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Histopathological Effects of Excess Dietary Iron on the Liver, Spleen, Kidney, and Cardiac Tissues of Rats

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ABSTRACT

Background: *Iron is an essential micronutrient that numerous physiological processes require. However, iron overload can cause tissue damage through iron accumulation and oxidative stress.* ***The study aimed*** *to investigate the histopathological changes in the liver, spleen, kidney and cardiac tissues of rats caused by excess dietary iron.* ***Methods:*** *Thirty-five adult male albino rats were randomly divided into five groups (n=7). The control group was fed a standard diet and the experimental groups were fed diets supplemented with 40, 80, 120 and 160 mg iron/kg feed for 28 days. Blood samples were collected at the end of the experimental period for hematological analysis and tissue specimens from liver, spleen, kidneys and heart were examined histologically with Hematoxylin and Eosin staining.* ***The results:*** *Our study showed dose-dependent increase in the concentration of hemoglobin (Hb) and packed cell volume (PCV) values in groups treated with iron as compared to the control group. Histopathological studies revealed progressive deposition of hemosiderin, cellular degeneration, congestion and focal necrosis in hepatic tissues particularly at higher concentrations of iron. Similar changes occurred in splenic tissue, with increased hemosiderin deposition and loss of normal splenic architecture. Kidney and cardiac tissues showed comparatively milder lesions such as tubular degeneration, inflammatory infiltration, myocardial degeneration and vascular congestion.* ***Conclusion****, despite improvement of hematological parameters, excessive dietary iron supplementation induced significant histopathological alterations in several vital organs. The liver and spleen were the most affected organs, suggesting their important role in iron storage and metabolism. These findings emphasize the importance of keeping iron intake within dietary recommendations to avoid the adverse effects of iron overload.*

Keywords: Iron overload, Spleen, Kidney, Heart, Rats, Hemosiderin deposition.

Article Information

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1. INTRODUCTION

The iron participates in a variety of physiological functions. It is involved in oxygen transport, cellular respiration, DNA synthesis and several enzymatic reactions necessary to sustain normal metabolic functions [1]. This mineral is a part of hemoglobin and other iron-containing proteins [2] and is vital for tissue oxygenation, energy production and cell growth. Dysregulation of iron homeostasis is thus of great significance for general health and biological performance. The regulation of iron metabolism is a tightly controlled process balancing the absorption, storage, utilization, and recycling [3]. In normal physiological conditions, iron absorption from the gastrointestinal tract is regulated according to the body needs and then transported through the bloodstream to the various tissues. Most excess iron is stored in intracellular ferritin protein, a protective reservoir that prevents the accumulation of free iron ions [4]. However, when iron intake exceeds the storage and regulatory capacity of the body, excess amounts may accumulate within tissues causing cellular and organ damage. Iron deficiency and iron-deficiency

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anemia are major public health problems worldwide.

During the past decades, iron supplementation and food fortification programs have been widely implemented as strategies to reduce their global burden [5]. These interventions have been effective in improving hematological status in many populations and have contributed to better public health outcomes, However, increasing attention has been paid to the possible consequences of excessive exposure to iron. Iron differs from many nutrients in that there is no specific physiological mechanism for its excretion, thereby increasing the chance for progressive accumulation with prolonged excessive intake [6]. Excessive iron deposition is known to promote formation of reactive oxygen species by oxidation-reduction reactions. These highly reactive molecules can damage membrane lipids, structural proteins, and nucleic acids, and thus affect cellular integrity [7]. The oxidative stress resulting from this has been implicated in the pathogenesis of many different organ system disorders. There is experimental and clinical evidence that iron overload may lead to inflammatory responses, tissue degeneration, fibrosis and functional impairment in several organs [8].The liver is an organ in which iron-induced injury is a concern due to its central role as the primary site of iron storage and metabolism. Increased hepatic iron concentrations have been associated with cellular degeneration, hepatocellular necrosis, chronic inflammation and fibrotic changes [9].

