%) when compared to Gr. I sham control animals. BAFE treatment in Gr. IV & V animals increased hemoglobin, total count of RBC and hematocrit ($P < 0.05$) when compared to Gr. II anemia control animals. Standard drug (iron-sucrose) treatment in Gr. III animals increased hemoglobin, TC of RBC and hematocrit ($P < 0.05$) when compared to group 2 anemia control animals. (Fig. 2)

In Gr. II anemia control animals, there was a significant ($P < 0.05$) decrease in serum iron concentration (67.1%) and increase in total iron binding capacity (TIBC) (35.0%) when compared to Gr. I sham control animals. BAFE treatment in Gr. IV & V animals increased serum iron concentration and decreased TIBC ($P < 0.05$) when compared to group 2 anemia control animals. Standard drug (iron-sucrose) treatment in Gr. III animals increased serum iron concentration and decreased TIBC ($P < 0.05$) when compared to Gr. II anemia control animals. (Fig. 2)

Antioxidant markers

In Gr. IIanemia control animals, there was a significant ($P < 0.05$) decrease in glutathione reductase (GSH) (20.7%), superoxide dismutase (SOD) (32.7%), catalase (26.9%) and increase in lipid peroxidation (LPO) (39.3%) when compared to Gr. I sham control animals. BAFE treatment in Gr. IV & V animals increased GSH, SOD, catalase and decreased LPO ($P < 0.05$) when compared to Gr. II anemia control animals. Standard drug (iron-sucrose) treatment in Gr. III animals increased SOD ($P < 0.05$) when compared to Gr. II anemia control animals. (Fig. 2).

RBC morphology

Scanning electron microscopy (SEM) showed that sham control animals had RBC size of 5.31±0.22 µm with normal bi-concave architecture. In anemic animals, the RBCs became flattened, their normal architecture was lost. Its diameter was reduced to 4.42±0.19 µm (15% reduction when compared to Gr. I sham control animal). Many white patches were also seen in the membrane of RBCs. Treatment with BAFE increased RBC diameter to 5.43±0.22 µm (17% when compared to Gr. II anemia control RBCs). A few grooves in the membrane of RBCs were found, but there were no white patches. It showed almost normal bi-concave architecture. (Fig. 3)

Cytokine level

In Gr. II anemia control animals, there was a significant ($P < 0.05$) decrease (54.3%) in interleukin 1β (IL 1β) when compared to Gr. I sham control animals. BAFE treatment in GR. IV & V animals increased IL 1β ($P < 0.05$) when compared to Gr. II

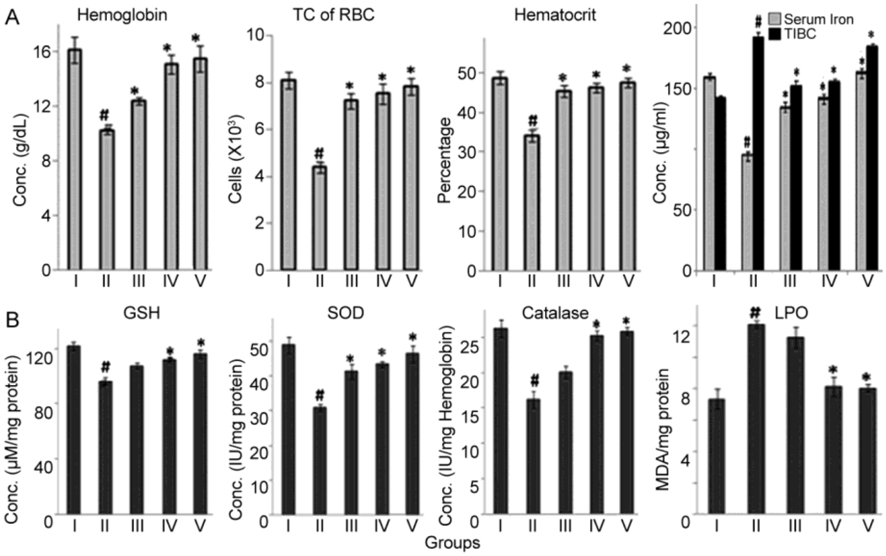

Fig. 2 — Effect of *Basella alba* fruit extract on (A) hematopoietic; and (B) antioxidant parameters in iron deficiency anemia *in vivo* model. [Values are expressed as mean ± standard error of mean (n=6). $^{\#}P < 0.05$ when compared to Gr. I, $^{*}P < 0.05$ when compared to Gr. II. Gr. I: Control, Gr. II: iron deficiency anemia control, Gr. III: standard drug iron-sucrose treated (1.5 mg/kg/i.p.), Gr. IV: BAFE treated (75 mg/kg/p.o.), and Gr. V: BAFE treated (125 mg/kg/p.o.)]

