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Sonneratia apetala (Buch.-Ham.) Fruit Extracts Ameliorate Iron Overload and Iron-Induced Oxidative Stress in Mice

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ABSTRACT: Iron overload results in oxidative damage to various biomolecules including DNA, proteins and lipids which ultimately leads to cell death. The *Sonneratia apetala* fruit contains a high content of antioxidants and displays several bioactive properties. Therefore, the powder of the *S. apetala* fruit was successively fractionated into *n*-hexane (Hex), chloroform (Chl), and methanol (Met) fractions to evaluate their efficiency in ameliorating iron overload. *In vitro*, a colorimetric method was used to assess the Fe-chelating activity of the fractions using ferrozine. The fractions were also used *in vivo* to examine their efficacy in ameliorating iron overload and iron-induced oxidative stress in mice induced by intraperitoneal injection of ferric carboxymaltose at 100 mg/kg body weight (bw). Among the fractions, Met showed the highest Fe-chelation ability with an inhibitory concentration 50 of 165 μg/mL followed by Hex (270 μg/mL), and Chl (418 μg/mL). *In vivo*, the results showed a significantly ($P<0.05$) lower iron profile (iron and ferritin concentrations in serum and liver tissue and total iron-binding capacity of serum) in the Met and the Hex treated mice groups than in the iron-overloaded group. Met at 1,000 μg/kg bw completely ameliorated iron overload in the blood and the liver tissue of mice. At this concentration, Met also prevented iron-induced oxidative stress in the liver tissue of iron-overloaded mice by restoring reducing power, total antioxidant capacity, and total protein. Thus, the *S. apetala* fruit, especially its Met fraction can be used in treating iron overload and associated toxicity.

Keywords: Fe-chelation, fruit, iron-overload, *Sonneratia apetala*, the Sundarbans

INTRODUCTION

Hemochromatosis or iron overload is a disorder in the body that stores excess iron in tissues, joints, and organs such as the liver, heart, and pancreas. In hemochromatosis, the body absorbs excess iron from red blood cell transfusions or intestinal dietary substances into the bloodstream. Primary hemochromatosis is commonly caused by an inherited change in DNA, whereas secondary hemochromatosis is produced by medical treatments or other medical conditions. Iron is a transition metal that participates in hydroxyl radical formation, lipid peroxidation, and ferroptosis (Dixon et al., 2012). Under iron overload conditions, non-transferrin-bound iron (NTBI) is generated and accumulates mostly in the liver (Wang and Knutson, 2013) and also enters the blood circulatory system. Free iron is harmful because of its ability to easily change oxidation state to participate in Fenton reactions and generates hydroxyl radicals (OH·), which affect various biomolecules in the body. Excessive iron accumulation and the consequent generation of toxic reactive oxygen species (ROS) cause diseases including hepatocellular necrosis, inflammation, fibrosis, hyperlipidemia, cancer, diabetes, cardiomyopathy, atherosclerosis, osteoporosis, and joint pain (Jang et al., 2014; Kim et al., 2017; Wang et al., 2021). Iron overload is treated with iron chelators, which expedite iron excretion in feces and urine by forming a stable complex with tissue iron. Currently, three iron-chelating agents-deferrioxamine, deferiprone, and deferasirox are mainly used in clinical practice (Wang et al., 2021). However, these chelators show several side effects including gastrointestinal disturbance, renal insufficiency, rash, liver function abnormalities, and neutropenia (Habib et al., 2021). Therefore the search for an ideal iron chelator is a major focus of current research. Phytochelators such as polyphenols bind to excess iron, decrease the concentration of iron ions in serum and, due to their antioxidant effects, reduce iron deposits. Lesjak and Srai (2019) reported that flavonoids are promising novel iron chelators and regulators of iron homeostasis.

Various fruits are common sources of dietary bioactive components such as polyphenols, flavonoids, anthocya-

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nins, vitamins, antioxidants and alkaloids, which can be used as nutraceuticals in public health and treating various diseases. Antioxidant, anti-amylase, anti-glucosidase and anti-allergic activities of common edible fruits in Bangladesh have been reported (Hossain et al., 2008; Alam et al., 2021). Edible mangrove fruits with potentially high antioxidant, anti-bacterial, anti-diarrheal, analgesic and anthelmintic activities were also reported from the Sundarbans mangrove forest of Bangladesh (Hossain et al., 2013, 2016a, 2017; Hosen et al., 2020, 2021; Biswas et al., 2023). Among 68 mangrove species occurring in the Sundarbans forest is *Sonneratia apetala*, whose fruit, called keora in Bengali and mangrove apple in English, is extensively consumed by coastal people mainly in Bangladesh, India, Sri Lanka, Myanmar, and Vietnam. The fruit contains a high content of nutrients, polyphenols, flavonoids, vitamin C, and antioxidants. The fruit exhibits the following health-promoting activities: anti-diarrheal, analgesic, hepatoprotective, anti-aging, and inhibition of elastase-induced lung injury (Hossain et al., 2016a, 2017; Liu et al., 2019; Yi et al., 2020; Jiang et al., 2022; Sengupta et al., 2022). Moreover, *S. apetala* fruit is second to *Emblica officinalis* in its content of polyphenols [48 mg gallic acid equivalent (GAE)/g powder] among the edible fruits in Bangladesh (Hosen et al., 2021). Various food products such as jam, jelly, pickles, tonic, and soup are prepared using the fruit. As the fruit contains high contents of polyphenols, flavonoids, antioxidants, and various bioactive compounds, it may be useful in treating iron overload and associated complications and therefore, *n*-hexane, chloroform, and methanol fractions of the *S. apetala* fruit were assayed both *in vitro* and *in vivo*.

