BIOCHEMICAL STUDY ON CONTRIBUTION OF IRON OVERLOAD AND DIETARY FATS ON MODULATION OF LIPID PEROXIDATIN IN LIVER OF RATS
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ABSTRACT
This study aimed to follow the effect of iron overload and hypercholesterimia in inducing lipid peroxidation in liver of rats. Eighty white male albino rats 12-16 weeks old divided in to 4 groups (20 each): the first group used as control group, the second group is iron group (I), the third group is cholesterol group (CH) and the fourth group is iron and cholesterol group (I&ch). This study takes 8 weeks. Liver samples were collected 2, 4, 6 and 8 weeks after the onset of experiment. Liver homogenate used for determination of malondialdehyde (MDA), glutathione reduced (GSH) and catalase. iron overload and hypercholesterimia lead to a significant increase in hepatic Malondialdehyde (MDA), a significant decrease in glutathione reduced (GSH) and catalase (CAT).

KEY WORDS: Catalase, Glutathione reduced, Liver, Malondialdehyde, Peroxidation

1. INTRODUCTION
Liver is the main storage site for iron in the body. Hepatic iron overload results in fibrosis and cirrhosis and may be complicated by the development of hepatocellular carcinoma. The mechanisms by which iron induced malignant transformation is by the generation of lipid peroxidation due to oxidative stress [10]. It is well known that the toxicity of iron is largely based on its ability to catalyze the formation of radicals via the well-known Fenton reaction. Catalytic amounts of iron are sufficient to yield hydroxyl radicals (OH) from superoxide (O2) and hydrogen peroxide (H2O2), jointly known as reactive oxygen intermediates (ROIs). Free radicals are highly reactive species, which can affect antioxidant enzymatic activities, peroxidation of membrane lipids, modification of nucleic acids, and eventually cause cell death and tissue injury [16] increase the susceptibility of animal body to lipid peroxidation by high dietary lipid with different fatty acid profiles of polar and non-polar fractions of tissue lipids. Liver microsomes of soft-shelled turtles were injured by lipid peroxidation [12].
Non-alcoholic fatty liver disease (NAFLD), a condition in which lipid accumulates inside liver cells, Hepatic lipid accumulation act as powerful oxidative stressors, causing overproduction of reactive oxygen species and depleting the concentration of natural cellular antioxidants. The plasma of patients with NAFLD has been found to
have increased lipid peroxidation, impaired redox balance, and lowered antioxidant capacity [20].

2. MATERIAL AND METHODS

2.1. Experimental animals:
A total number of 80 male albino rats, weighting 200-250 gm were used in this experimental investigation of this study. Rats were obtained from the laboratory animal research center, faculty of veterinary medicine, Moshtohor, Benha University. The animals were divided to four groups as follow:
- **Group (I)**-Control group (CN): consisted of 20 rats, were fed normal diet.
- **Group (II)**-Iron group (I): composed of 20 rats were fed normal diet and injected with poly maltose iron at dose of (63mg/kg) every second day for 8 weeks.
- **Group (III)**-Cholesterol group (CH): composed of 20 rats, received diet contains 0.75% cholesterol and 1.5 bile salts for 8 weeks.
- **Group (IV)**-Iron and cholesterol group (I&ch): consist of 20 rats, received diet containing 0.75% cholesterol, 1.5 bile salts and injected with poly maltose iron at doses of 63mg/kg every second day for 8 weeks.

2.2. Tissue samples:
Liver specimens were collected 2, 4, 6 and 8 weeks after the onset of experiment. Immediately after killing the animals by decapitation, the liver was removed by dissection, washed with ice cold isotonic saline and blotted between 2 filter papers. Then we take 1.0gm liver tissue homogenized in 10ml of 50mM potassium phosphate buffer (pH 7.5). Homogenate used for determination of malondialdehyde (MDA) [2], Glutathione reduced (GSH) [21] and catalase [9] determination.

2.3. Statistical analysis:
The obtained data were statistically analysed and the significant difference between groups was evaluated by student t-test as explained by Snedecor and Cochran [19].