Likewise, the spleen is a major site of erythrocyte turnover and iron recycling, and thus another major site of iron accumulation. Increased iron in splenic tissues may disrupt normal splenic structure and may affect physiological functions of the spleen in blood filtering and immune regulation.Iron overload may also affect the kidneys because of their function in filtration and maintenance of systemic homeostasis. Oxidative stress caused by excess iron can damage the cellular structures of the kidney and result in pathological changes in the renal tissues. Likewise, cardiac tissues are highly susceptible to oxidative damage by iron because of their high metabolic demand and continuous functional activity . Iron accumulation in the myocardium has been associated with structural changes, impaired contractility and changes in cardiovascular function [10].

Animal models are useful for the study of biological consequences of nutritional imbalances and toxicological exposures. Rats are commonly used in biomedical research due to their physiological similarity to humans and the ability to expose them to controlled experimental conditions. Histopathological examination of rat organs provides a detailed description of microscopic tissue changes caused by dietary interventions and provides valuable information on mechanisms of toxicity and organ specific responses.Therefore, the present study was carried out to assess the histopathological effects of excessive dietary iron on some vital organs in rat such as liver, spleen, kidneys and heart. Studies of tissue changes in iron overload may teach us more about iron toxicity and its effects on the health of humans and animals.

2: MATERIALS AND METHODS

2.1: Experimental Animals

The present study was carried out on thirty five healthy adult male albino rats of Rattus norvegicus species weighing between 180-220 g and 8-10 weeks old. Animals were obtained from the Animal House of the College of Science and were kept under standard laboratory conditions (temperature 22 ± 2°C, relative humidity 50–60%, and 12 h light/12 h dark cycle).

The rats were provided free access to food and drinking water during the period of

experiment. Before the experiment all animals were acclimatized for 1 week under the same environmental conditions[11].

2.2: Experimental Design

The rats were randomly assigned to five groups of seven animals each. Group I was the control and was fed a normal laboratory diet without iron supplement. Groups II, III, IV and V were fed diets supplemented with ferrous sulphate at 40, 80, 120 and 160 mg iron /kg feed respectively. The iron source was ferrous sulfate, which was chosen because of its high bioavailability and frequent use in food fortification studies [12]. The feeding period was 28 consecutive days.

2.3: Sample Collection

Animals were fasted overnight and anesthetized prior to sample collection at the end of the treatment period. Blood samples for hematological analysis were obtained by cardiac puncture. The rats were killed after the blood had been obtained and samples of tissue from the liver, spleen, kidneys and heart were carefully dissected out and washed with physiological saline solution to remove any blood residue.

2.4:Histopathological Examination

The collected tissues were fixed in 10% neutral buffered formalin for 48 h. The specimens were then dehydrated in an ascending ethanol series, cleared in xylene and embedded in paraffin wax. Sections of tissue, 5 μm thick, were cut with a rotary microtome and put on clean glass slides. The sections were stained with Hematoxylin and Eosin (H&E) and examined microscopically for histopathological changes such as cellular degeneration, necrosis, infiltration of inflammatory cells, vascular congestion and hemosiderin accumulation.[11].

2.5:Statistical Analysis

The obtained data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS software version 26. Differences among groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Values of $P < 0.05$ were considered statistically significant [12].

3: RESULTS AND DISCUSSION

The present study was designed to investigate the histopathological effects of excessive dietary iron supplementation on liver, spleen, kidney and cardiac tissues of rats. The results showed that the increase in dietary iron concentrations resulted in dose-dependent changes in both hematological parameters and tissue morphology. As shown in **Table 1 and Figure 2**, values of hemoglobin (Hb) concentration and packed cell volume (PCV) increased progressively with increasing dietary iron level. The highest values were found in group IV, which received 160 mg/kg of dietary iron. These results suggest increased erythropoiesis and increased iron availability for hemoglobin synthesis. The hematological parameters improved after iron supplementation, however, the excess iron intake was associated with significant pathological alterations in several organs. Histopathological examination showed the liver as the most affected organ.

