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Evaluation of the Protective Effect of Echinacea purpurea on Iron Overload-Induced Multi-Organ Damage in Male Rats: In Comparison with Vitamin E

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Abstract

By generating reactive hydroxyl radicals and other reactive oxygen species, iron overload may contribute to cellular damage and change the integrity of essential organelles. "*Echinacea purpurea*, a medical plant with a root liquid extract, includes a variety of phytochemical substances that are physiologically active. The purpose of this study was to assess Echinacea purpurea's preventive efficacy against bone marrow suppression, with a focus on apoptosis of liver and spleen cells induced by iron overload. Fifty albino male rats were divided into five groups: **Group (I)** [negative control), intraperitoneally injection with (0.2 ml) normal saline solution every 3 days; with daily oral gavage of (1 ml) normal saline for 4 weeks. **Group (II)** [Iron-overloaded]: Rats intraperitoneally injected with (200mg/kg iron dextran every 3 days); with daily oral gavage of (1 ml) normal saline solution for 4 weeks. **Group (III)** Rats intraperitoneally injected with iron dextran (200mg/kg every 3 days) +*Echinacea purpurea* (100mg/kg/day) orally administered for 4 weeks. **Group (IV)** Rats intraperitoneally injected with iron dextran (200mg/kg every 3 days) +*Echinacea purpurea* (200mg/kg/day) orally administered for 4 weeks. **Groups (V)** Rats intraperitoneally injected with iron dextran (200mg/kg every 3 days) and an oil solution of vitamin E (200mg/kg/day) orally administered for 4 weeks. Twenty-four hours following the treatment duration; the animals were euthanized, sacrificed and blood was drawn from the carotid artery in the neck of each male rat to be utilized for the estimation of serum Ferritin, and Hepcidin; liver tissue homogenate utilized for malondialdehyde, superoxide dismutase2, and glutathione biomarkers estimation, liver tissue for histopathological examination; spleen tissue homogenate to estimate Nuclear factor erythroid 2- related factor 2 and Caspase-3 protein expression. *Echinacea purpurea* in 200mg showed a significant elevation *($p<0.05$)* in the serum hepcidin and antioxidant superoxide dismutase2, glutathione, and nuclear factor erythroid 2 related factor 2 protein expression levels; with a significant reduction in Ferritin and Malondialdehyde levels and a non-significant elevation in Caspase-3" level.

Keywords: Antioxidant, Bone marrow suppression, *Echinacea purpura*, Iron overload, Malondialdehyde.

Introduction

Since iron is a component of myoglobin and hemoglobin, it is a vital trace element in the human body, as well as of enzymes engaged in the respiration of cells, including catalase (CAT), cytochromes, and cytochrome oxidase; these enzymes are essential for processes like electron transport, redox reactions, and cell development and discrepancy[(1)]. Excess free iron can create reactive oxygen species (ROS) by speeding up Haber-Weiss reduction and Fenton reactions, primarily reactive hydroxyl radicals, which can cause cellular damage and change the integrity of essential organelles [(2)(3)]. Additionally, Excessive iron interferes with significant buildup of free iron in parenchymal organs and iron hemostasis, such as the spleen, which serves as a phagocytic filter by eliminating damaged and senescent cells [(4)]. Iron overload syndrome (IOL) was the result of them [(5)]. Genetic deficiencies in iron absorption, particularly those caused by hereditary hemochromatosis, significantly worsen IOL by increasing the amount of dietary iron absorbed by the duodenal enterocytes; pathological conditions marked by moderate iron deposits or dysregulation of body iron transport; frequent parenteral iron delivery in transfusion-dependent anemia [(6)]. The purple coneflower, or *Echinacea purpurea* (E.P.), is a medicinal plant that is a member of the Asteraceae

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family [7]. There are nine species in the genus Echinacea, and Echinacea purpurea is frequently used medicinally[8]. The most widely utilized preparation is a liquid extract derived from *Echinacea purpurea* roots [9]. Numerous physiologically active substances are included in it, such as polysaccharides, polyacetylenes, flavonoids, alkamides, derivatives of caffeic acid, and the main active ingredient, cichoric acid [2][10][11]; It possesses antibacterial, anti-inflammatory, psychotropic, immunomodulatory, mutagenic, and anticancer properties [2]. *Echinacea purpurea's* pharmacological actions are caused by its main ingredients, which include polysaccharides, alkamides, and derivatives of caffeic acid [11] [12]. In the treatment of a variety of illnesses, flavonoids can be essential as agents that are anti-inflammatory, anti-bacterial, anti-allergic, and anti-mutagenic, as demonstrated by several clinical trials as well as various *in vitro* and *in vivo* epidemiological investigations [13]. Several medical Herbs and botanicals are employed as immunomodulators[9]; Many pathological disorders have been prevented and treated with *Echinacea purpurea.* It possesses antioxidant, anti-inflammatory, and wound-healing qualities [7].

Aim of study

This study's objective was to compare the anti-apoptotic and antioxidant effects of two distinct dosages of *Echinacea purpurea* supplementation, which significantly ameliorated iron overload-induced bone marrow suppression.

Materials and Methods

Materials

Echinacea "Purpura tablets (The Jordanian Pharmaceutical Manufacturing Co./Jordan), Iron Dextran injections (LYKA LABA Limited/INDIA), and Vitamin E soft gelatin capsule (Adriengagnon/Canada).

