Evaluation of The Protective Effect of Echinacea Purpurea on Iron Overload-Induced Bone Marrow Suppression in Male Rats: In Comparison with Vitamin E

By:

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طالبة دكتوراه

فرع الأدوية والسموم

- Iron is an essential trace element in the human body.
- It is a vital component of:

-haemoglobin and myoglobin.

-enzymes involved in cell respiration like cytochromes, cytochrome oxidase, and catalase (CAT).

- enzymes play a crucial role in vital processes like electron transport, redox reactions, and cell discrepancy and development.

- Iron overload contribute to cellular damage and change the integrity of essential organelles; By producing reactive oxygen species, primarily reactive hydroxyl radicals.

- excess free iron can produce reactive oxygen species (ROS), primarily reactive hydroxyl radicals, which can possibly cause cellular damage and change the integrity of essential organelles

- Too much iron interferes with iron hemostasis and excessive free iron accumulation in parenchymal organs, such as the spleen, which serves as a phagocytic filter by eliminating damaged and senescent cells.
- 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.

- Echinacea Purpurea (E.P.), the purple coneflower, a medicinal plant that is a member of the Asteraceae family.

- The genus Echinacea has nine different species, Echinacea purpurea is commonly employed for medicinal purposes.

- The most widely utilized preparation is a liquid extract derived from E. purpurea roots.

- 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.

- It possesses antibacterial, anti-inflammatory, psychotropic, immunomodulatory, mutagenic, and anticancer properties.

- Echinacea purpurea's pharmacological actions are caused by its main ingredients, which include polysaccharides, alkamides, and derivatives of caffeic acid.

- In the treatment of a variety of illnesses, flavonoids can be essential as antioxidant, antibacterial, anti-allergic, anti-mutagenic, and anti-inflammatory agents, as demonstrated by several clinical trials as well as various in-vitro and in-vivo studies.

Materials and Methods

- Fifty albino male rats aged six-eight weeks with weight of (180 – 240) g were utilized, divided into fife groups:
- Group (I) normal control rats (negative control) take an intraperitoneal (IP) injection of (0.2 ml) normal saline 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 (200 mg/kg/day iron dextran every 3 days for 4 weeks); 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 (200 mg/kg/day every 3 days for 4 weeks); and E.P. (100mg/kg/day) to be orally administered by rats oral gavage for 4 weeks.

- Group (IV) Rats would be IP injected with iron dextran (200 mg/kg/day every 3 days for 4 weeks), and E.P. (200mg/kg/day) to be orally administered by rats' oral gavage for 4 weeks.

- Groups (V) Rats would be IP injected with iron dextran (200 mg/kg/day every 3 days for 4 weeks), and an oil solution of vitamin E (200mg/kg/day) would be orally-administered by rats' oral gavage for 4 weeks.

- Twenty-four hours after the end of the treatment duration.
- The animals euthanized, sacrificed and blood obtained from the carotid artery in the neck of each male rat.
- The jugular vein blood gathered in non-heparinized tubes; kept at room temp to clot For ½ hr.
- Centrifuged for 15 minutes at 4000 rpm to obtain serum supernatant.
- blood collected in an EDTA tube for blood film preparation to detect neutrophil, eosinophil, monocyte, and lymphocyte cell count.

- A 1g of liver tissue placed in 10 ml tube of phosphate buffer saline solution (pH 7.4).

- The tissue homogenized to produce a 10% tissue homogenate.

- centrifuged for 15 minutes at 1500×g (or 5000 revolutions per minute (rpm)) at 4 0C.

- A piece of the spleen tissue preserved in RIPA buffer for the examination of oxidative and proapoptotic parameters.

- Including protein expression quantitatively; using western blot technique for Nrf2 and Casp-3 proteins.

- A small piece of the spleen tissue from each animal, about 50 mg placed in an appropriate volume of TRIZOL and kept frozen at −20 °C for later quantification of mRNA expression.

- Serum supernatant utilized to estimate Hepcidin, ferritin, LDH, and AST biomarkers.

- Fresh blood in EDTA tube utilized for CBC.

- The live tissue homogenate supernatant used to estimate MDA, SOD2, and GSH using their respective commercially available kits for rats in compliance with the manufacturer's guidelines.

- Spleen tissue homogenate used to estimate Nrf2 and Casp-3 proteins.

- Spleen tissue homogenate used to estimate Sirt3, Foxo1, AKT1, TNF-alpha, Interleukin 1beta, and NF-κB using RT-qPCR.

- Liver tissue, spleen tissue, and bone marrow samples used for histopathological examination.

Results:

- Echinacea purpurea showed anti-oxidant activity noticed by the increase in Nrf2 protein level and Sirt-3 mRNA expression in spleen tissue and elevation in liver tissue SOD2 and GSH levels.
- Echinacea purpurea minimizes Ferritin, LDH, and AST serum levels and MDA live tissue level with considerable elevation in hepcidin serum level (reduce liver injury).
- Echinacea purpurea showed an anti-apoptotic effect by reducing Casp-3 protein level and Akt-1, Foxo-1 mRNA expression.

Results:

- Echinacea purpurea showed an anti-inflammatory effect noticed from the significant reduction in NFκB, TNF-α, and IL-1β mRNA expression.

- Echinacea purpurea showed a manifest immunomodulatory effect noticed by the significant reduction in neutrophil and monocyte cell count and a significant elevation in eosinophil, and lymphocyte cell count.

Conclusion:

- E.P. concomitant treatments restored serum parameters and liver antioxidants.
- Amelioration of antioxidant protective mechanisms to minimize iron-overload-induced oxidative stress in vivo.
- E.P. treatment prevents the apoptosis of bone marrow and spleen cells.
- Potentiate anti-inflammatory and immunomodulatory effects.

Thank You