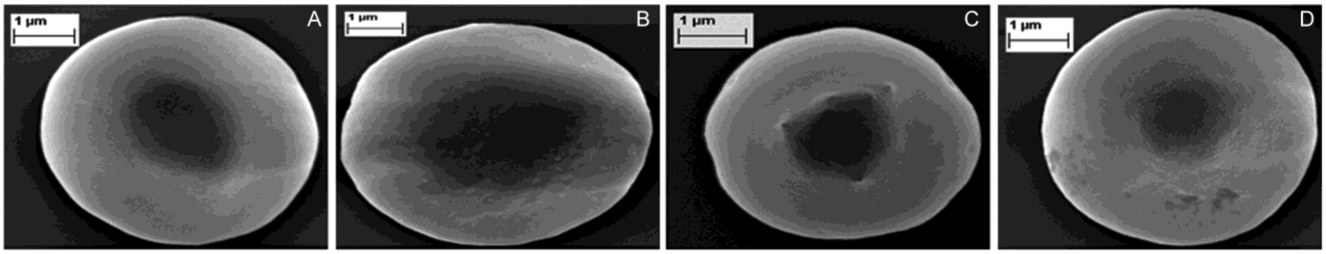

Fig. 3 — Effect of *Basella alba* fruit extract on RBC morphology in iron deficiency anemia *in vivo* model. (A) sham control; (B) anemia control; (C) standard drug iron-sucrose treated (1.5 mg/kg/p.o.); and (D) BAFE treated (125 mg/kg/p.o.).

anemia control animals. Standard drug (iron-sucrose) treatment in Gr. III animals increased IL 1β (P <0.05) when compared to group 2 anemia control animals.

Effect of BAFE on CCl_4 induced *in vivo* hepatotoxicity model

Hepatotoxicity markers

In group 2 hepatotoxic control animals, there was a significant (P <0.05) increase in serum AST (86.8%), ALT (82.2%), γ-GT (49.8%), ACP (86.8%), ALP (82.2%) and total bilirubin (49.8%) when compared to Gr. I sham control animals. BAFE treatment in Gr. IV & V animals decreased AST, ALT, γGT, ACP, ALP and total bilirubin (P <0.05) when compared to Gr. II hepatotoxicity control animals. Standard drug (silymerin) treatment in Gr. III animals decreased AST, ALT, γGT, ACP, ALP and total bilirubin (P <0.05) when compared to Gr. II hepatotoxicity control animals. (Fig. 4)

Antioxidant markers

In Gr. II hepatotoxic control animals, there was a significant (P <0.05) decrease in GSH (27.2%), SOD (46.1%), catalase (45.9%) and increase in LPO (80.5%) when compared to Gr. I sham control animals. BAFE treatment in Gr. IV & V animals increased GSH, SOD, catalase and decreased LPO (P <0.05) when compared to Gr. II hepatotoxic control animals. Standard drug (silymerin) treatment in Gr. III animals increased GSH, SOD, catalase and decreased LPO (P <0.05) when compared to GR. II hepatotoxic control animals. (Fig. 4)

Cytokine markers

In Gr. II hepatotoxic control animals, there was a significant (P <0.05) increase in interleukin 17 (IL 17) (83.3%), IL 1β (102.2%), tumor necrosis factor α (TNF α) (50.3%), decrease in IL 10 (35.2%), and cathepsin K (70.4%) when compared to Gr. I sham control animals. BAFE treatment in Gr. IV & V animals decreased IL 17, IL 1β, TNF α, increased IL 10 and cathepsin K (P <0.05) when compared to Gr. II anemia control animals. Standard drug (silymerin) treatment in group 3 animals decreased IL 17, IL 1β, TNF α, increased IL 10 and cathepsin K (P <0.05) when compared to Gr. II anemia control animals. (Fig. 4)

