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Research Article

Preventive Effect of Hydroethanolic Extract of *Aerva lanata* Leaf on Iron Overload-Induced Oxidative Stress

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ABSTRACT

Excess iron is a major cause of iron-induced oxidative stress and a number of diseases. Plants like *Aerva lanata*, offer a mitigative potential against iron-induced oxidative damage. This study aimed at determining the antioxidant enhancing potential of hydroethanolic extract of *Aerva lanata* (HEAL) leaf against iron overload-induced oxidative damage. Thirty-six male Wistar rats were randomised into six groups. Group 1 received 0.5 mL of distilled water while groups 2-6 were given treatments consisting of intraperitoneally administration of iron dextran (100 mg/kg) plus oral administration of 0.5 mL of distilled water, 20 mg/kg deferasirox, 50, 100 and 200 mg/kg of the extract respectively for 21 days. The extract was assessed for a number of secondary metabolites as well as its *in-vitro* antioxidant capacity while biochemical parameters of oxidative stress and iron overload were assessed in the rats. Six secondary metabolites were found in the extract, with cardiac glycosides (270.60 mg/L) being the most abundant while flavonoids (2.56 mg/L) were the least prevalent, exhibiting antioxidant and free radical scavenging capabilities, with the inhibitory concentrations (IC_{50}) of 41.19 g/mL (DPPH), 258.154 g/mL (ABTS). Treatment with HEAL significantly reduced the increased serum iron level in iron-overload oxidative stressed animals, and reduced AST, ALP, MDA levels, and increased antioxidant activities. The antioxidant and free radical-scavenging activities of HEAL discovered in this study have shown that HEAL treatment can strengthen the antioxidant defenses against iron overload-induced oxidative stress in rats and might contribute to the mechanism by which HEAL prevents the liver from iron loaded-induced oxidative stress.

Keywords: *Free radical-scavenging activities, Iron-induced oxidative, Deferasirox, Aerva lanata*

INTRODUCTION

Iron is essential for multiple cell functions including oxygen transport, biosynthesis, detoxification, DNA synthesis, and energy metabolism, due to its redox potential and flexible chemical coordination (Sönmez-Aydın *et al.*, 2021; Ogłuszka *et al.*, 2022). Lack of an effective excretory mechanism may, however, impede the maintenance of normal iron levels which may result in iron accumulation, and in turn, iron toxicity due to oxygen radical generation. Iron overload can manifest through a heterogeneous group of either inherited or idiopathic conditions which may affect many vital organs, including the liver, heart, endocrine glands, skin, and joints (Camaschella *et al.*, 2022). When iron excess occurs in the liver, it encourages the overproduction of free radicals, which eventually causes oxidative stress and hepatic damage. Iron overload in males may be indicated by serum ferritin levels higher than 300 ng/mL. Iron removal with the iron-chelating agent is the primary strategy for the treatment of iron overload (Sheikh *et al.*, 2021). Deferoxamine has been used in humans for

nearly half a century, it is however, less favored by patients due to intravenous administration (Pinto *et al.*, 2020). Oral deferasirox and deferiprone have higher lipid solubility and thereby have a more significant potential for the chelation of cytosolic iron; yet, these drugs have been associated with certain renal toxicity (Ghosh and Ghosh, 2018).

Hepcidin, a critical negative regulator of the plasma-iron level and a regulator of iron distribution, was proposed to decrease the system iron overload. Although hepcidin can increase iron deposition in parenchymal cells including those in the liver and kidney, a high amount of hepcidin lowers ferroportin-1-dependent cellular iron efflux (Nemeth and Ganz, 2021). Therefore, research into novel drugs or therapeutic approaches from the plant source for the management of hepatic damage caused by iron excess is crucial. Plants with remarkable antioxidant property such as *Aerva lanata*, becomes vital in this case as antioxidants can effectively captures free iron and thus prevents iron-induced oxidative stress.