Table 2 summarizes and **Figure 1** illustrates progressive hemosiderin deposition, hepatocellular degeneration, congestion and focal necrosis in liver sections of iron-treated rats. The severity of these lesions was increased with increasing iron concentration indicating excessive accumulation of iron within the hepatic tissues. The liver is considered the main storage of iron and is thus especially vulnerable to iron-induced oxidative damage. Splenic tissues showed similar marked pathological changes.

As shown in **Table 2 and Figure 1**, higher dietary iron levels increased hemosiderin accumulation in splenic macrophages, sinusoidal congestion and white pulp architectural changes. Taken together, our results show the critical role of the spleen in

iron recycling and storage and indicate that excess iron intake can be harmful to the normal splenic architecture and function.The lesions in the kidneys were relatively milder than those of the liver and spleen. As shown in **Table 3 and Figure 1**, tubular degeneration, glomerular congestion and mild infiltration of inflammatory cells were mainly observed in the groups exposed to higher concentrations of iron. These results indicate that prolonged iron overload may be harmful to renal tissues through oxidative stress mechanisms.Cardiac tissues also showed mild pathological changes. Myocardial degeneration and vascular congestion were observed in rats fed high levels of dietary iron **(Table 3; Figure 1)**. The cardiac lesions were less pronounced than those in the hepatic and splenic tissues, but suggest that excessive iron exposure may contribute to structural changes in the myocardium.The present study showed that, in general, histopathological changes are induced in several vital organs by excessive dietary iron supplementation in a dose-dependent manner. Iron supplementation improved hematological indices, but excess iron accumulation caused adverse tissue effects particularly in liver and spleen. These findings highlight the importance of maintaining dietary iron intake within the recommended limits to prevent the harmful effects of iron overload.[13-17].

Table 1. Experimental Design and Hematological Parameters of Rats Exposed to Dietary Iron.

Groups	No. of Rats	Iron Concentration (mg/kg feed)	Hb (g/dL) Mean ± SD	PCV (%) Mean ± SD
Control	7	0	10.2 ± 0.4	31.4 ± 1.2
Group I	7	40	11.5 ± 0.5	34.8 ± 1.3
Group II	7	80	12.7 ± 0.6	38.2 ± 1.5
Group III	7	120	13.9 ± 0.7	42.6 ± 1.8
Group IV	7	160	15.1 ± 0.8	46.9 ± 2.0

Table 2. Histopathological Changes in Liver and Spleen Tissues of Experimental Rats.

Groups	Liver Hemosiderin Deposition	Hepatocellular Degeneration	Necrosis	Splenic Hemosiderin Deposition
Control	-	-	-	-
Group I	+	+	-	+
Group II	++	++	+	++
Group III	+++	+++	++	+++
Group IV	++++	++++	+++	++++

Table 3. Histopathological Changes in Kidney and Cardiac Tissues of Experimental Rats.

Groups	Tubular Degeneration	Glomerular Congestion	Inflammatory Infiltration	Myocardial Degeneration
Control	-	-	-	-
Group I	-	+	-	-
Group II	+	+	+	+
Group III	++	++	+	+
Group IV	+++	++	++	++

Figure 1. Histopathological effects of excessive dietary iron on the liver, spleen, kidney, and cardiac tissues of rats. *Photomicrographs show normal hepatic architecture in the control group and pathological alterations including hemosiderin deposition, cellular degeneration, congestion, inflammatory infiltration, and necrosis in iron-treated animals (H&E stain, ×400).*

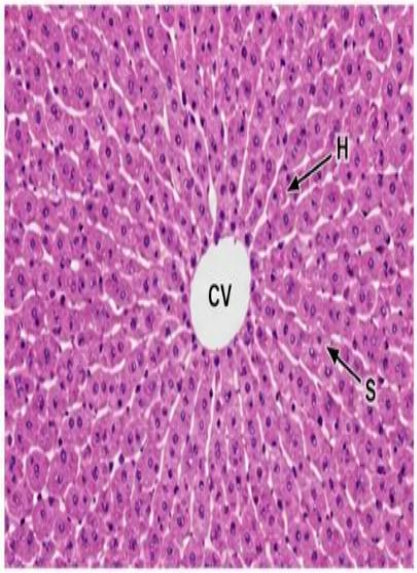

Figure 1. Normal histological architecture of rat liver tissue in the control group (H&E stain, ×400).
CV: central vein; H: hepatocytes; S: sinusoids.