Methods

Animals and treatment procedures

In the present study, fifty albino male rats aged six to eight weeks with a weight of (180 – 240) g were utilized; they were acquired and housed at the University of Baghdad's College of Pharmacy Experimental Animal House, Iraq. The animals were kept in cages made of plastic (every 5 animals per cage) under regulated light/dark cycle conditions (12 hours), the humidity (50 ± 5%), and temperature (23 ± 2 °C); in plastic cages with enough ventilation. permitted unlimited access to pellets and water. Before the trial began, the animals were acclimated for a week. Committees for Graduate Studies and Ethics at the University of Baghdad's College of Pharmacy approved the study protocol; the study approval" No. 349 on 13/2/2024.

Experimental design

Five equal groups of ten rats each would be created from the experimental animals:

- Group (I) normal control rats (negative control) take an injection of 0.2 ml of normal saline intraperitoneally (IP) every 3 days for 4 weeks, with daily oral gavage administration of 1 ml normal saline solution/rat/day for 4 weeks.
- Group (II) [The iron-overloaded group]: Rats would be IP injected with 200mg/kg for four weeks, take iron dextran every three days[14], with daily oral gavage administration of (1 ml) normal saline solution/rat/day for 4 weeks.
- Group (III) Rats would be IP injected with the iron dextran (200mg/kg every 3 days for 4 weeks) [14], and E.P. (100mg/kg/day) to be orally administered by oral gavage for 4 weeks [7].
- Group (IV) Rats would be IP injected with iron dextran (200mg/kg every 3 days for 4 weeks) [14], and E.P. (200mg/kg/day) to be orally administered to rats by oral gavage for 4 weeks [7].
- Groups (V) Rats would be IP injected with iron dextran (200mg/kg every 3 days for 4 weeks) [14], and an oil solution of vitamin E (200mg/kg/day) would be orally administered by rats' oral gavage for 4 weeks [15]. Twenty-four hours, the animals were killed, slaughtered, and blood drawn from each male rat's carotid artery in the neck after the treatment period to estimate hepcidin and ferritin serum levels. The spleen tissue was prepared for Nrf2 and Casp-3 protein expression, and the liver tissue homogenate was obtained to estimate the oxidative stress biomarkers, and the histological analysis was performed on the liver tissue.

Determination of hepcidin and ferritin serum levels.

The jugular vein blood was "gathered in non-heparinized tubes "specialized serum separator collection tube; gel tube", and allowed to coagulate at room temperature for ½ hour. The serum supernatant was then extracted by centrifuging it for 15 minutes at 4000 rpm. It was kept in an aliquot location at -20°C to be used for estimation of hepcidin and ferritin serum biomarkers by using an ELISA colorimetric assay kit from Cloud Clone Corp. (CCC) USA. For ferritin determination antibody that is specific to ferritin has already been applied to the microplate that comes with this kit. The matching microplate wells containing the standards or samples are then filled with ferritin-specific biotin-conjugated antibodies. The plates are incubated after Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well. Only the wells containing ferritin,

biotin-conjugated antibody, and enzyme-conjugated Avidin will change color when the TMB substrate solution is introduced. The enzyme-substrate reaction is stopped by adding sulfuric acid solution, and spectrophotometry at 450 nm ± 10 nm is used to detect the color shift. The ferritin concentration in the samples is then determined by comparing their optical density to the standard curve. Concerning the manufacturing procedure for hepcidin, the assay employs the competitive inhibition enzyme immunoassay technique, in which an antibody specific to hepcidin has been pre-coated" onto a microplate. A competitive inhibition reaction is launched between "biotin-labeled hepcidin and unlabeled hepcidin (Standards or samples) with the pre-coated antibody specific to hepcidin. The unbound conjugate is removed by washing after incubation. Then, horseradish was conjugated with avidin. Peroxidase (HRP) is added to each microplate well, and the wells are then incubated. There is an inverse relationship between the amount of bound HRP conjugate and the concentration of hepcidin in the sample. When the substrate solution is added, the amount of hepcidin in the sample is inversely related to the colour intensity" that develops when evaluated spectrophotometrically at 450 nm.

Determination of Nuclear factor erythroid 2-related factor 2 (Nrf2) and caspase-3 (Casp-3) protein level in the spleen tissue homogenate.

For the western blot analysis, weighing 50 g of spleen tissue allowed us to separate proteins from the samples.