A. lanata, a perennial plant that belongs to the Amaranthaceous family. It is also known by the name Gorakha Ganga (Prajapati *et al.*, 2022). The name of the plant varies depending on the language; it is known as Mountain Knotgrass in English, *Obompa* (Igbo), and *Ewe Owo* (Yoruba). The leaf of *A. lanata* has been shown to provide a variety of therapeutic benefits, including those for urolithiasis, diuretic effect, and antibacterial activity. In many parts of the world, the plant is utilized for a wide range of traditional and folkloric purposes. A leaf decoction is used to treat diabetes and inflammation brought on by kidney stones (Wasana, *et al.*, 2022). Cholera, skin conditions, asthma, biliousness, dyspepsia, typhoid, chest discomfort, malaria fever, headaches, piles, bleeding, and snake venom are all treated using the entire plant and its roots.

In order to cure osteoporosis, *A. lanata* is used in combination with other herbs (Kalam *et al.*, 2022). To treat leukorrhea, a pinch of salt is combined with the plant's crushed roots (Meenakshi *et al.*, 2019).

MATERIALS AND METHODS

Plant material and authentication

A. lanata leaves were obtained at a local market in Ilorin West local government, Kwara State, Nigeria. The leaf was then identified and authenticated by a botanist, at University of Ilorin Herbarium, Ilorin, Nigeria, where a voucher sample was deposited under UILH/006/1106.

Experimental animals

Thirty-six healthy Wister rats weighing 125 ± 5.37g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Nigeria. The animals were housed in plastic cages placed in a well-ventilated Animal House and maintained at room temperature. The animals were fed with rat chow and tap water. All the animals were handled strictly according to the instructions Guidelines on Care and Use of Laboratory Animals.

Drugs, assay kits and chemicals

Iron dextran was a product of Alliance Biotech Company Limited. Village Katha, Baddi, India, Ethanol was a product of Qualikems manufactured in India, DMSO (Dimethyl Sulphoxide) was a product of Loba Chemie Laboratory Reagents and Fine Chemicals. Wodehouse Road, Colaba, India. Assay kits for bilirubin, albumin, alkaline phosphatase, alanine aminotransferase and gamma glutamyl transferase were products of Fortress Diagnostic Limited, United Kingdom. The standard oral-iron chelating drug, deferasirox, was obtained from Norvatis Pharma Stein AG, Switzerland.

Methods

Preparation of plant extract

Dried leaf of *A. lanata (*100 g) were pulverized into a fine powder and extracted in 1000 mL (1/10w/v) of hydroethanolic solvent (50:50 v/v) at room temperature for 48 h with intermittent shaking. Using Zirbus freeze-dryer (Model VaCo 5-11, Stephensonstraat, Germany), the filtrate that resulted was freeze dried to obtain yield of 9.61%. The extract was reconstituted in 2% v/v DMSO to obtain the desired doses of 50, 100, and 200 mg/kg body weight used for the study.

In vitro study

Phytochemical screening, antioxidant and free radical scavenging potential of HEAL

Flavonoids, phenols, tannins, saponins, alkaloids, and cardiac glycosides were assayed for using the procedures outlined by Odebiyi and Sofowora (1978), Trease and Evans (1989), Sofowora, (1993), Edeoga *et al.* (2005), and Harborne 1973), and were subjected to quantitative analysis using known techniques: saponins (Obadoni and Ochuko, 2002), alkaloids (Adeniyi *et al.*, 2009), tannins (Makkar et al., 1993), flavonoids (Boham and Kocipai, 1974) and cardiac glycosides (El-Olemy *et al.*, 1994). ABTS radical cation decoloriztaion assay, DPPH radicals scavenging assay, and reducing power assays were carried out as previously reported (Re *et al.*, 1999; Mensor *et al.*, 2001). The FRAP of HEAL was also done using previously published protocol of Benzie and strain (1996).

Induction of iron overload in male Wistar rats

Induction of iron overload in rats was done by intraperitoneal injection of ten doses of 100mg/kg body weight of iron dextran every other day for 20 days (Jensen *et al.*, 2020).

Animal grouping and administration of chemical compounds

A total of 36 male Wistar rats were allocated into six groups (1 to 6) in a totally randomized manner, with six animals in each group, as follows:

Group 1: Rats + 0.5 mL of distilled water.

Group 2: Rats + 100 mg/kg body weight of iron dextran (ip) + 0.5 mL distilled water (oral).