MATERIALS AND METHODS

Chemicals and reagents

Acetic acid, ammonium molybdate, chloroform, ethylenediaminetetraacetic acid (EDTA), ferric chloride, ferrous chloride, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate (ferrozine), *n*-hexane, methanol, potassium ferricyanide, sodium acetate, sodium phosphate, sulfuric acid, trichloroacetic acid were purchased from Sigma-Aldrich. Feripon (deferiprone) and maltofer (ferric carboxymaltose) were purchased from Drug International Ltd. and Incepta Pharmaceuticals Ltd., respectively.

Fruit sample preparation

Fruits of *S. apetala* (Buch.-Ham.) were collected from the world's largest mangrove forest, the Sundarbans, Bangladesh in August 2021. The fruits were washed before they were chopped into small pieces and sun-dried until the water had completely evaporated. After they were dried, they were ground into fine powder using a mechanical grinder. The powder was sealed in an airtight container and stored in a cool and dry place until analysis. The powder was successively extracted into *n*-hexane (Hex), chloroform (Chl), and methanol (Met) fractions. Briefly, 20 g of powder was extracted in 200 mL Hex at 30°C, 0.32 *g* for 3 days. The extract was filtered through Whatman no. 1 filter paper and the filtrate was air dried, weighed, and stored at 4°C as Hex fraction. The residue on the filter paper was used for preparing Chl, and then Met fractions, respectively. The weight of the Hex, Chl, and Met fractions were 5.3, 3.5 and 21%, respectively, of the powder.

Animals

Male Swiss-albino mice (16～22 g) were purchased from the International Center for Diarrheal Disease Research, Bangladesh (ICDDR'B). ICDDR'B formulated pellet diet and tap water were provided *ad libitum* to the mice. Six mice were housed in a separate polypropylene cage (30×15×15 cm) and kept in the natural day-night cycle related to the sunrise/set. Guidelines of the Animal Ethics Committee of Khulna University which adheres to the EU Directive 2010 for animal experiments, were strictly followed in conducting the experiments (Research ref. no. KUAEC-2022/09/17).

In vitro Fe-chelating activity

The Fe-chelating activity was determined using the colorimetric method (Carter, 1971). Various concentrations of each fraction were added to 50 μL of $FeCl_2$ (2 mM) and 2 mL of sodium acetate buffer (0.1 M, pH 4.9). After mixing, the mixture was incubated in the dark at room temperature for 30 min. Then 0.2 mL of ferrozine (5 mM) was added before the mixture was vigorously shaken. After incubating for 30 min, absorbance was measured at 562 nm using a spectrophotometer. EDTA served as a positive control (PC). The percentage of inhibition of Fe^{2+}-ferrozine complex formation was calculated as follows:

$$\text{Chelation percentage } (\%) = [(A_0 - A_1)/A_0] \times 100$$

where A_0 and A_1 were the absorbance of the control and the fraction, respectively.

In vivo Fe-chelating activity

Mice were divided into eleven groups of six mice each (n=6), which were housed separately for one week for adaptation. The method described by Chaudhuri et al. (2015) was used for the experiment with some modifications. The control group (C) received normal saline only. The other groups were given five doses of ferric carboxymaltose saline at 100 mg/kg body weight (bw) by intra-

peritoneal injection as one dose every alternate day. After the first iron injection, oral administration of normal saline was begun the next day to one iron-overloaded group (IO); another IO was treated with deferiprone (PC) at 20 mg/kg bw, a medication that chelates iron. The other groups were orally treated with the Met and the Hex at 250, 500, 750, and 1,000 μg/kg bw for 21 consecutive days and designated for Met as Met 250, Met 500, Met 750, Met 1,000 and for Hex as Hex 250, Hex 500, Hex 750, Hex 1,000, respectively. On the 22nd day, all the animals were starved overnight, mildly anesthetized with diethyl ether, and blood was drawn via cardiac puncture before they were sacrificed. Serum was separated from blood by centrifugation at 460 *g* for 15 min and stored at −20°C until further analysis.