"RIPA lysis buffer was added with phenylmethylsulfonyl fluoride PMSF and sodium orthovanadate Na3VO4 in the following ratio: (0.3 g spleen mL RIPA lysis buffer: 10 µL of both PMSF and Na3VO4". Thus, 50 g of spleen tissue should be combined with 333 µl of RIPA lysis buffer, 3.3 µl of PMSF, and Na3VO5. To guarantee that all of the protein has been eliminated from the tissue, spin the sample and collect the supernatant layer after fully homogenizing it. Utilize the BCA Protein Assay Kit (Elabscience, USA) to determine the protein content, and measure each sample's absorbance at 563 nm using the Mobi-apparatus (Bio-Rad Laboratories, USA), utilizing a regression equation to convert absorbance to concentration. Following protein concentration normalization and conversion to SDS-loading samples, 10 µL of the sample was added to each lane. The SDS PAGE gel was then used to separate the samples, and the gels were then placed onto PVDF membranes. The membrane was blocked with 5% milk, fat-free, for an hour at room temperature, and then it was incubated with antibodies against Nrf2, Casp-3, and beta-actin for the entire night at 4 °C. The membrane was then washed three times using TBST. Membranes were then rinsed three times with TBST after being incubated for one hour with coated anti-rabbit antibody. Lastly, the PVDF membrane was photographed using the "ChemiDoc MP Imaging System (Bio-Rad Laboratories, USA) and ECL-A and ECL-B detection reagents from Elabscience, USA, which are used to identify protein expression signals. Image-J (NIH) was used to quantify band intensities scanned by densitometry". When analyzing the samples, use beta actin as an internal control to estimate nuclear factor erythroid 2 related factor 2 (Nrf2) and caspase-3 (Casp-3) protein expression in the spleen tissue [16].

Determination of selected levels of oxidative stress (OS) parameters in the liver tissue homogenate.

Following the "scarification of each animal or group, each rat's liver was removed and cleaned using ice-cold PBS pH (7.0 +/- 0.05). The liver tissues were weighed and finely minced after any blood or debris had been removed. One gram of liver was put in a tube with nine ml of PBS (pH 7.0 ± 0.05) to make a 10% liver tissue homogenate. A homogenizer was used to homogenize the liver tissue on ice at 4 0C for 15 minutes at 1500×g (or 5000 rpm); the supernatant layer was then collected and stored at -20 °C after being centrifuged for 5 minutes"; then it was used to estimate malonaldehyde (MDA), superoxide dismutase 2 (SOD2), and glutathione (GSH) level in the liver tissue homogenate using their respective commercially available kits from Mybiosource (USA) for rats in compliance with the manufacturer's guidelines [16]; The pre-coated Ab plate was filled with the samples, standards, and blank, and it was incubated for one hour at 37 °C. The color turned blue once each parameter's The chromogenic substrate, enzyme conjugate, and particular Ab reagent were added. The reaction was then stopped, and the color changed to yellow by successively adding stop solutions. Next, "the optical density was measured at 450 nm". The liver tissue homogenate's MDA, SOD2, and GSH concentrations were expressed.

Histopathological examination

A small part of the liver tissues was obtained from every group of rats; histopathological assessment was performed following Junqueira et al. 1995[17]. After being cleaned with PBS, the tissues were dried and preserved for 72 hours in a 10% buffered formalin saline solution, then processed according to the paraffin embedding technique, and stained using the Eosin and Hematoxylin stain from Harries (18). "Light microscopy was used to study the tissue, and a Future Win Joe microscopic camera was used for microphotography" (Suad et al., 2018) [19].

Statistical analysis

"GraphPad Prism software (version 9.5.1) was used to present the study's results as Mean ± standard deviation (SD). The statistical significance between groups was evaluated using a one-way analysis of variance (ANOVA) test and Tukey's post-hoc test for multiple comparisons. The threshold for statistical significance was set at $P < 0.05$".

Results

The effects of Iron-overload, Echinacea purpurea, and Vitamin E on ferritin and hepcidin serum levels.

Table 1 demonstrates that the ferritin serum level increased significantly ($P<0.05$) in male rats given 200 mg/kg of iron dextran (IP) "Group II, in contrast to Group I; the negative control (44.09 ± 3.04 vs. 7.83 ± 0.78). When compared to Group II, the serum ferritin levels in Groups III, IV, and V were significantly lower ($P<0.05$) (20.31 ± 1.75, 14.05 ± 2.98, and 18.01 ± 1.51 vs. 44.09 ± 3.04), respectively. According to data analysis, the serum ferritin levels of Groups III, IV, and V were significantly higher ($P<0.05$) than those of Group I (20.31 ± 1.75, 14.05 ± 2.98, and 18.01 ± 1.51 vs. 7.83 ± 0.78), respectively. Groups III and IV demonstrate non-significant variations in serum levels ($P>0.05$) of ferritin as compared with each other (20.31 ± 1.75 vs.14.05 ± 2.98). Concerning hepcidin, Table 1 demonstrates a highly significant decrease ($P<0.05$) in hepcidin serum level in group II (induction group) compared to the negative control group (group I) (17.98±1.41 vs. 528.9±35.53) was observed. Group III's serum hepcidin level was elevated non-significantly ($P>0.05$) compared to Group II (67.48±27.45 vs. 17.98±1.41). In contrast, Groups IV and V showed a significant elevation in hepcidin serum level in comparison with the induction group (284.4±108.8 and 120.3±30.97 vs.17.98±1.41). Data analysis showed that Groups III, IV, and V have a significant reduction ($P<0.05$) in serum level of hepcidin compared to Group I (67.48±27.45, 284.4±108.8, and 120.3±30.97 vs. 528.9±35.53), respectively. Group III's serum hepcidin level was significantly lower ($P<0.05$)" as compared with group IV (67.48±27.45 vs. 284.4±108.8).

Table 1. The effect of various treatments on serum levels of Ferritin and Hepcidin in male rats.