Group 3: Rats + 100 mg/kg body weight of iron dextran (ip) + 20 mg/kg body weight deferasirox
Group 4: Rats + 100 mg/kg body weight of iron dextran (ip) + 50 mg/kg body weight of HEAL.
Group 5: Rats + 100 mg/kg body weight of iron dextran (ip) + 100 mg/kg body weight of HEAL.
Group 6: Rats + 100 mg/kg body weight of iron dextran (ip) + 200 mg/kg body weight of HEAL.

In vivo study

The serum and the tissue supernatant were prepared following the procedure described by Yakubu *et al.* (2009) was adopted. Iron storage capacity was done as described by Fasciolo *et al.* (2022), ferritin concentration as described by bleicher *et al* (2018), serum transferrin as described by (Edwards and Edwards, 2016), total iron binding capacity as described by (Lee *et al.*, 2022). Superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), reduced glutathione (GSH) and malondialdehyde (MDA) were evaluated as described respectively by Beers and Sizer (1952), Misra and Fridovich (1972), Habig *et al.* (1974), Gornall *et al.* (1949). Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), albumin, total and direct bilirubin and gamma-glutamyl transferase (GGT) levels were evaluated following procedures from the kits acquired from Fortress Diagnostic Limited. The method of Drury and Wallington (1980) was employed for liver histopathology.

Statistical analysis

Data were expressed as the mean ± SEM of six determinations except in *vitro* studies where the sample size was three. Data were analyzed using a one-way analysis of variance followed by Tukey's *post-hoc* test for multiple comparisons. Statistical significance was set at a 95% confidence interval ($p < 0.05$) and graph Pad statistical Package version 6.0 was used for the statistical analysis.

RESULTS

In vitro Study

Among the phytochemicals screened, cardiac glycosides had the highest concentration (270.60 ± 0.1495 mg/mL) while flavonoids had the least concentration (2.563 ± 0.0319 mg/mL) presented in Table 1. The *in vitro* antioxidant activity of HEAL shows that the extract has lower antioxidant activity compared with the standard (Figure 1). This is shown in the assay of DPPH with an IC_{50} of 41.19 against the used standard, BHT with an IC_{50} value of 6.20 mg/mL (Figure 1), FRAP with a lower activity of ferric reducing antioxidant power (Figure 1) while ABTS has an IC_{50} of 258.154 mg/ml against the IC_{50} of 10.33 mg/ml of BHT standard used (Figure 1).

In vivo study

A. lanata normalized the liver iron, transferrin, serum ferritin levels and total iron binding capacity

When compared to the normal group (Control), the iron levels in the liver, transferrin, serum ferritin and total iron binding capacity were shown to have significantly increased in the iron-overloaded. Oral administration of HEAL at 50, 100 and 200 mg/kg bw significantly decreased the liver iron content, transferrin, serum ferritin level and total iron binding capacity, this normalizing effect was superior to that of the standard iron chelator, deferasirox with the exception of total iron binding capacity which it decreased more effectively (Table 2).

Antioxidant Enzymes

Iron-overload in the liver lowers the concentration of antioxidant enzymes like SOD, CAT and GST, along with antioxidant molecules like GSH, causing significant oxidative stress. The results showed that the iron-induced depletion of the antioxidant enzymes SOD, CAT, GST, and the levels of non-enzymic antioxidant GSH were significantly increased when treated with the different doses of *A. lanata* (Table 3). This increment is similar to that shown by the standard drug, deferasirox. The concentration of malondiadehyde was significantly increased in iron overloaded rats, this reduced when treated orally with different doses of *A. lanata* with the highest reduction at the highest dose level of 200 mg/kg bw.

Serum marker enzymes

Table 4 shows the serum indices of liver injury, such as alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma albumin, and bilirubin. The activities of the enzymes and concentration of albumin and bilirubin were higher in the iron overload group when compared to the normal group. *A. lanata* orally significantly reversed this effect.