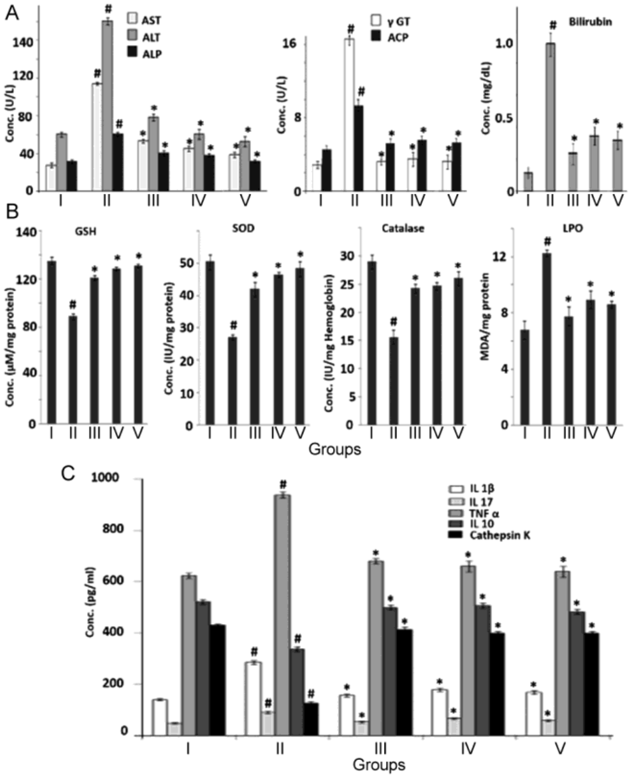

Fig. 4 — Effect of *Basella alba* fruit extract on (A) biochemical; (B) antioxidant; and (C) inflammatory parameters in CCl_4 induced *in vivo* hepatotoxicity model. [Values are expressed as mean ± standard error of mean (n=6). $^{\#}P$ <0.05 when compared to Gr. I, $^{*}P$ <0.05 when compared to Gr. II. Gr. I: Control, Gr. II: hepatotoxicity control (CCl_4 1 mL/kg/p.o.), Gr. III: standard drug silymerin treated (10 mg/kg/p.o.), Gr. IV: BAFE treated (75 mg/kg/p.o.), Gr. V: BAFE treated (125 mg/kg/p.o.)]

Liver tissue histology

Histology of liver tissue of Gr. I sham control animals showed normal cellular architecture, intact central canal and hepatocytes. Group II CCl_4-induced hepatotoxic animal liver tissues showed disruption of central canals, centrizonal necrosis, nuclear disintegration, vacuolization and fatty infiltration.

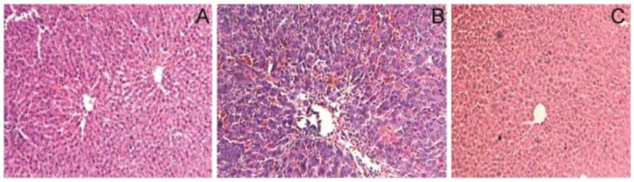

Fig. 5 — Effect of *Basella alba* fruit extract on liver tissue histology in CCl_4 induced *in vivo* hepatotoxicity model. (A) sham control; (B) hepatotoxic control; and (C) BAFE treated (125 mg/kg/p.o.). CCl_4-induced hepatotoxic animal liver tissues showed disruption of central canals, centrizonal necrosis, nuclear disintegration, vacuolization and fatty infiltration. Treatment with BAFE showed partial recovery of cellular architectures (less necrosis, less vacuolization, intact nucleus and less fatty infiltration).

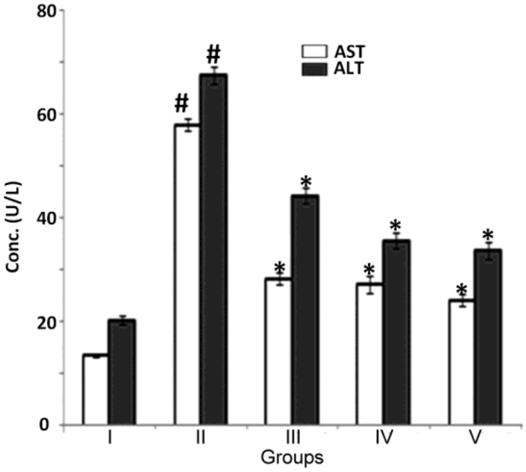

Fig. 6 — Effect of *Basella alba* fruit extract on biochemical parameters in paracetamol induced *in vitro* hepatotoxicity model. [Values are expressed as mean ± standard error of mean (n=6). $^{\#}P$ <0.05 when compared to Gr. 1, *P <0.05 when compared to Gr. II. Gr. I: Control, Gr. II: hepatotoxicity control (paracetamol 1 mM), Gr. III: standard drug silymerin treated (50 µg), Gr. IV: BAFE treated (50 µg), and Gr. V: BAFE treated (100 µg).