Livers were collected and washed in saline water to remove the blood and other debris. After removing the water, livers were preserved at −20°C until use. A mass of 1 g of liver tissue of each group was minced and dissolved in 10 mL lysis buffer (20 mM Tris-HCl, 1 mM sodium azide, pH 7.4). The suspension was homogenized with a tissue homogenizer before centrifugation at 7,375 *g* for 10 min. The supernatant was collected and stored at −20°C until analysis.

Measurement of iron, ferritin, and total iron-binding capacity (TIBC)

Serum and liver supernatants were used to determine iron and ferritin contents and TIBC. Iron content in serum and liver supernatants and serum TIBC were quantitatively determined using an iron chromazurol assay kit (Agappe Diagnostics Ltd.). Ferritin in serum and liver supernatants was measured by a turbidimetric immunoassay kit (Spinreact).

Determination of reducing power, total antioxidant capacity (TAC), and total protein in the liver tissue

The reducing power of the liver supernatants was determined following the method described by Oyaizu (1986) with some modification. Various concentrations of the liver supernatant were mixed with 2.5 mL of 0.2 M phosphate buffer, pH 6.6, and 2.5 mL of 1% potassium ferricyanide solution. After incubation at 50°C for 20 min, the mixtures were each mixed with 2.5 mL of 10% trichloroacetic acid followed by centrifugation at 650 *g* for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance of this solution was measured at 700 nm. Results were presented as optical density (OD) for mg liver tissue/mL.

The assay was done according to the method described by Prieto et al. (1999) with some modifications. The tubes containing the liver supernatants and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) were incubated at 90°C for 90 min. After cooling the supernatants at room temperature, their absorbances were measured at 695 nm against a blank. The TAC was presented as mg ascorbic acid (AAE/g liver tissue) and gallic acid (GAE/g liver tissue) equivalents.

The prepared liver supernatants were used to determine total protein content in the liver tissue following the method described by Lowry et al. (1951) using bovine serum albumin as a standard and presented as mg protein/g liver tissue.

Statistical analysis

Values are presented as mean±standard deviation. Statistical differences among multiple groups were tested for by the analysis of variance followed by Tukey's multiple comparison post-hoc test. In all the analyses, P-value< 0.05 were considered significant. All statistical analyses were performed in SPSS (version 16, SPSS Inc.).

RESULTS

In vitro Fe-chelating activity

The Fe-chelation activity of Met, Chl, and Hex of *S. apetala* fruits was determined *in vitro*. All the fractions increased Fe-chelation dose-dependently, with Met and Hex showed higher Fe-chelating ability than Chl (Fig. 1A). From the dose-dependent curves, the concentration of the fractions at which 50% iron became chelated (IC_{50}) was determined (Fig. 1B). The Met had the lowest IC_{50} (165 μg/mL) followed by the Hex (270 μg/mL) and Chl (418 μg/mL) whereas it was 5.6 μg/mL for the PC, EDTA.

Serum iron and ferritin contents and TIBC

Based on the *in vitro* results, various concentrations of Met and Hex were used to treat IO mice. Intraperitoneal injection of five doses of ferric carboxymaltose saline at 100 mg/kg bw on every alternate day increased iron and ferritin accumulations by 84.8% and 189.2%, respectively, whereas it decreased TIBC by 44.8% in the serum of the IO mice relative to the control at day 22 (Fig. 2). The Met and Hex dose-dependently decreased iron (Fig. 2A) and ferritin (Fig. 2B) accumulations but increased the TIBC (Fig. 2C) in the serum of the IO mice group. IO groups treated with Met (1,000 μg/kg bw) and deferiprone (20 mg/kg bw, a drug used as the PC) showed similar results to the control mice group with respect to iron and ferritin contents and TIBC in the serum (Fig. 2).

Liver iron and ferritin contents

Liver tissue of the IO mice showed 178.8% and 66.5% higher iron and ferritin contents, respectively, than that of the control on day 22 (Fig. 3). Treatment of the IO