Histopathological evaluation of Liver

There is no significant inflammation or features of acute or chronic damage (Plate 1a). Treatment with iron dextran revealed hepatic tissue with distortion of the architecture by numerous foci of bile plugs (bile lakes) expanding the sinusoids. The portal tracts show moderate to dense lymphocytic infiltration (Plate 1b). The cross section of the liver of the group administered 20 mg of deferasirox showed preserved architecture; the portal tracts show mild lymphocytic infiltration (Plate 1c). The cross section of the liver of the group administered 50mg of the extract following iron dextran administration showed hepatic tissue with distortion of the architecture by numerous foci of bile plugs (bile lakes) expanding the sinusoids. Also seen are pigment laden macrophages and the portal tracts appear largely normal (Plate 1d). The cross section of the group administered 100 mg revealed hepatic tissue with distortion of the architecture by numerous foci of bile plugs (bile lakes) expanding the sinusoids. Also seen are pigment laden macrophages. The portal tracts appear largely normal (Plate 1e) while the examination of the 200 mg treated group revealed hepatic tissue with distortion of the architecture by numerous foci of bile plugs (bile lakes) expanding the sinusoids. Also seen are pigment laden macrophages. There is periportal lymphocytic inflammation with extension to the lobules; few focal hepatocyte dropouts is seen (Plate 1f)

Table 1. Phytochemicals Constituents of Hydroethanolic *A. Lanata* Leaf

Phytochemicals	Concentration (mg/ml)
Cardiac glycosides	270.60 ± 0.15
Alkaloids	140.66 ± 0.20
Saponins	95.04 ± 0.16
Tannins	65.33 ± 0.19
Phenols	15.39 ± 0.01
Flavonoids	2.56 ± 0.03

Values are mean of 3 replicates ± S.E.M

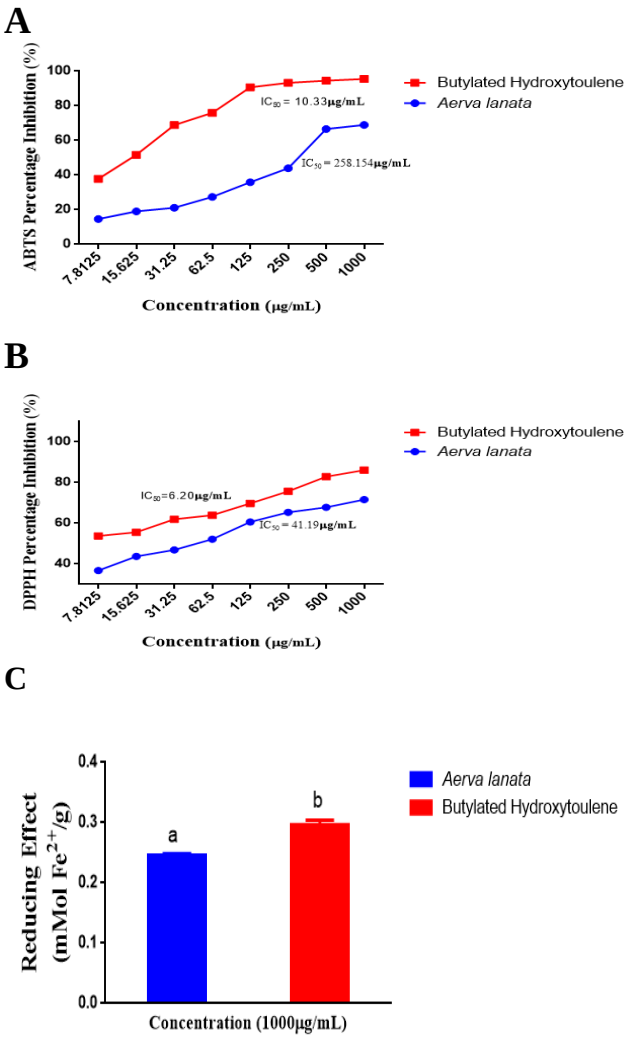

Figure 1: Total Antioxidant and Reducing Power Activity of *A. lanata* (A) Total antioxidant activity using ABTS•+ radical cation decolorization assay, (B) DPPH radical scavenging assay, and (C) reducing power activity.

The results are mean ± S.D. of eight measurements for ABTS and DPPH, two measurements for FRAP.