Treatment with BAFE (in Gr. IV & V animal liver tissue) showed partial recovery of cellular architectures (less necrosis, less vacuolization, intact nucleus and less fatty infiltration) (Fig. 5).

Effect of BAFE on paracetamol induced *in vitro* hepatotoxicity model

In the incubate of Gr. II hepatotoxic liver slices, there was a significant (P <0.05) increase in AST (86.8%) and ALT (82.2%) in when compared to Gr. I normal liver slice incubate. BAFE treatment in Gr. IV & V liver slice incubate decreased AST and ALT (P <0.05) when compared to Gr. II hepatotoxic control liver slice incubate. Standard drug (silymerin) treatment in group 3 liver slice incubate decreased AST and ALT (P <0.05) when compared to Gr. II hepatotoxic control liver slice incubate. (Fig. 6)

Discussion

Anemia and hepatotoxicity are the two major burdens for healthy lifestyle, leading to manpower loss and a large number of casualties worldwide. The economic burden is also a long-term issue to the victim and their families. The limitations of the present treatments for anemia and hepatotoxicity result in increased number of incidences worldwide. There is a need for better therapeutic interventions in terms to decrease mortality, morbidity, economic burden and to improve lifestyle.

Traditional medicinal system has a long history of use in health maintenance, chronic disease prevention and treatment[32]. Medicinal plants contain numerous biologically active compounds such as carbohydrates, proteins, enzymes, fats and oils, minerals, vitamins, alkaloids, quinones, terpenoids, flavonoids, carotenoids, sterols, simple phenolic glycosides, tannins, saponins, polyphenols, etc.[33,34]. Over the years, medicinal plants have been found useful in the treatment and management of pathophysiological conditions. Ayurveda, one of the traditional medicinal systems of India is associated with the natural sources and natural product derived preparations for the treatment of various diseases. It utilizes herbs, metals and minerals for medicinal purposes. In traditional systems of medicine, the fruit of *B. alba* has long been used against iron deficiency anemia in the eastern parts of Indian Subcontinent[4]. The present study was an attempt to establish the therapeutic potential of *B. alba* fruit extract against iron deficiency anemia and hepatoxicity in experimental animal model.

In iron deficiency anemia (IDA), hemoglobin level becomes significantly low due to the absence of enough amount of iron in blood. It has been observed that hemoglobin, total count of RBC and hematocrit became significantly higher than that of anemia control group after treatment with BAFE. Serum iron is transported by binding with transferrin glycoprotein. The amount of transferrin-bound circulating serum iron concentration is measured through determining serum iron concentration. Total iron binding capacity (TIBC) is the measurement of maximum amount of iron that transferrin can carry. Hence, it indirectly measures the transferrin concentration in serum. In IDA, serum iron concentration becomes low due to lack of iron. But TIBC increases in IDA, because liver produces more transferrin to maximize the use of small amount of iron present in serum[35]. BAFE showed increase in serum iron concentration and a reduction in TIBC, probably by increasing dietary uptake of iron, and promoting hematopoiesis. IDA enhances oxidative stress in red blood cells which plays an important role in the pathogenesis of iron deficiency anemia[36].

Reduced glutathione (GSH) acts as an antioxidant in cellular systems. After scavenging, GSH converts to GS-SG and reduces free radicals into nontoxic byproducts[37]. SOD catalyses the scavenging of superoxide anion into hydrogen peroxide and molecular oxygen[37]. Catalase eliminates toxic hydrogen peroxide in cell to form non-toxic water and oxygen[38]. Lipid peroxidation is also a good marker to determine oxidative stress[39]. In anemia control group, there was decreased GSH, SOD and catalase and increased lipid peroxidation, suggesting IDA-induced oxidative stress in animal model. Treatment with BAFE increased GSH, SOD, catalase and decreased lipid perooxidation, restored the antioxidant status in the animals, likely due to the presence of antioxidants in the extract. Inlerleukin 1β (IL 1β) inhibits gastric acid secretion, which is essential for iron absorption in gastrointestinal tract[40]. It has been observed that IL 1β level in Gr. II anemia control mice was significantly lowered when compared to Gr. I sham control mice. This data may be explained by the body's ability to adopt in low serum iron environment. In anemia control mice, decreased IL 1β promotes gastric acid secretion, which in turn accelerates iron absorption from the gut. In standard drug (iron-sucrose) and BAFE treated animals, IL 1β level was significantly increased, leading to decreased gastric acid secretion and increased iron absorption from guts. IDA is associated with microcytic and hypochromic RBCs. In hypochromic RBCs, increased central pallor of RBC occupies more than 1/3rd of the total RBC diameter. Microcytosis is characterized by a decrease in RBC size[35]. Induction of IDA decreased RBC diameter, flattened the RBCs, caused abnormal architecture and induced white patches in anemia control mice RBC. This might be due to hypochromicity of RBCs. BAFE treatment showed almost normal RBC architecture with less white patches, and increase in RBC diameter.