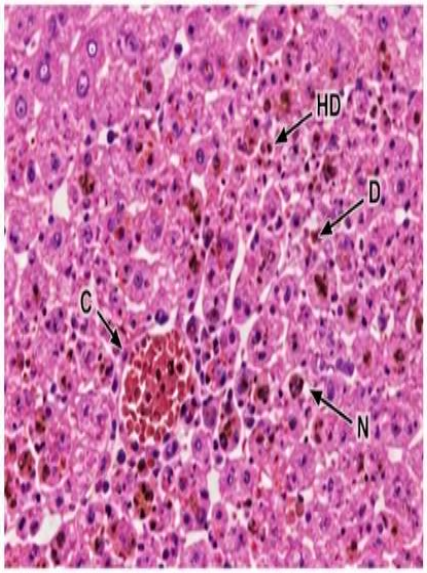

Figure 2. Liver tissue showing hemosiderin deposition, hepatocellular degeneration, congestion, and focal necrosis in iron-treated rats (H&E stain, ×400).
HD: hemosiderin deposition; D: degeneration; C: congestion; N: necrosis.

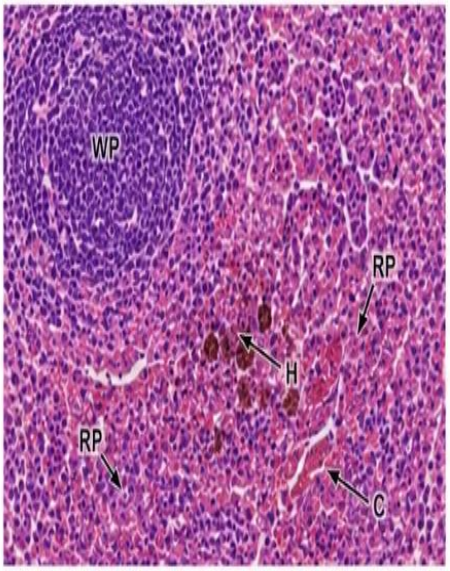

Figure 3. Histopathological changes in rat spleen following excess dietary iron exposure (H&E stain, ×400).
H: hemosiderin deposits; WP: white pulp; RP: red pulp; C: congestion.

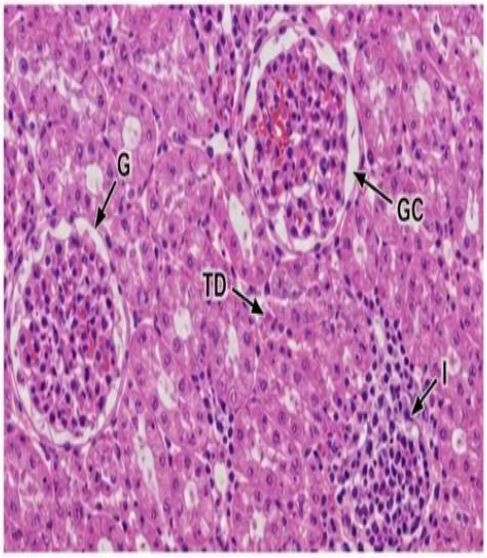

Figure 4. Kidney tissue demonstrating tubular degeneration, glomerular congestion, and inflammatory infiltration in iron-treated rats (H&E stain, ×400).
G: glomerulus; GC: glomerular congestion; TD: tubular degeneration; I: inflammatory infiltration.