Discussion

Phytochemicals are natural bioactive compounds that constitute important sources of new therapeutic agents for several diseases (Xiao and Bai, 2019; Gong *et al.*, 2020;). Thousands of phytochemicals including saponins, flavonoids, tannins, phenols, cardiac glycosides, alkaloids have been reported to have various pharmacological actions (Gong *et al.*, 2020). Cardiac glycosides and flavonoids are the most abundant and least abundant phytochemicals in the hydroethanolic extract of *A. lanata* leaf, respectively, and they may be responsible for the plant extract's well-known pharmacological effects. This agrees with the research conducted by Al-Anasri *et al.* (2019) which shows the presence of some phytochemicals such as flavonoids, alkaloids, and saponins in *A. lanata* leaf. It is possible that the phyto-constituents are working singly for various disease conditions or working in synergy with each other. Oxidative stress is defined as an imbalance between pro-oxidant and antioxidant species (Pisoschi *et al.*, 2021). Oxidative stress occurs when the production of reactive oxygen species outnumbers the body's natural antioxidant defenses. In order to keep the cell's equilibrium with its immediate environment, reactive oxygen species are used as second messengers in several intracellular signaling cascades. At greater doses, they can harm biological molecules in an indiscriminate manner, causing function loss and even cell death (Burton and Jauniaux, 2011). Superoxide is detoxified by the superoxide dismutase enzymes, which convert it to hydrogen peroxide. Hydrogen peroxide is not a free radical, and so is less reactive than O_2^-. However, it comes under the term ROS as it is intimately involved in the generation and detoxification of free radicals. Because it is non-polar, it may diffuse through the membranes of cells and organelles and hence plays a significant role as a second messenger in signal transduction pathways. The enzyme catalase then detoxifies hydrogen peroxide into water (Pisoschi *et al.*, 2021). The non-significant difference in SOD activity of all groups as evident in this study is an indication that the extract and the standard drug (deferasirox) were able to combat the generation of superoxide radicals generated due to iron overload. The observed decrease in the catalase activity of the group administered ID + DW is suggestive of the repressive effect of the iron dextran used for the induction of iron overload. Following extract treatment, all groups that received extract doses performed favorably compared to the control group that wasn't stimulated. This shows that the extract had the ability to change hydrogen peroxide into catalytic oxygen and water at different dosages. One of the main antioxidant defense mechanisms that protect mammalian tissues from oxidative stress is GSH. It plays a homeostatic function in fundamental biological processes such DNA synthesis, cell development, protein maturation, redox management, and cellular signaling (Sonmez-Aydın *et al.*, 2021). MDA is the final product of the lipid peroxidation process. The decrease in GSH, GST, and increase in MDA levels due to iron overload in the ID + DW group is suggestive of oxidative stress and lipid peroxidation respectively. The increased GSH and GST activities in comparison to the decreased MDA level after the administration of *A. lanata* leaf suggest that the hydroethanolic extract of *A. lanata* leaf not only prevented oxidative stress but also protected macromolecules from electrophilic attack and promoted protein synthesis, cell

growth, and repair. Oxidative stress may however be provoked by Iron accumulation which can cause DNA and protein damage and accelerates lipid oxidation, resulting in the loss of function in critical organs, such as the liver (Sonmez-Aydın *et al.*, 2021).

Table 2. The Iron Storage and Transporters Capacity Parameters in Rats Following Administration of Hydroethanolic Extract of *Aerva lanata* Leaf

Treatment	Ferritin (ng/dl)	Transferrin (mg/dl)	Total iron binding capacity (mg/dl)	Liver Iron (mg/dl)
DW (control)	0.48 ± 0.02 [b]	2.46 ± 0.04 [b]	39.79 ± 2.19 [a]	9.32 ± 1.09 [c]
ID + d H_2O	0.85 ± 0.02 [a]	3.44 ± 0.03 [a]	32.49 ± 1.26 [b]	18.63 ± 0.95 [a]
ID + Deferasirox	0.27 ± 0.02[c]	2.38 ± 0.04 [b]	39.70 ± 1.51 [a]	4.95 ± 0.35 [d]
ID + 50 mg/kg b.w of HEAL	0.51 ± 0.04 [b]	2.51 ± 0.02 [b]	39.57 ± 0.81 [a]	14.38 ± 1.20 [b]
ID + 100 mg/kg b.w of HEAL	0.44 ± 0.01 [b]	2.50 ± 0.01 [b]	42.37 ± 1.38 [a]	15.98 ± 0.86 [b]
ID + 200 mg/kg b.w of HEAL	0.47 ± 0.07 [b]	2.46 ± 0.02 [b]	43.77 ± 0.31 [a]	12.80 ± 0.65 [b]