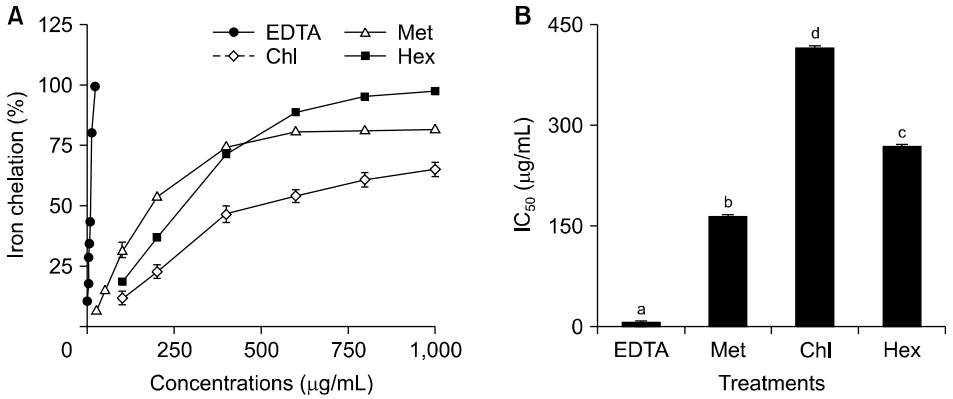

Fig. 1. *In vitro* Fe-chelating activity of various solvent fractions [methanol (Met), chloroform (Chl), and *n*-hexane (Hex)] of *Sonneratia apetala* fruits. (A) Dose-dependent Fe-chelation (%) activity of the fractions and (B) inhibitory concentration 50 (IC_{50}) of the fractions for Fe-chelation. Ethylenediaminetetraacetic acid (EDTA) served as a positive control. Data are presented as mean±SD (n=3~5). Different letters (a-d) indicate significant differences at $P<0.05$.

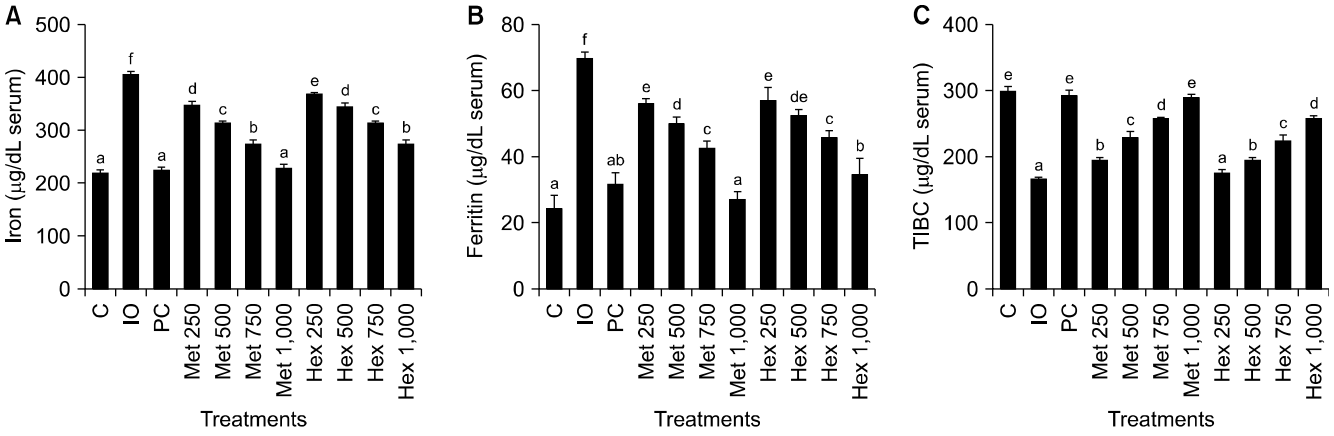

Fig. 2. Effects of solvent fractions [methanol (Met) and *n*-hexane (Hex)] of *Sonneratia apetala* fruits on the iron profile of mice serum. Dose-dependent effects of Met and Hex on (A) serum iron, (B) serum ferritin contents, and (C) total iron-binding capacity (TIBC). Data are presented as mean±SD (n=6). Different letters (a-f) indicate significant differences at $P<0.05$. C, control group; IO, iron-overloaded group; PC, positive control.

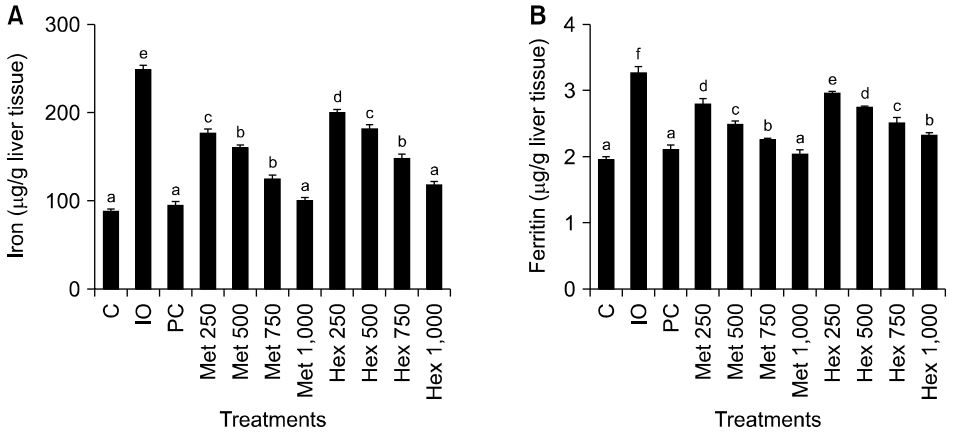

Fig. 3. Effects of solvent fractions [methanol (Met) and *n*-hexane (Hex)] of *Sonneratia apetala* fruits on the iron profile of mice liver tissue. Dose-dependent effects of Met and Hex on (A) iron and (B) ferritin contents in liver tissue. Data are presented as mean±SD (n=6). Different letters (a-f) indicate significant differences at $P<0.05$. C, control group; IO, iron-overloaded group; PC, positive control.