Groups	Treatment	Serum Ferritin Levels (ng/ml)	Serum Hepcidin Levels (pg/ml)
Group I	Negative control	7.83 ± 0.78	528.9±35.53
Group II	Induction group (The Iron-Overload at a dose of 200mg/kg of Iron Dextran.)	44.09 ± 3.04*	17.98±1.41*
Group III	The Iron-Overload at a dose of 200mg/kg of Iron Dextran +100mg/kg of Echinacea	20.31 ± 1.75#*	67.48±27.45Ψ*
Group IV	The Iron-Overload at dose 200mg/kg of Iron Dextran +200mg/kg of Echinacea	14.05 ± 2.98#*	284.4±108.8#*
Group V	The Iron-Overload at a dose of 200mg/kg of Iron Dextran + 200 mg/kg of Vitamin E	18.01 ± 1.51#*	120.3±30.97#*

N "is the number of animals, and each value is the mean ± standard deviation (STD). * Differs significantly (P<0.05) from the negative control group. # Differs significantly (P<0.05) from the induction control group. Significantly different (P<0.05) from the g IV (200 mg/kg echinacea + 200 mg/kg iron dextran).

The effect of Iron Overload, Echinacea purpurea, and Vitamin E on the MDA level in liver tissue homogenate.

MDA results in Table 2 demonstrated a notable rise ($P<0.05$) in liver tissue homogenate MDA level in group II (induction group) as compared to the negative control group (group I) (3.57±0.54 vs. 2.17±0.83). At the same time, Group IV showed a significant reduction ($P<0.05$) in the level of MDA in the liver tissue homogenate compared to Group II (1.87±0.54 vs. 3.57±0.54). In contrast, Groups III and V showed a non-significant reduction in liver tissue homogenate MDA level as compared to group II (2.99±0.05 and 2.74±0.41 vs. 3.57±0.54). The results of the data analysis indicated that the levels of MDA in the liver tissue homogenate in Groups III, IV, and V were not significantly different from those in Group I (2.99±0.05, 1.87±0.54, and 2.74±0.41 vs. 2.17±0.83)". Liver tissue homogenate MDA levels in Groups III and IV differ from one another in non-significant ways ($P>0.05$) (2.99±0.05 vs. 1.87±0.54).

Table 2. Effects of various treatments on liver tissue homogenate levels of Malonaldehyde (MDA) in male rats.

Groups	Treatment	MDA Levels (nmol/ml)
Group I	Negative control	2.17±0.83
Group II	Induction group (The Iron-Overload at a dose of 200mg/kg of Iron Dextran.)	3.57±0.54*
Group III	The Iron-Overload at a dose of 200mg/kg of Iron Dextran +100mg/kg of Echinacea	2.99±0.05
Group IV	The Iron-Overload at dose 200mg/kg of Iron Dextran +200mg/kg of Echinacea	1.87±0.54#
Group V	The Iron-Overload at a dose of 200mg/kg of Iron Dextran+200mg/kg of Vitamin E	2.74±0.41

- N is the number of animals, and each value is the mean ± standard deviation (STD).
- *Differentially significant (P<0.05) from the negative control group.
- # Differs significantly (P<0.05) from the induction control group.

The effects of Iron-overload, Echinacea purpurea, and Vitamin E on levels of SOD2 and GSH in the liver tissue homogenate.

In this study, Table 3 "showed a significant reduction (*P<0.05*) in liver tissue homogenate SOD2 level in group II (induction group) as compared to the negative control group (group I) (80.35±4.86 vs. 118.9±7.946). At the same time, Groups III, IV, and V showed significant elevation (*P<0.05*) in the level of SOD2 liver tissue homogenate compared to Group II (102.6±12.15, 115.9±12.26, 112.7±10.28 and vs. 80.35±4.86) respectively. Data analysis showed that Groups III, IV, and V have a non-significant decrease (P>0.05) in the liver tissue homogenate concentration of SOD2 as compared to Group I (102.6±12.15, 115.9±12.26, and 112.7±10.28 vs. 118.9±7.95) respectively. Groups III and IV show non-significant differences (*P>0.05*) in the level of SOD2 liver tissue homogenate as compared with each other (102.6±12.15 vs. 115.9±12.26). Additionally, in this study, there was a significant reduction (*P<0.05*) in liver tissue homogenate GSH level in group II (induction group) as compared to the negative control group (group I) (166.6±5.37 vs. 254.1±53.4). Table (3) shows that Groups III and V have a non-significant elevation (*P>0.05*) in the level of GSH liver tissue homogenate compared to Group II (189.6±5.76 and 227.7±46.17 vs.166.6±5.37) in contrast, Group IV showed a significant increase in GSH level in comparison to Group II (244.1±57.41 vs. 166.6±5.37). Data analysis showed that Groups III, IV, and V have a non-significant reduction (*P>0.05*) in liver tissue homogenate GSH level compared to Group I (189.6±5.76, 244.1±57.41, and 227.7±46.17 vs. 254.1±53.4), respectively. Groups III and IV show non-significant differences (*P>0.05*) in the levels of GSH liver tissue homogenate as compared with each other (189.6±5.76 vs. 244.1±57.41)".

Table 3. Effects of various treatments on the levels of superoxide dismutase2 (SOD2) and Glutathione (GSH) in the liver tissue homogenate.