Values are means of six replicates ± SEM. Values with different superscripts are significantly different from each other ($p < 0.05$). Where: ID represents iron dextran, DW represents distilled water, HEAL represents hydroethanolic extract of *A. lanata*.

Table 3. Specific Activity and Some Antioxidant and Lipid Peroxidation Parameters in Rats Following the Administration of Hydroethanolic Extract of *A. lanata* Leaf

Treatment	Superoxide dismutase (mmol/min/mg protein)	Catalase (mmol/min/mg protein)	Reduced Glutathione (µmol/mL)	Glutathione-S-transferase (µmol/mL)	Malondialdehyde (µmol/mL)
Distilled water (control)	1.16 ± 0.11[a]	3.20 ± 0.49 [b]	66.61 ± 7.91 [b]	0.17 ± 0.02 [b]	6.27 ± 0.24 [b]
ID + DW	0.58 ± 0.03 [b]	1.95 ± 0.01 [c]	51.22 ± 1.67 [c]	0.16 ± 0.01 [b]	9.15 ± 0.03 [a]
ID + Deferasirox	0.95 ± 0.04 [a]	4.44 ± 0.09 [a]	74.02 ± 0.77 [a]	1.11 ± 0.03 [a]	5.21 ± 0.34 [c]
ID + 50 mg/kg b.w of HEAL	1.07 ± 0.09 [a]	2.83 ± 0.24 [b]	67.76 ± 6.32 [b]	0.20 ± 0.03 [b]	7.29 ± 0.21 [b]
ID + 100 mg/kg b.w of HEAL	1.04 ± 0.05 [a]	3.06 ± 0.39 [b]	73.95 ± 6.88 [a]	0.17 ± 0.01 [b]	6.75 ± 0.52 [b]
ID + 200 mg/kg b.w of HEAL	1.09 ± 0.04 [a]	3.28 ± 0.06 [b]	76.90 ± 9.85 [a]	0.15 ± 0.02 [b]	7.50 ± 0.37 [b]

Values are means of six replicates ± SEM. Values with different superscripts are significantly different from each other ($p < 0.05$). Where: ID represents iron dextran, DW represents distilled water, HEAL represents hydroethanolic extract of *A. lanata*.

Table 4. The Level of Liver Function Indices in Rats Following the Administration of Hydroethanolic Extract of *A. lanata* Leaf

Treatment	Alanine aminotransferase (U/Lmg/protein)	Alkaline phosphatase (U/Lmg/protein)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)	Albumin(mg/dl)	Gamma-glutamyl transferase (U/mg protein)
Distilled water (control)	18.15 ± 1.75 [a]	10.94 ± 1.58 [a]	65.01 ± 1.69 [c]	34.40 ± 2.35 [b]	9.26 ± 0.91[c]	5.06 ± 0.33 [a]
ID + DW	9.92 ± 0.76 [c]	12.73 ± 1.73 [a]	108.47 ± 9.25 [a]	44.90 ± 1.75 [a]	6.26 ± 0.40[a]	4.85 ± 0.09 [a]
ID + Deferasirox	12.57 ± 0.91 [b]	13.43 ± 0.36 [a]	43.64 ± 0.77 [d]	24.40± 2.03[c]	12.84 ± 0.17 [a]	3.90 ± 0.15 [a]
ID + 50 mg/kg b.w HEAL	15.90 ± 4.13 [b]	13.35 ± 0.94 [a]	80.03 ± 10.12 [b]	38.70 ± 1.51 [b]	10.47 ± 0.87 [b]	4.65 ± 0.45 [a]
ID + 100 mg/kg b.w HEAL	14.46 ± 4.94 [b]	13.12 ± 1.16 [a]	63.95 ± 4.18 [c]	34.40 ± 2.59 [b]	10.68 ± 0.30 [b]	4.65 ± 0.19 [a]
ID + 200 mg/kg b.w HEAL	12.09 ± 1.26 [b]	12.29 ± 1.45 [a]	64.31 ± 1.38 [c]	35.20 ± 1.73 [b]	11.40 ± 0.58 [b]	4.88 ± 0.22 [a]