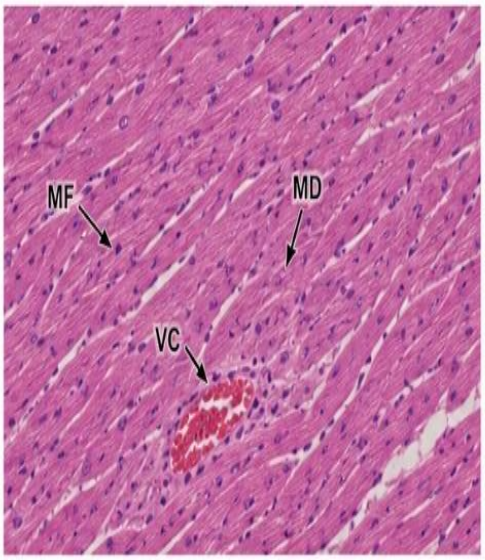

Figure 5. Cardiac tissue showing mild myocardial degeneration and vascular congestion following iron overload (H&E stain, ×400).
MF: myocardial fibers; MD: myocardial degeneration; VC: vascular congestion.

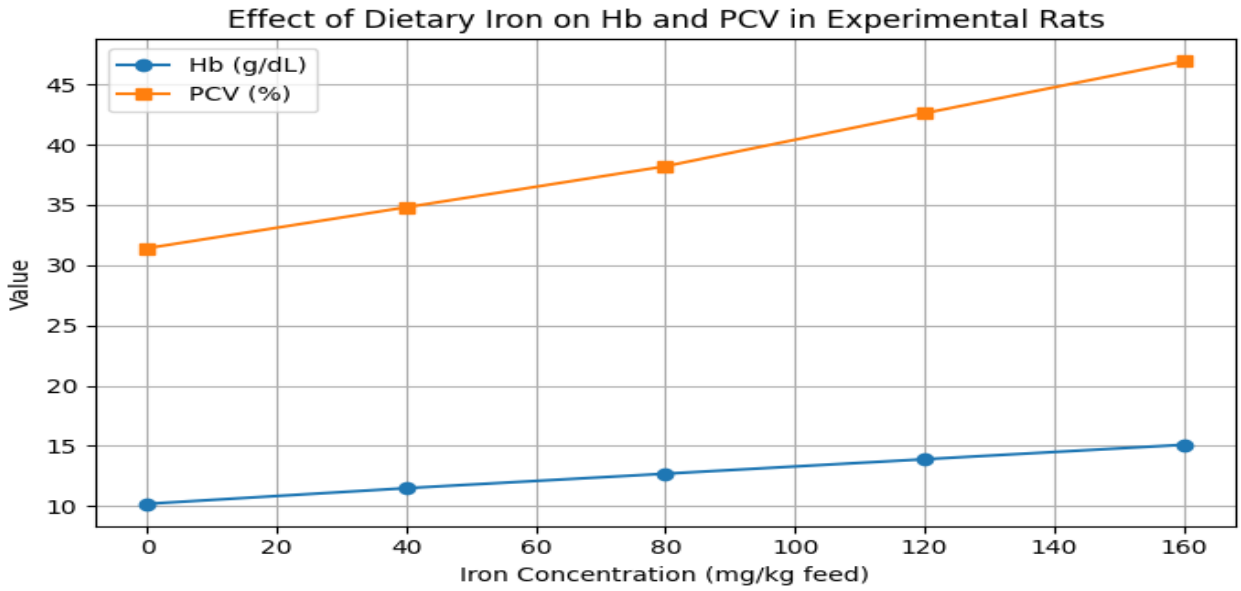

Figure 2. Effect of increasing dietary iron concentration on hemoglobin (Hb) and packed cell volume (PCV) in experimental rats.

4: CONCLUSION

Extra iron in the food changed the histopathology of rats in a way that depended on the dose. The liver and spleen were the organs most affected. In larger amounts of iron, a great deal of hemosiderin accumulation, cell death and tissue congestion were observed. Iron supplements increased hemoglobin and packed cell volume but excess iron caused bad changes in tissues. The findings suggest that while iron is necessary for proper body functioning, excess iron can cause organ damage and should be closely monitored.

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