Groups	Treatment	SOD2 Levels (ng/ml)	GSH Levels (µg/ml)
Group I	Negative control	118.9±7.95	254.1±53.4
Group II	Induction group (The Iron-Overload at a dose of 200mg/kg of Iron Dextran.)	80.35±4.86*	166.6±5.37*
Group III	The Iron-Overload at a dose of 200mg/kg of Iron Dextran +100mg/kg of Echinacea	102.6±12.15#	189.6±5.76
Group IV	The Iron-Overload at dose 200mg/kg of Iron Dextran +200mg/kg of Echinacea	115.9±12.26#	244.1±57.4#
Group V	The Iron-Overload at a dose of 200mg/kg of Iron Dextran+200mg/kg of Vitamin E	112.7±10.28#	227.7±46.17

Each value represents mean ± standard Deviation (STD), N = number of animals. *Significantly different (*P*<0.05) with respect to the negative control group. # Significantly different (*P*<0.05) with respect to the induction control group.

The Effects of Iron-overload, Echinacea purpurea, and Vitamin E on the levels of Nuclear factor erythroid 2-related factor 2 (Nrf2) and Caspase-3 protein in the spleen tissue homogenate.

The relative protein levels of "Nrf2 and Caspase were measured using western blotting analysis; Figure. 1A, 1B exhibited that the Iron overload induction group exposed a non-significant (*P*>0.05) elevation in the spleen

tissue homogenate Nrf2 protein level; the elevation was (1.38±0.47) folds of the control. The administration of 200mg Echinacea Purpurea concomitantly with 200mg/kg Iron Dextran (Group IV) significantly (*P*<0.05) elevated the Nrf2 protein level of the spleen tissue homogenate by (3.84±0.29) folds of the control; in addition concomitant administration of 200mg Vitamin E with 200mg Iron in Group V showed a significant increase in spleen tissue homogenate Nrf2 protein level by (3.49±0.03) folds of the control; in contrast the administration of 100mg Echinacea Purpurea concomitantly with 200mg/kg Iron Dextran (Group III) showed non-significant elevation (*P*>0.05) in the Nrf2 protein level of the spleen tissue homogenate (1.87±0.03) folds of the control. The spleen tissue's homogenate caspase-3 protein level was significantly higher (*P<0.05*) in the iron overload induction group gII compared to the control group (7.06±0.12); Figure. 1C, 1D showed a non-significant increase in the casp-3 protein level of the spleen tissue homogenate in groups III, IV, and V; the group elevated by (3.9±1.24, 2.11±0.99, and 2.46±0.62)" folds of the control.

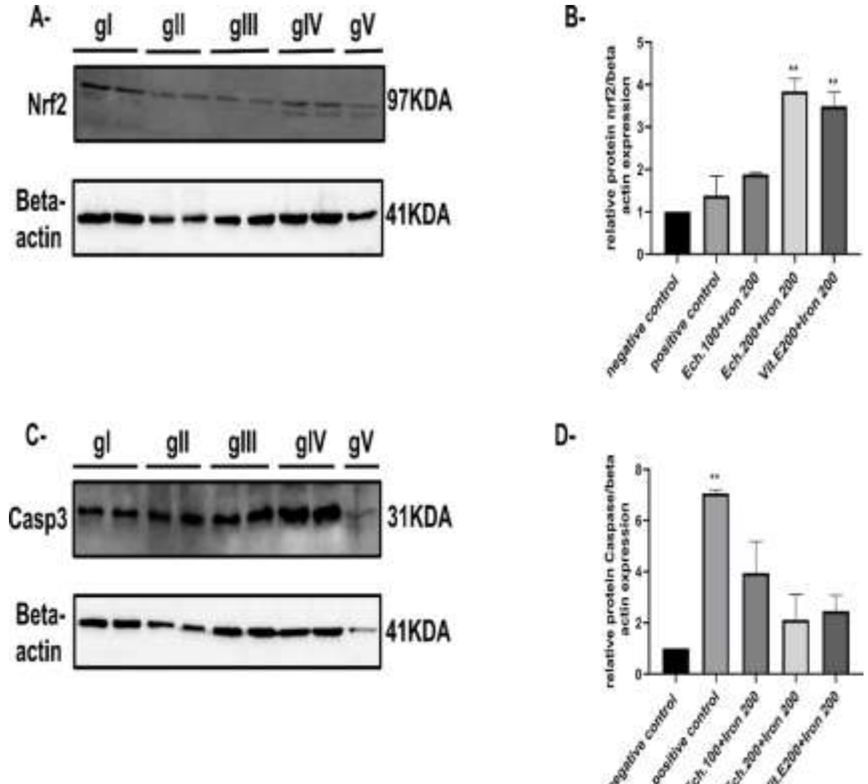

Figure 1. "Effect of Iron Dextran overload induces bone marrow suppression in spleen tissue on the Nrf2 and Levels of Casp-3 proteins in Wister rats; A) Illustration of the western blot bands of the proteins Nrf2 and beta-actin in spleen tissues. B) Relative protein expression of Nrf2. C) Representative western blot bands image of Casp-3 and beta-actin protein in spleen tissues. D) Relative protein expression of Casp-3. The data is presented as (mean of fold change ± SD) in relation to the control; (n=4). ** (*P<0.01*) vs. control group".