Hepatotoxicity is concerned with unhealthy lifestyle, alcohol consumption, pollution, unhealthy diet habit and drug metabolism. When the amount of toxic by-products accumulate inside the liver becomes more than the amount liver can metabolize, it gets damaged. AST, ALT, γGT, ACP, ALP and total bilirubin are the classical hepatic markers that are increased during liver damage. Carbon tetrachloride (CCl_4) causes fatty degeneration of liver, fibrosis, hepatocyte death, and hepatic carcinogenicity. Normally transaminases and other enzymes are present inside the hepatocytes and they do not appear in circulation. Hepatotoxicity causes membrane rupture of hepatocytes which in turn causes the enzymes to be leaked out from the cells, following the increase in blood transaminase levels. Induction of CCl_4 in mice increased serum AST, ALT, γGT, ACP, ALP and total bilirubin levels, indicating hepatotoxic events. Treatment with BAFE decreased the hepatic parameters (both *in vivo* and *in vitro*). Earlier studies showed that induction of hepatotoxicity contributes in the pathogenesis of hepatotoxicity[41-43]. Induction of CCl_4 in mice increased oxidative stress in hepatotoxic control animals. After treatment with BAFE, GSH, SOD and catalase levels were increased, whereas, LPO was decreased, most likely due to antioxidants present in BAFE extract. The association of proinflammatory and anti-inflammatory cytokines in progression of hepatotoxicity is well established[44,45]. Induction of CCl_4 raised proinflammatory cytokines (IL 17, IL 1β and TNF α) and decreased anti-inflammatory cytokines (IL 10 and cathepsin K), suggesting a proinflammatory environment inside the liver. Treatment with BAFE caused increase in proinflammatory cytokines and decrease in anti-inflammatory cytokines level, probably due to anti-inflammatory compounds present in BAFE extract. Histopathological changes in liver tissue are well known after CCl_4 induction[46]. In the present study, there was disruption and necrosis of central canal, fatty infiltration, vacuolization and nuclear disintegration in CCl_4 control animals. BAFE treatment partially recovered the hepatocellular architecture. There was intact nucleus, less fatty infiltration, less vacuolization and less necrosis of central canal after treatment with BAFE.

Use of herbs and herbal constituents has been reported earlier against anemia and hepatotoxicity[47,48]. Presence of terpenoids, flavonoids and alkaloids in these medicinal plants were proposed to exert their hematopoietic/hepatoprotective activities. The active compounds, especially betacyanin (already proved as hematopoietic, hepatoprotective, antioxidant and anti-inflammatory in nature) are supposed to have therapeutic potentials in the earlier studies[21,22]. In the present study, the same active compound(s) may exert their hematopoietic/hepatoprotective activities in animal model, probably through: (i) antioxidant properties of herbal compounds present in the BAFE, that halts the pathogenesis of diseases; (ii) anti-inflammatory potential of herbal compounds in BAFE may balance the pro/anti-inflammatory equilibrium *in vivo*; (iii) interference with the molecular and cellular markers in the progression of disease; (iv) interference with intestinal absorption of iron, leading to increased serum iron concentration; (v) promoting erythropoiesis; (vi) upregulating transferring receptor for carrying more iron through serum; and (vi) decreasing lipid peroxidation of the cell membrane, and causing minimum cell damage in hepatoxicity. The future research emphasis will be the enhancement of the potency of *B. alba* fruit extract against anemia and hepatotoxicity using nanotechnology, as it has been observed that nanotechnology increases the efficacy of herbs[49,50].

Conclusion

The above results have demonstrated that *Basella alba* fruit extract improves the hematopoietic and antioxidant parameters in the experimental anemia model, and hepatic, antioxidant, cytokine and histopathology parameters in the experimental animal model. The hematopoietic and hepatoprotective efficacy of *B. alba* fruit extract were mediated through the antioxidant and anti-inflammatory properties of the phytoconstituents present in the extract. Further investigation on the isolation of active components from *B. alba* fruit extract and its possible protection against anemia and hepatotoxicity is warranted.

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Conflict of interest

Authors declare no competing interests.

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