3. RESULTS AND DISCUSSION

The metal accumulates in its main storage site in liver. Dietary iron overload may be complicated by hepatic fibrosis, cirrhosis, and, less often, hepatocellular carcinoma. Iron causes cleavage of lipid hydroperoxides, producing aldehydes, such as 4-hydroxy-2-nonenal (4-HNE), malondialdehyde, crotonaldehyde and acrolein, which are capable of diffusing from their production sites to more distant sites to damage hepatic DNA and proteins [2].

Hypercholesterolemia is regarded as an important factor in the development of the primary cause of mortality in the United States, Europe and most parts of Asia. Free radical-induced lipid peroxidation has been implicated in the pathogenesis, and reactive oxygen species (ROS) are known to be the initiators of lipid peroxidation [21].

Fig 1 Effect of iron overload and high cholesterol in diet on hepatic MDA activity (mmol/g tissue)

The obtained data demonstrated in figure (1.2.3) revealed that iron injection to normal rats exhibited a significant increase in MDA, a significant decrease in GSH and catalase all over the experimental period. These results were nearly similar to the reported studies of Briunet et al. [6]
who recorded a significant increase in hepatic malondialdehyde and a significant decrease in activity of catalase after administration of iron (Fe)/ascorbate at doses 50 and 200 µM/kg to rats. Also, Cornejo et al. [7] investigated that carbonyl-iron-treated rats have a significant increase in MDA and a significant decrease in GSH and GSSG. Similarly, Sutton et al. [18] and Asare et al. [2] showed that iron overload causes a significant increase in MDA and a significant decrease in antioxidant system. Free iron is a catalyst for the formation of reactive oxygen and nitrogen species and consequently causes oxidative damage to hepatocytes, DNA, protein, and lipids. Increased LPO is an important contributor to hepatocarcinogenesis in iron overload. Trans-4-hydroxy-2-nonenal [4-HNE] is a major electrophilic by-product of lipid peroxidation caused by oxidative stress [1].

Iron overload caused hepatic oxidative stress. Iron overload induced liver injury in mice. Iron overload induced lipid peroxidation in liver due to increasing oxidative stress and reactive oxygen species, which in turn cause the oxidation of lipids [22].

Iron catalyzed microsomal membrane lipid peroxidation in rat liver. An increase in intracellular transit pool of iron can catalyze peroxidation of lipids to produce reactive aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE). Covalent binding of such lipid aldehydes with proteins cause impairment in cellular function and integrity [8].

In the liver of iron load mouse, total antioxidant status level was significantly decreased, while thiobarbituric acid reactive substance level was increased. These results indicated that there was significant oxidative stress on liver. Hepatocellular lipid peroxidation of polyunsaturated fatty acids in membranes has been implicated as a mechanism by which iron causes liver damage. Iron-induced peroxidation of intracellular membranes may lead to cellular dysfunction and eventually sideronecrosis. Iron over load caused a significant decrease on catalase activity in mouse liver [23].

Increased LPO is an important expression of both acute and chronic iron toxicity and is a proposed driving force for hepatocarcinogenesis in hereditary haemochromatosis. Iron over load in rat liver cause increasing oxidative stress which leads to lipid peroxidation and increasing MDA in liver tissue [4]. Also the obtained data demonstrated that increase cholesterol in diet of normal rats exhibited a significant increase in MDA, a significant decrease in GSH and catalase all over the experimental period. These
results were nearly similar to the reported studies of Balkan et al. [5] who demonstrated that feeding a cholesterol rich diet to rabbits exposed the animals to oxidative stress. Hypercholesterolemia causes a significant increase in plasma and liver lipid peroxidation, Erythrocyte glutathione peroxidase (GSH-Px) activity significantly decreased GSH-Px and catalase activities in the liver significantly decreased. Also, Maggi-Capeyron et al. [14] stated that high-cholesterol diet leads to a significant increase in hepatic MDA, antioxidant activity were significantly decreased. Similarly, Lebedinsky et al., [11] and Yao et al., [21] stated that high cholesterol diet HC diet cause a significant increase in plasma and liver MDA level, a significant decrease in antioxidant defense systems including glutathione reduced (GSH), glutathione peroxidase (GSH-Px), glutathione-S-transferase (GST) in liver homogenate.