Changes in histopathology

Histopathological analysis of the liver tissue in group I using H&E-stained transverse sections showed a normal central vein along with minor nuclear pyknosis and zonal cellular enlargement of the hepatocytes (Figure. 2A, 2 B). Male rats IP-injected with 200 mg/kg iron dextran (induction group) showed histological alterations in their livers, which included mild hepatic hemosiderosis and a normal - looking central vein , normal arrangement of hepatic cords with mild proliferation of sinusoidal macrophages laden hemosiderin (Figure. 2C,2D). The histological examination of the liver section of rats in groups III and IV showed that hepatic lobules revealed a normal appearance of the central vein, a normal arrangement of hepatic cords with normal hepatocytes and sinusoids (Figure .2E,2F); Rats in group V's liver sections underwent histological analysis, which demonstrated that the central vein

seemed normal in the hepatic lobules, normally arrangement of hepatic cords, mild vascular degeneration without necrosis and the hepatic sinusoids revealed hemosiderin laden macrophages (Figure. 2G,2H).

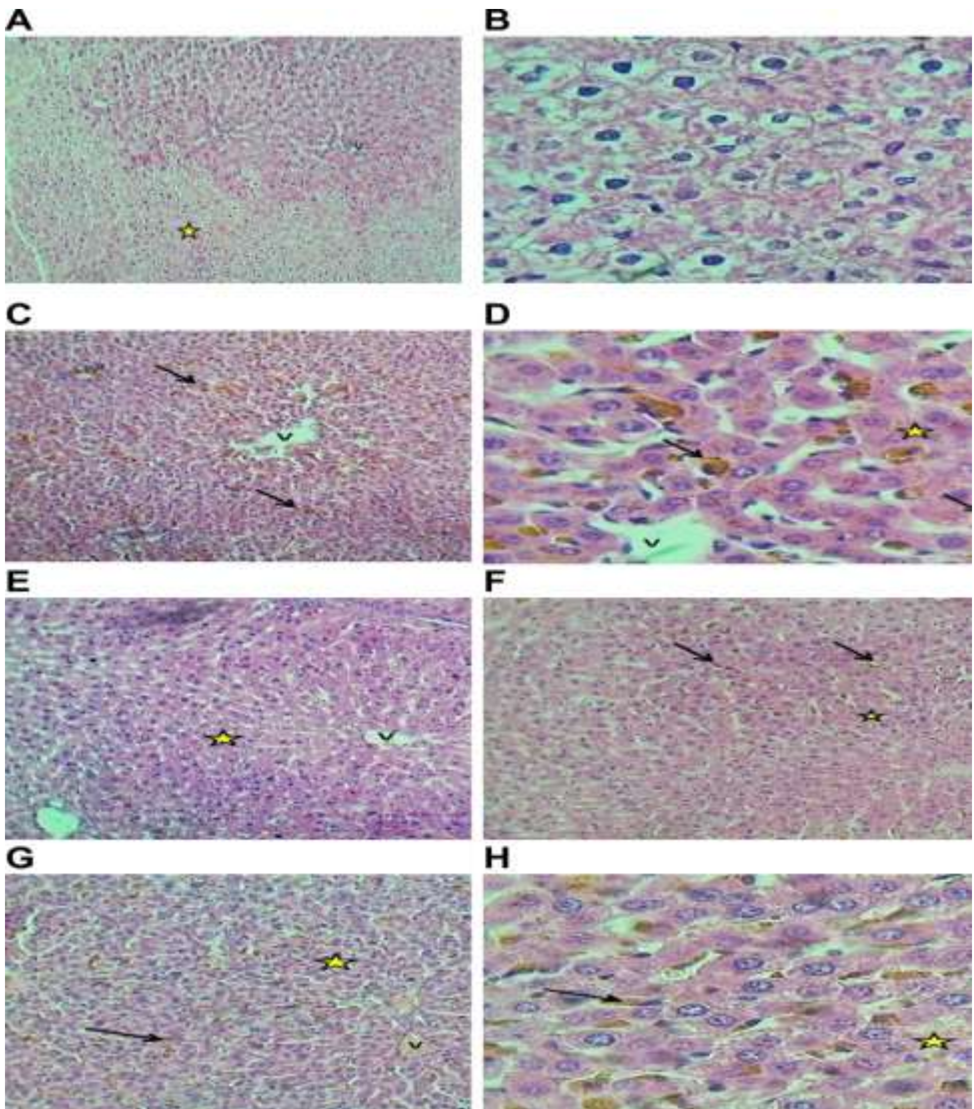

Figure 2. "Rat liver section stained with hematoxylin and eosin and seen under a high-power light microscope: (A) section of the hepatic lobule (Group-I) shows normal central vein (V), with mild cellular swelling (Asterisk), 100x. (B) The hepatic lobule section (Group-I) exhibits nuclear pyknosis and moderate hepatocyte cellular edema, 400x. (C) section of hepatic lobule (Group-II) shows: mild hepatic hemosiderosis that revealed normal central vein (V), normal arrangement of hepatic cords (Asterisk) with mild proliferation of macrophages laden with hemosiderin (Arrows), 100x. (D) section of hepatic lobule (Group-II) shows: a normal central vein (V), a normal arrangement of hepatic cords with normal sinusoids (Asterisk), there was a mild proliferation of macrophages-laden hemosiderin, this is in the iron-overload treated group (Arrows), 400x. (E) represents the section of hepatic lobule (Group-III) shows: normal central vein (V), normal arrangement of hepatic cords, this is in a low dose of *Echinacea purpura* concomitant treated group (Asterisk), 100x. (F) represents the section of hepatic lobule (Group-IV) shows: normal central vein (V), normal arrangement of hepatic cords (Asterisk) with mild hemosiderin-laden macrophages, this is in a high dose of *Echinacea purpura* concomitant treated group (Arrows), 100x. (G) section of hepatic lobule (Group-V) shows: normal central vein (V), normal arrangement of hepatic cords (Asterisk) with mild hemosiderin-laden macrophages (Arrows), 100x. (H) section of hepatic lobule (Group-V) shows: mild vascular degeneration of hepatocytes (Asterisk) with marked sinusoidal hemosiderin-laden macrophages cords, this is in the vitamin E concomitant treated group (Arrows), 400x".