mice with Met and Hex dose-dependently decreased iron (Fig. 3A) and ferritin (Fig. 3B) contents in liver tissue. The Met showed a stronger effect than the Hex. Met at 1,000 μg/kg bw, when used to treat IO mice, decreased iron and ferritin contents in their liver tissue to a level almost similar to those of the negative control and PC (deferiprone, 20 mg/kg bw; Fig. 3).

Reducing power, TAC, and protein content in the liver tissue

Reducing power is concomitant with the antioxidant potential. Liver supernatants of the various mice treatment groups showed a dose-dependent increase in reducing power (OD; Fig. 4A and 4B). At all doses, the liver supernatant of the IO mice had the lowest reducing power, which was significantly (P<0.05) lower than that of the control mice (C). The oxidative stress generated by iron overload was the cause of the low reducing power. IO mice groups treated with different concentrations of Met and Hex increased the reducing power dose-dependently (Fig. 4A and 4B). Mice with an iron overload when treated with Met at 1,000 μg/kg bw restored reducing power to the same level as that of the negative control group and the PC group (deferiprone, 20 mg/kg bw; Fig. 4A).

TAC was presented as mg AAE or GAE/g liver tissue. Liver tissue of IO mice showed the lowest TAC and it was decreased to 38% AAE or 32% GAE of that of the control mice (C). Met and Hex dose-dependently increased TAC in the liver tissue of IO mice groups. Met at 1,000 μg/kg bw restored TAC to a level similar to that of the control group (Fig. 4C).

Protein content in the liver tissue of the control mice group was 146.5 mg protein/g liver tissue (14.7%). It was significantly (P<0.05) decreased to 79.4 mg protein/g liver tissue (8%) in the IO mice (Fig. 4D). Both Met and Hex dose-dependently restored the protein content in the liver tissue of the IO mice groups. Met (1,000 μg/kg bw) and deferiprone (20 mg/kg bw, PC) treated IO mice, and control mice groups showed similar amounts of protein in their liver tissue (Fig. 4D).

DISCUSSION

In vitro iron-chelating activity was assayed using ferrozine, which binds with Fe^{2+} resulting in the formation of a violet-colored complex which was measured at 562 nm spectrophotometrically. Under acidic conditions, transferrin-bound iron is released and reduces to ferrous ions. The presence of Fe^{2+}-chelating components in the fractions interrupted the Fe-ferrozine complex formation and decreased the intensity of the violet color. Among the fractions, Met showed the strongest Fe^{2+}-chelating activity as indicated by the lowest IC_{50} value of 165 μg/mL (Fig. 1B). This suggests that the Met is contained highly active or a high content of Fe^{2+}-chelating components followed by Hex. Hossain et al. (2016a) reported a high content of polyphenols and antioxidants in the Met frac-