The increase of plasma and liver MDA in case of high cholesterol diet due to the animals’ severe hypercholesterolemia and under supraphysiologic cholesterol concentration the antioxidant protection by plasma vitamin E may be insufficient to scavenge free radicals generated within a large substrate pool which cause oxidative stress. The total plasma oxysterol concentration significantly increased in rabbits fed cholesterol-rich diet [17]. GSH is the most important biomolecule against chemically induced toxicity and can participate in the elimination of reactive intermediates by reducing hydroperoxides in the presence of GSH-Px , GSH also functions as a free radical scavenger and in the repair of free radical-induced biological damages so its activity decreased in case of lipid peroxidation [13].

The decrease in the GSH level represents increased utilization of GSH molecules due to oxidative stress to eliminate high amount of free radicals [3]. High cholesterol diet was able to increase the level of peroxidized lipids. The oxidative stress in the liver results from antioxidant deficiency. High cholesterol diet induces RO (reactive oxygen) overproduction which could in turn initiate lipid peroxidation and cause liver injury by initiating lipid, protein and DNA oxidative modifications, which could be involved in hypercholesterolemia [16].

Feeding a cholesterol supplemented diet to rabbits produced severe hypercholesterolemia and increase in oxidative stress. It is known, oxidative stress results from an imbalance between the production of free radicals and the scavenger antioxidant system [5]. In contrast, high Cholesterol diet caused an increase in antioxidant enzyme activities in the rat liver. These investigators suggested that these adaptive increases in antioxidant enzyme activities protect the liver against lipid peroxidation in rats [17].

4. REFERENCES

تأثير زيادة جرعة الحديد وزيادة الدهون الغذائية في تعديل أكسدة دهون الكبد في الفئران

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الملخص العربي

أجريت هذه الدراسة بهدف معرفة تأثير زيادة الحديد وزيادة الدهون الغذائية وخاصة الكوليسترول في تعديل أكسدة دهون الكبد في الفئران. لقد استخدم لهذا الهدف 80 فأر من ذكور الفئران قسمت على النحو التالي: (1) المجموعة الأولى (المجموعة الضابطة). (2) المجموعة الثانية (مجموعة الحديد) تم حقنها في العضل بجرعات عالية من الحديد 63 ملجم/كمجم يوم بعد يوم. (3) المجموعة الثالثة (مجموعة الكوليسترول) تم تغذيتها على الغذاء عالي الكوليسترول بنسبة 75% واملاح الصفرا بنسبة 1.5%. (4) المجموعة الرابعة (مجموعة الحديد والكوليسترول) تم حقنها في العضل بجرعات عالية من الحديد 63 ملجم/كمجم يوم و يوم بالإضافة الى تغذيتها على الغذاء عالي الكوليسترول بنسبة 75% واملاح الصفرا بنسبة 1.5%. و استمرت الدراسة لمدة 8 أسابيع ثم جمعت عينات الكبد بعد اسبوعين و اربع و ثمانية أسابيع. وقد تم تقدير كل من الأكسدة الفوقية للدهون (ال- مالون داي الدهيد) والجموتاثيون المختزل و إنزيم الكتاليز. وقد أسفرت نتائج هذا البحث عن أن اعطاء جرعات عالية من الحديد عن طريق الحقن العضلي أدى إلى زيادة نسبة الأكسدة الفوقية لدهون الكبد بالإضافة إلى علاجه عالي الكوليسترول أدأ إلى زيادة نسبة الأكسدة الفوقية لدهون الكبد، و انخفاض في تركيز الجموتاثيون المختزل و إنزيم الكتاليز. كما أسفرت نتائج هذه الدراسة عن أن الغذاء عالي الكوليسترول أدأ إلى زيادة نسبة الأكسدة الفوقية لدهون الكبد بالإضافة إلى انخفاض في تركيز الجموتاثيون المختزل و إنزيم الكتاليز. وإضافة إلى أن اعطاء جرعات عالية من الحديد عن طريق الحقن العضلي بالإضافة إلى الغذاء عالي الكوليسترول أدأ إلى زيادة نسبة الأكسدة الفوقية لدهون الكبد، و انخفاض في تركيز الجموتاثيون المختزل و إنزيم الكتاليز. و من النتائج المستخزنة بعد أن زيادة الحديد و زيادة الكوليسترول يؤدي إلى زيادة الأكسدة الفوقية لدهون و انخفاض في الانزيمات المضادة للاكسدة في الكبد.