Discussion

Chronic blood transfusions without routine iron burden monitoring or iron-induced organ damage result in iron overload, which ultimately causes end-organ damage and high morbidity and death[20][21]. Commercially accessible synthetic ion-chelating drugs, such as deferoxamine, deferasirox, and deferiprone, work involves constructing complexes that eliminate extra iron from the bloodstream through feces or urine [22]. "Despite the fact that these compounds are helpful in treating IOL syndrome, their use is limited because of a number of issues, chief among them being their short plasma half-life and low oral bioavailability. They are also unable to prevent iron-mediated oxygen reduction[1]. This study evaluates the effects of two different doses of *Echinacea purpurea* therapy on bone marrow suppression brought on by iron excess. Bioactive substances; naturally occurring flavonoids have antioxidant qualities and can prevent cell damage [23]; In place of traditional iron chelators, *Echinacea purpurea* can trap free radicals and could be utilized as an iron-removable treatment [6]. A common serum test for assessing the body's overall iron stores is serum ferritin; the blood level of ferritin, a protein that stores iron, indicates iron storage; an increased ferritin level indicates a danger of iron overload [24]. It has a role in diagnosing and managing iron overload and iron deficiency [1]. Serum ferritin levels in a group that received a lot of Echinacea purpurea treatment in this study were significantly lower. This is supported by additional research, which found that the vitamin E-treated group's serum ferritin levels significantly decreased[25]. Mutations in hemojuvelin, transferrin receptor 2, HFE, and the hepcidin gene itself have all been connected to hepcidin deficiency and hereditary hemochromatosis. It was repressed in patients with thalassemia syndromes and congenital dyserythropoietic anemia type 1, but it was undetectable in patients with juvenile hemochromatosis who had HAMP mutations. By binding to ferroportin, the iron export channel, it causes internalization and destruction. Hepcidin prevents cellular iron efflux [26]. In this study, the hepcidin level was significantly elevated in the high-dose *Echinacea purpurea-treated* group (g IV); similarly, in the vitamin E-treated group. Oxidative stress is the result of an imbalance between the production of free radicals and the antioxidant system, which maintains the body's equilibrium [27]. By triggering inflammation, secreting proteases, and producing copious amounts of oxidative chemicals, oxidative stress can cause harm [1][28]. Because electrons escape from the electron transport chain and produce superoxide anion (O2 •−), mitochondria are the location of ROS generation [29]; SOD2 and GSH are two examples of antioxidant enzyme systems that catalyze processes to counteract ROS and free radicals. The last byproduct of the peroxidation between lipids and free radicals, MDA, can serve as a direct indicator of the level of lipid oxidation [1]. In contrast to the *Echinacea purpurea*-treated groups, iron overload raised the MDA concentrations in the induction group and reduced the antioxidant ability of the liver tissues. The Fenton reaction can be started or catalyzed by iron, which is a key factor in the creation of ROS, which can kill liver cells. At the same time, oxidative damage and lipid peroxidation will be made worse by ROS generated by too much iron [30][31]. In this study, *Echinacea purpurea* showed significant elevation in both SOD2 and GSH levels as compared with the induction group. Reactive oxygen species (ROS) accumulation promotes cellular damage and contributes to various complications in development and progression. It can directly damage lipids, proteins, or DNA and modulate intracellular signaling pathways, producing permanent oxidative alterations by altering the expression of proteins[27]. Ferroptosis; Iron buildup and lipid peroxidation are two biological characteristics linked to oxidative stress-induced cell death. According to recent research, ferroptosis is closely linked to the pathophysiological processes of numerous illnesses, including organ damage, degenerative diseases (such as ischemic organ damage, liver and lung fibrosis, and neurodegeneration), and several cancer types [32]. Numerous ferroptosis-related genes, including those of iron control (heme synthesis, hemoglobin catabolism, iron storage, and iron export), are Nrf2 target genes. Ferroptosis and the Nrf2 pathway have been linked in studies [31]. The results of this investigation were consistent with those of others; where the protein expression level of Nrf2 showed a significant (P<0.05) elevation in Group IV in the spleen tissue compared to the control group. Iron-induced ROS generation is a crucial mechanistic step in apoptotic cell death; immunofluorescent colocalization of cytochrome C indicates that iron promotes the release of cytochrome c from depolarized mitochondria; this, in turn, increases apoptosis, as indicated by the level of caspase-3 protein expression [33]. As compared with vitamin E were several studies have shown that vitamin E has efficient anti-oxidant biological activity during the peroxidation of unsaturated lipids [34]. Natural antioxidants, which are involved in the antioxidant defence system and oxidative stress, reduced iron-induced cell death. In this study, the plant's antioxidant action is attributed to its phenolic components and cichoric acid content. It was

proposed that, because of its capacity to scavenge peroxyl radicals, *Echinacea purpurea* may function as a potent antioxidant that breaks chains in biological systems [(2)]. In this investigation, there was a non-significant" rise in the Casp-3 protein level of the spleen tissue in groups III, IV, and V relative to the control group; this indicates that there was an anti-apoptotic effect detected from the *Echinacea purpurea* and vitamin E concomitant use.