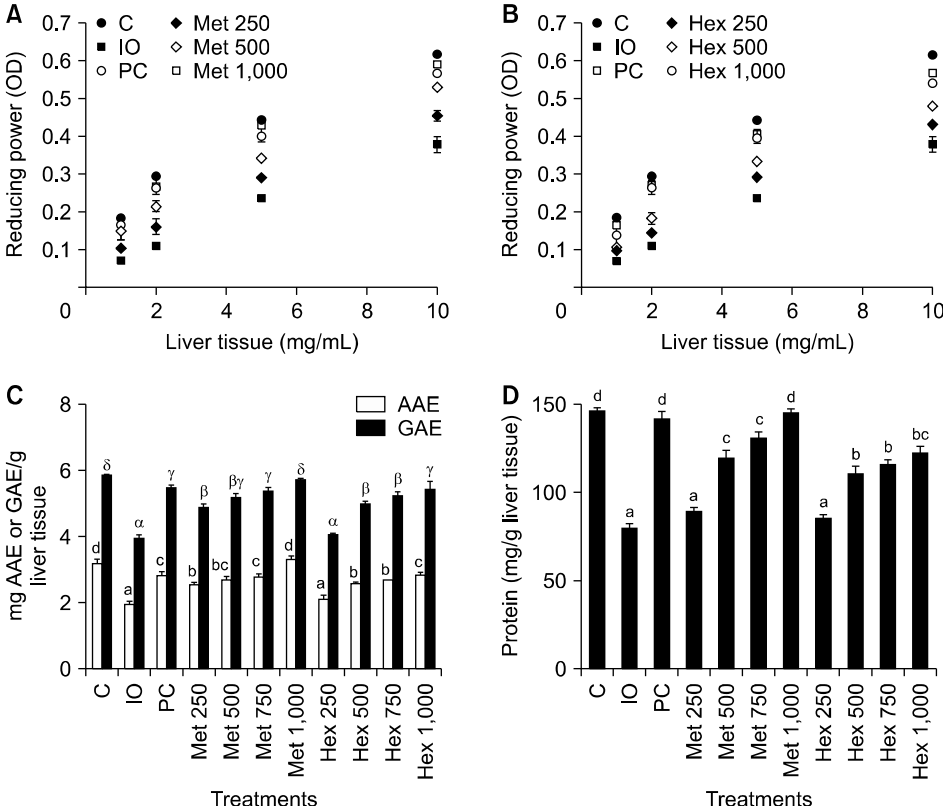

Fig. 4. Effects of solvent fractions [methanol (Met) and *n*-hexane (Hex)] of *Sonneratia apetala* fruits on reducing power, total antioxidant capacity, and total protein content in liver tissue of mice. Dose-dependent restoration of reducing power (optical density, OD) by (A) Met and (B) Hex, (C) dose-dependent restoration of total antioxidant capacity (mg ascorbic acid, AAE or gallic acid, GAE, equivalents) by Met and Hex, and (D) dose-dependent restoration of total protein content in liver tissue of mice treated by Met and Hex. Data are presented as mean±SD (n=6). Different letters (a-d; α-δ) indicate significant differences at P<0.05.

tion of *S. apetala* seed and identified polyphenols including caffeic acid, (+)-catechins, quercetin, which are powerful in iron chelation (Mandel et al., 2006; Leopoldini et al., 2006; Kitsati et al., 2012).

Intraperitoneal injection of ferric carboxymaltose in mice elevated iron and ferritin in the serum and the liver tissue but decreased TIBC of the blood serum (Fig. 2 and 3), indicating an increased absorption of iron. Ferritin, an intracellular protein that stores iron, is a marker of iron status and plays a major role in iron homeostasis in the body (Anderson and Frazer, 2017). It is the primary tissue iron storage protein in the liver, where most extra body iron is stored. Expression of ferritin is induced in primary or secondary iron-overload disorders, resulting in increased hepatic and circulating ferritin levels (Arosio et al., 2009). Iron can enter cells either by binding with the transporter protein, transferrin, or as NTBI through the divalent metal ionic transporter. Under iron-overloading conditions, the iron-binding capacity of transferrin is exceeded resulting in NTBI entering into circulation mainly in hepatocytes and the parenchymal cells of the pancreas, thyroid, heart, and central nervous system. Deposition of iron is associated with complications such as fibrosis, atherosclerosis, carcinogenesis, and organ dysfunction through cell death (Kohgo et al., 2008; Fleming and Ponka, 2012). NTBI is highly reactive and produces a many free radicals through the Fenton reaction, involved in the pathogenesis of diseases such as heart failure (Münzel et al., 2015), liver failure (Wang et al., 2019), and neurodegeneration (Konno et al., 2021). The fruit of *S. apetala* is highly recognized for its antioxidant properties (Hossain et al., 2013, 2016a, 2016b). Increasing concentrations of Met and Hex decreased iron and ferritin contents in serum and liver tissue of IO mice and, additionally, both fractions increased TIBC of blood serum (Fig. 2 and 3). This was possibly due to the effective chelation of iron from the blood serum and the liver tissue by chelating agents in the Met and Hex fractions.

The liver tissue of IO mice showed the lowest reducing power, TAC, and protein content (Fig. 4). This can be attributed to the presence of free iron ions, Fe^{2+}, which generated oxidative stress through the creation of ROS. Antioxidant status (reducing power and TAC) in the liver tissue of IO mice was restored dose-dependently when the mice were treated with Met and Hex fractions (Fig. 4A～4C). This was probably caused by the antioxidant activity of Met and Hex as well as preventing iron from entering the Fenton reaction. Harmful hydroxyl free radicals are generated through Fenton reactions involved in the oxidation of proteins, lipids, DNA, and other biomolecules. Probably, Met and Hex dose-dependently inhibited the oxidation of liver biomolecules including proteins and thereby preventing their deformation and degradation in the liver tissue of IO mice. Protein oxidation through iron overload contributes to morphological and functional modifications of various proteins leading to the pathogenesis of diseases such as diabetes, cystic fibrosis, ulcerative colitis, and atherosclerosis (Dalle-Donne et al., 2003). Sarkar et al. (2015) reported that the fruit of *E. officinalis* effectively reduced protein carbonyl formation in IO mice.