Values are means of six replicates ± SEM. Values with different superscripts are significantly different from each other ($p < 0.05$). Where: ID represents iron dextran, DW represents distilled water, HEAL represents hydroethanolic extract of *A. lanata*.

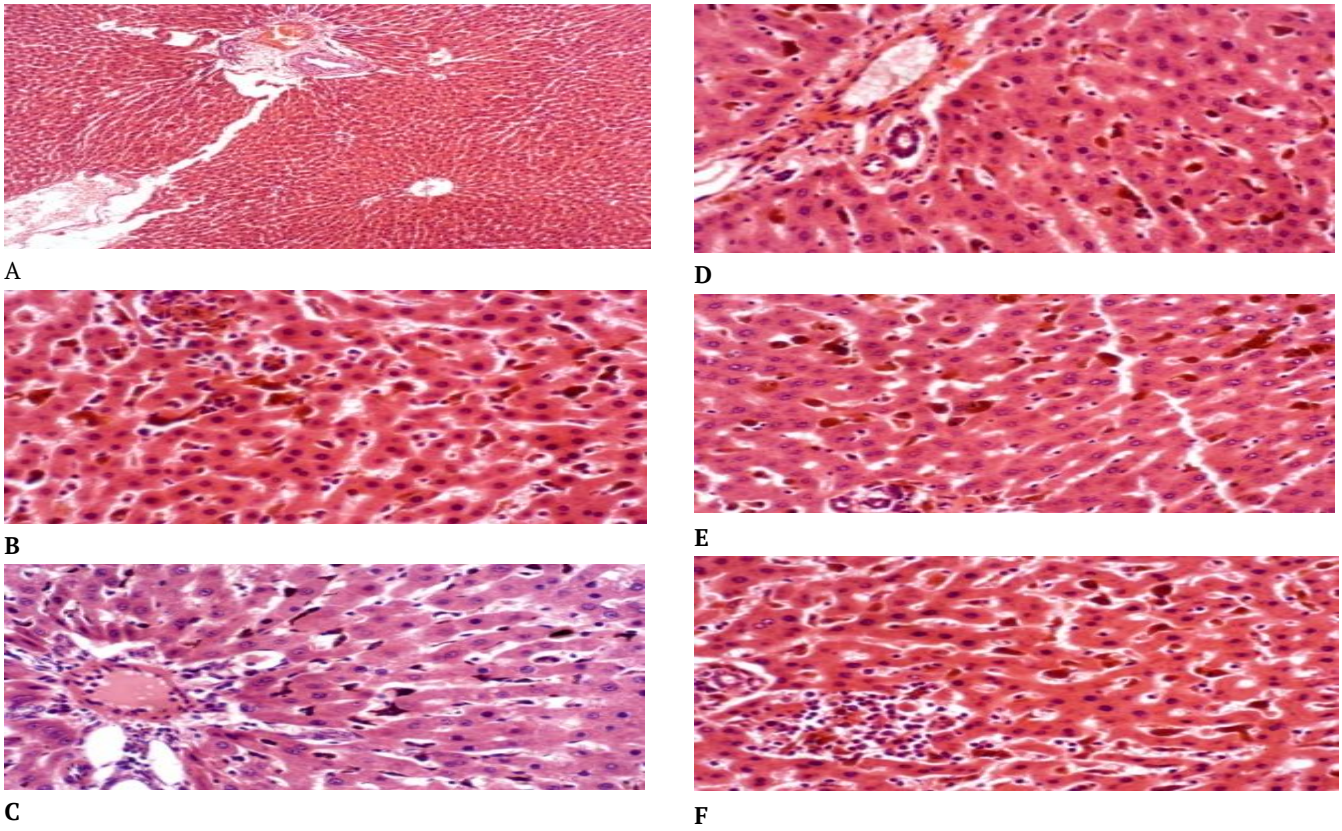

Plate 1: Histopathological Evaluation of Liver Sections with Hematoxylin and Eosin Staining at 40X and 400X Magnification.