Conclusion

Echinacea purpurea high-dose concomitant treatment restored serum parameters and liver antioxidants; it resulted in a considerable reduction of the ferritin level with an elevation in hepcidin concentration; in addition to amelioration of antioxidant protective mechanisms to minimize iron-overload-induced oxidative stress *in vivo* as compared with the vitamin E-treated group. Furthermore, *Echinacea purpurea* treatment prevents the apoptosis of bone marrow and spleen cells

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Conflicts of Interest

No conflicts of interest are disclosed by the writers.

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Ethics Statements

The University of Baghdad's College of Pharmacy's Ethics Committee gave its approval to the study (acceptance number 349 on 13/2/2024).

Author Contribution

Equally contributed.

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تقييم التأثير الوقائي للقنفذية الارجوانية على تثبيط نقي العظم الناجم من جرع الحديد الزائدة على ذكور الجرذان بالمقارنة مع فيتامين ه

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الخلاصة

يؤدي ارتفاع نسبة الحديد إلى موت الخلايا وتلف العديد من العضيات الحيوية وذلك بسبب توليد أنواع مختلفة من جذور الأوكسجين الحرة، وخاصة الجذور الهيدروكسيلية الفعالة. القنفذية الارجوانية هي نبات طبي يحتوي على العديد من المركبات الكيميائية تستخلص من جذور النبات والتي تمتاز بفعالياتها البيولوجية المتنوعة. كان الهدف من هذه الدراسة هو تقييم التأثير الوقائي للقنفذية الارجوانية على تثبيط نخاع العظم مع الموت المبرمج لخلايا الكبد والطحال الناجم من زيادة نسبة الحديد داخل الجسم. تم تقسيم خمسين جرذ إلى خمس مجموعات تحتوي كل مجموعة على عشر جرذان: المجموعة (الأولى) [التحكم السلبي] تم حقن الجرذان بحقنة داخل الصفاق بـ (٠,٢ مل) من محلول ملحي كل ٣ أيام؛ مع حقنة فموية يومية بـ (١ مل) من محلول ملحي لمدة ٤ أسابيع. المجموعة (الثانية) [التحكم الايجابي]: تم حقن الجرذان داخل الصفاق بـ (٢٠٠ مجم/كجم)من دكستران الحديد كل ٣ أيام)؛ مع حقنة فموية يومية بـ (١ مل) من محلول ملحي لمدة ٤ أسابيع. المجموعة (الثالثة) تم حقن الجرذان داخل الصفاق بـ (٢٠٠مجم/كجم) من دكستران الحديد كل ٣ أيام؛ مع القنفذية الارجوانية (١٠٠ مجم/كجم/يوم) عن طريق الفم لمدة ٤ أسابيع.المجموعة (الرابعة) تم حقن الجرذان داخل الصفاق بـ (٢٠٠ مجم/كجم) من دكستران الحديد كل ٣ أيام؛ مع القنفذية الارجوانية (٢٠٠مجم/كجم/يوم) عن طريق الفم لمدة ٤ أسابيع. المجموعة(الخامسة) تم حقن الجرذان داخل الصفاق بجرعة (٢٠٠ مجم/كجم) كل ٣ أيام دكستران الحديد ومحلول زيت فيتامين هـ (٢٠٠ مجم/كجم/يوم) عن طريق الفم لمدة ٤ أسابيع. بعد أربع وعشرين ساعة من انتهاء مدة العلاج، تم ذبح الحيوانات للحصول على الدم من الشريان السباتي في عنق كل جرذ لاستخدامه في تقييم مستويات الفيريتين والهبسيدين في مصل الدم؛ تم استخدام متجانس أنسجة الكبد لتقييم نسبة المؤشرات الحيوية كحمض المالونالدهيد، السوبر أكسيد ديسميوتاز٢، والجلوتاثيون بالإضافة الى الفحص النسيجي للكبد؛ تم استخدام متجانس أنسجة الطحال لتقييم نسبة بروتين عامل النسج المرتبط بالاريثرويد ٢ وبروتين الكاسبيز ٣. أظهرت القنفذية الارجوانية بجرعة ٢٠٠ مجم ارتفاعًا ملحوظًا في مستويات الهيبسيدين في المصل ومضاد الأكسدة سوبر أكسيد ديسميوتاز ٢، والجلوتاثيون و عامل النسج المرتبط بالاريثرويد ٢؛ مع انخفاض ملحوظ في مستويات الفيريتين والمالونديالدهيد وارتفاع غير ملحوظ في مستوى بروتين الكاسبيز ٣.

الكلمات المفتاحية: مضاد للأكسدة، تثبيط نخاع العظم، القنفذية الارجوانية ، زيادة الحديد، مالونالدهيد.