The findings of this study reveal that the *S. apetala* fruit, especially Met and Hex fractions contains hydrophilic and lipophilic bioactive components, which strongly chelate iron from the serum and the liver tissue of IO mice. The Met at 1,000 μg/kg bw, also effectively restored the reducing power, TAC, and total protein content in the liver tissue of IO mice and protected the liver from iron toxicity and oxidative stress. Therefore, the fruit is potentially useful in therapeutic applications as 1.3 mg Met produced from 6.6 mg powder of *S. apetala* fruit was equivalent to 25 mg deferiprone, the lowest dose available in Bangladesh markets. Thus, the consumption of a few milligrams of the *S. apetala* fruit can potentially prevent iron overload. Hence, the *S. apetala* fruit can be used for the development of functional foods, food supplements, and nutraceuticals to ameliorate iron overload and treat associated diseases.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: SJH. Analysis and interpretation: MM, MRI, MSG. Data collection: MM, MRK, MRI. Writing the article: SJH, MM. Critical revision of the article: SJH. Final approval of the article: all authors. Statistical analysis: MRI, MM. Obtained funding: MSG, SJH. Overall responsibility: SJH.

REFERENCES

Alam HMI, Biswas A, Hosen MZ, Islam MR, Hossain SJ. Antioxidant properties and potentiality of silver nanoparticles biosynthesis of thirty-five edible Bangladeshi fruits. Bangladesh J Bot. 2021. 50:445-451.

Anderson GJ, Frazer DM. Current understanding of iron homeostasis. Am J Clin Nutr. 2017. 106:1559S-1566S.

Arosio P, Ingrassia R, Cavadini P. Ferritins: a family of molecules for iron storage, antioxidation and more. Biochim Biophys Acta. 2009. 1790:589-599.

Biswas A, Islam MR, Hosen MZ, Ahmed KS, Hossain H, Hossain SJ. Analgesic and anthelmintic activities in common fruits of the Sundarbans mangrove forest, Bangladesh. Bangladesh J Bot. 2023. 52:79-86.

Carter P. Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). Anal Biochem. 1971. 40:450-458.

Chaudhuri D, Ghate NB, Panja S, Das A, Mandal N. Wild edible fruit of *Prunus nepalensis* Ser. (Steud), a potential source of antioxidants, ameliorates iron overload-induced hepatotoxicity and liver fibrosis in mice. PLoS One. 2015. 10:e0144280. https://doi.org/10.1371/journal.pone.0144280

Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl groups as biomarkers of oxidative stress. Clin Chim Acta. 2003. 329:23-38.

Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell. 2012. 149:1060-1072.

Fleming RE, Ponka P. Iron overload in human disease. N Engl J Med. 2012. 366:348-359.

Habib HM, Ibrahim S, Zaim A, Ibrahim WH. The role of iron in the pathogenesis of COVID-19 and possible treatment with lactoferrin and other iron chelators. Biomed Pharmacother. 2021. 136:111228. https://doi.org/10.1016/j.biopha.2021.111228

Hosen MZ, Biswas A, Islam MR, Nazrul M, Bhuiyan I, Hossain SJ. Comparison of physicochemical and antioxidant properties of edible fruits in the Sundarbans' mangrove forest, Bangladesh. Bangladesh J Bot. 2020. 49:671-678.

Hosen MZ, Biswas A, Islam MR, Hossain SJ. Anti-bacterial, anti-diarrheal, and cytotoxic activities of edible fruits in the Sundarbans mangrove forest of Bangladesh. Prev Nutr Food Sci. 2021. 26:192-199.

Hossain SJ, Basar MH, Rokeya B, Arif KMT, Sultana MS, Rahman MH. Evaluation of antioxidant, antidiabetic and antibacterial activities of the fruit of *Sonneratia apetala* (Buch.-Ham.). Orient Pharm Exp Med. 2013. 13:95-102.

Hossain SJ, Iftekharuzzaman M, Haque MA, Saha B, Moniruzzaman M, Rahman MM, et al. Nutrient compositions, antioxidant activity, and common phenolics of *Sonneratia apetala* (Buch.-Ham.) fruit. Int J Food Prop. 2016a. 19:1080-1092.

Hossain SJ, Islam MR, Pervin T, Iftekharuzzaman M, Hamdi OAA, Mubassara S, et al. Antibacterial, anti-diarrhoeal, analgesic, cytotoxic activities, and GC-MS profiling of *Sonneratia apetala* (Buch.-Ham.) seed. Prev Nutr Food Sci. 2017. 22:157-165.

Hossain SJ, Pervin T, Suma SA. Effects of cooking methods at different time durations on total phenolics and antioxidant activities of fresh and dried-stored fruits of *Sonneratia apetala* (Buch.-Ham.). Int Food Res J. 2016b. 23:556-563.