Ferritin and transferrin often regulate the activity of many enzymes, and iron is a crucial component of cytochromes. In contrast to ferritin, which stores iron and releases it when the body requires it, transferrin transports iron throughout the body. The reactivity of excess iron, on the other hand, makes it exceedingly hazardous since it generates toxic free radicals that lead to oxidative stress. Transferrin also plays an important role by sequestering free iron ions and so inhibiting the Fenton reaction and production of hydroxyl radical (Burton and Jauniaux, 2011). Total iron binding capacity is a measure of effective binding of free iron to transferrin for distribution across the system. Albumin and bilirubin are commonly used in addition to marker enzymes to assess hepatic function (Abe and Kalantar-Zadeh, 2015). Administration of various doses of hydroethanolic extract of *A. lanata* leaf was able to reduce the accumulation of iron in the iron-induced increase in ferritin and transferrin which controls iron storage, and transferrin receptors, which control iron uptake in the blood suggesting that the extract might be a good chelator that can effectively binds and enhance iron transport to where it is needed in the body.

The elevated level of transferrin might be a response to the dextran assault with an attempt to inhibiting Fenton reaction and ultimately halting the formation of hydroxyl radicals. The extract was able to improve the total iron binding capacity and thus prevent the accumulation of iron in the tissue. The increase in bilirubin with a corresponding decrease in albumin level of the Iron dextran + Distilled water treated group suggests hepatobiliary injury. Administration of the extract however was able to prevent iron overload-induced hepatobiliary injury.

Alkaline phosphatase is a crucial biomarker for the diagnosis of hepatobiliary and skeletal diseases and it is commonly used in the assessment of membrane integrity (Zhang *et al.*, 2021). Gamma glutamyl transferase (GGT), a membrane-bound enzyme, contributes to the metabolism of glutathione (GSH), which plays a critical physiological role in protecting cells against oxidative stress (Takemura *et al.*, 2021). The lack of significance of the ALP and GGT activities found in this investigation suggests that administering the extract does not change the integrity of the membrane. One of the typical enzyme panels for evaluating liver function includes alanine aminotransferase. However, when iron excess occurs, the liver is one of the important organs that is most frequently impacted. Numerous factors might be responsible for iron buildup, a sickness that is characterized by an excess of iron. The imbalance between iron intake and excretion causes the body to gradually become iron-overloaded, which increases the quantity of non-transferrin-bound iron in plasma and may cause organ toxicity.

The increase in ALT activity with corresponding increase in iron deposit of the ID + DW treated group is an indication of liver damage arising from iron overload. Administration of the doses of the extract however reversed the iron overload induced oxidative stress that may initiate organ damage.

CONCLUSION

This research has proffered scientific validity for the indigenous usage of *A. lanata* leaf in reducing iron overload. The findings from our research suggests the antioxidant and free radical-scavenging activities of HEAL have shown that HEAL treatment can strengthen the antioxidant defenses against iron overload-induced oxidative stress in rats and might contribute to the mechanism by which HEAL prevents the liver from iron loaded-induced oxidative stress. The leaf also showed some improved results from the histopathology that resulted that the *A. lanata* leaf does not affect the histology of the liver sections treated with the plant. This further validates the popular choice for medicinal plants that might act through multiple mechanisms over conventional medicines that are constrained by their mechanism specificity.

AUTHORS' CONTRIBUTIONS

Conceptualization by ATA who also participated in methodology, Project administration, supervision and Writing original draft as well as editing the final manuscript. LOO contributed to data curation, carried out the research, and result analysis. BOY and IAM assisted in writing the original draft, and editing the manuscript. ZMY helped with data curation, investigation as well as preparation of the manuscript and submission of same for publication. All authors approved the final version for publication.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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