Hossain SJ, Tsujiyama I, Takasugi M, Islam MA, Biswas RS, Aoshima H. Total phenolic content, antioxidative, anti-amylase, anti-glucosidase, and antihistamine release activities of Bangladeshi fruits. Food Sci Technol Res. 2008. 14:261-268.

Jang HI, Do GM, Lee HM, Ok HM, Shin JH, Kwon O. *Schisandra chinensis* Baillon regulates the gene expression of phase II antioxidant/detoxifying enzymes in hepatic damage induced rats. Nutr Res Pract. 2014. 8:272-277.

Jiang S, Jiang CP, Cao P, Liu YH, Gao CH, Yi XX. Sonneradon A extends lifespan of *Caenorhabditis elegans* by modulating mitochondrial and IIS signaling pathways. Mar Drugs. 2022. 20:59. https://doi.org/10.3390/md20010059

Kim SH, Yadav D, Kim SJ, Kim JR, Cho KH. High consumption of iron exacerbates hyperlipidemia, atherosclerosis, and female sterility in zebrafish *via* acceleration of glycation and degradation of serum lipoproteins. Nutrients. 2017. 9:690. https://doi.org/10.3390/nu9070690

Kitsati N, Fokas D, Ouzouni MD, Mantzaris MD, Barbouti A, Galaris D. Lipophilic caffeic acid derivatives protect cells against H_2O_2-induced DNA damage by chelating intracellular labile iron. J Agric Food Chem. 2012. 60:7873-7879.

Kohgo Y, Ikuta K, Ohtake T, Torimoto Y, Kato J. Body iron metabolism and pathophysiology of iron overload. Int J Hematol. 2008. 88:7-15.

Konno T, Melo EP, Chambers JE, Avezov E. Intracellular sources of ROS/H_2O_2 in health and neurodegeneration: Spotlight on endoplasmic reticulum. Cells. 2021. 10:233. https://doi.org/10.3390/cells10020233

Leopoldini M, Russo N, Chiodo S, Toscano M. Iron chelation by the powerful antioxidant flavonoid quercetin. J Agric Food Chem. 2006. 54:6343-6351.

Lesjak M, Srai SKS. Role of dietary flavonoids in iron homeostasis. Pharmaceuticals. 2019. 12:119. https://doi.org/10.3390/ph12030119

Liu J, Luo D, Wu Y, Gao C, Lin G, Chen J, et al. The protective effect of *Sonneratia apetala* fruit extract on acetaminophen-induced liver injury in mice. Evid Based Complement Alternat Med. 2019. 2019:6919834. https://doi.org/10.1155/2019/6919834

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951. 193:265-275.

Mandel S, Amit T, Reznichenko L, Weinreb O, Youdim MB. Green tea catechins as brain-permeable, natural iron chelators-antioxidants for the treatment of neurodegenerative disorders. Mol Nutr Food Res. 2006. 50:229-234.

Münzel T, Gori T, Keaney JF Jr, Maack C, Daiber A. Pathophysiological role of oxidative stress in systolic and diastolic heart failure and its therapeutic implications. Eur Heart J. 2015. 36:2555-2564.

Oyaizu M. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr Diet. 1986. 44:307-315.

Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999. 269:337-341.

Sarkar R, Hazra B, Mandal N. Amelioration of iron overload-induced liver toxicity by a potent antioxidant and iron chelator, *Emblica officinalis* Gaertn. Toxicol Ind Health. 2015. 31:656-669.

Sengupta S, Abhinav N, Singh S, Dutta J, Mabalirajan U, Kaliyamurthy K, et al. Standardised *Sonneratia apetala* Buch.-Ham. fruit extract inhibits human neutrophil elastase and attenuates elastase-induced lung injury in mice. Front Pharmacol. 2022. 13:1011216. https://doi.org/10.3389/fphar.2022.1011216

Wang CY, Knutson MD. Hepatocyte divalent metal-ion transporter-1 is dispensable for hepatic iron accumulation and non-transferrin-bound iron uptake in mice. Hepatology. 2013. 58:788-798.

Wang X, Li Y, Han L, Li J, Liu C, Sun C. Role of flavonoids in the treatment of iron overload. Front Cell Dev Biol. 2021. 9:685364. https://doi.org/10.3389/fcell.2021.685364

Wang Y, Chen Q, Shi C, Jiao F, Gong Z. Mechanism of glycyrrhizin on ferroptosis during acute liver failure by inhibiting oxidative stress. Mol Med Rep. 2019. 20:4081-4090.

Yi X, Jiang S, Qin M, Liu K, Cao P, Chen S, et al. Compounds from the fruits of mangrove *Sonneratia apetala*: Isolation, molecular docking and antiaging effects using a *Caenorhabditis elegans* model. Bioorg Chem. 2020. 99:103813. https://doi.org/10.1016/j.bioorg.2020.103813