RUTIN ATTENUATES IRON OVERLOAD-INDUCED HEPATIC OXIDATIVE STRESS IN RATS

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A B S T R A C T

Iron is an essential element that participates in several metabolic activities of the cell. However, excess iron is a major cause of iron-induced oxidative stress and several human diseases. Natural flavonoids, as rutin, are well-known antioxidants, and could be efficient protective agents. Therefore, the present study was undertaken to evaluate the protective influence of rutin supplementation to improve rat antioxidant systems against iron overload (IOL)-induced hepatic oxidative stress. Sixty male albino rats were randomly divided to three equal groups. The first group, the control, the second group, iron overload group, the third group was used as iron overload+rutin group. Rats received six doses of ferric hydroxide polymaltose (100 mg/kg b.w.) as one dose every two days, by intraperitoneal injections (IP) and administerated rutin (50 mg/kg b.w.) as one daily oral dose until the sacrificed day. Blood samples and liver tissue specimens were collected three times, after three, four and five weeks from the onset of the experiment. Serum iron profile [iron, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), transferrin (Tf) and transferrin saturation% (TS%)], ferritin, albumin, total Protein, total cholesterol, triacylglycerols levels, as well as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined. Moreover, iron in the liver, L-malondialdehyde (L-MDA), glutathione (GSH), nitric oxide (NO) and total nucleic acid (TNA) levels and glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activities were also determined. The obtained results revealed that IOL resulted in significant increase in serum iron, TIBC, Tf, TS% and ferritin levels as well as AST and ALT activities. Moreover, it increased liver iron, L-MDA, and NO levels. Meanwhile, it decreased serum UIBC, albumin, total protein, total cholesterol, triacylglycerols levels, as well as liver GSH, TNA levels, and Gpx, CAT and SOD activities when compared with the control rats. Rutin administration to IOL-rats resulted in significant decrease in serum iron, TIBC, Tf, TS%, ferritin levels, and AST and ALT activities as well as liver iron, L-MDA, and NO levels. Rutin also induced significant increases in serum UIBC, albumin, total protein and total cholesterol levels, as well as liver GSH, CAT and SOD activities compared with the IOL-rats. This study provides in vivo evidence that rutin administration can improve the antioxidant defence systems against IOL-induced hepatic oxidative stress in rats. This protective effect in liver of iron loaded rats may be due to both the antioxidant and metal chelation activities.

Keywords: Iron overload, Oxidative stress, Iron profile, Antioxidants, Rutin, Rats.

(BVMJ-25 [2]: 84 -98, 2013)

1. INTRODUCTION

In the human body, excessive amounts of iron may become very toxic due to lacking of effective mechanisms to protect cells against iron overload (1). Iron overload is one of the most common metal-related toxicity (2). It may be caused by: 1) Defects in iron absorption, as increase in iron absorption from the diet as in hereditary hemochromatosis (3); 2) Parenteral iron administration in transfusion-dependent anaemias, as β-thalassemia; 3) Pathological conditions characterized by increases in iron (4). The liver is the main storage organ for iron, in iron overload, iron excess generate oxidative stress through increasing the rate of HO• formation by the Haber–Weiss

reaction (5). Free radical formation and generation of lipid peroxidation (LPO) products may result in progressive tissue injury as fibrosis (6) and eventually cirrhosis or hepatocellular carcinoma (1). Excessive iron deposition in hepatocytes also leads to further injuries such as hepatocellular necrosis (7). Cases of acute iron toxicity are rare and mostly related to hepatoxicity (8), which in turn cause the oxidation of lipids, proteins and nucleic acids (9) that may affect membrane fluidity and permeability, and subsequently cell structure, function and viability (10). Iron-removal therapy may be achieved by antioxidants, iron chelators and/or free radical scavenging compounds, as flavonoids (polyphenols) (11). They exert multiple biological effects including antioxidant and free radical scavenging activities (12). Rutin is a kind of flavonoid glycoside known as vitamin P, a polyphenolic compound that is widely distributed in vegetables and fruits (13). It is very effective free radical inhibitor in animal and human pathological states, such as IOL in rats (14), including hepatoprotective (15) in which its administration sharply suppressed free radical production in liver microsomes and by phagocytes in IOL animals (14). In addition, it could reduce iron content in mouse liver (16). Accordingly, the aim of the present study was to evaluate the protective role of rutin administration against hepatic oxidative stress induced in IOL rat models.

2. MATERIALS AND METHODS

2.1. Experimental animals:

Sixty white male albino rats of 8-10 weeks old and weighing 180- 220 g were used in the experimental investigation of this study. Rats were obtained from the Laboratory Animals Research Center, Faculty of Veterinary Medicine, Benha University, housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of the experiment. The animals provided with a constant supply of standard pellet diet and fresh, clean drinking water *ad libitum*.

2.2. Drug and antioxidants:

The drug and antioxidant compounds used in the present study were: 1-Haemojet$^{(R)}$: Haemojet ampoules were produced by Amriya Pharm. Ind. for European Egyptian Pharma. Ind., Alexandria, Egypt. Each ampoule contains elemental iron (100 mg) as ferric hydroxide polymaltose complex.
2-Rutin: Rutin is pale yellow crystalline powder (purity~99%). It was purchased from Egyption Pharmaceutical Industries Company (EIPICO), 10th of Ramadan city, Egypt. Rutin was dissolved in propylene glycol and administered to animals in daily oral dose of 50 mg/kg body weight (b.w.) (17).

2.3. Experimental design:

Rats were randomly divided into three equal groups: each group containe twenty rats as follows: Group I (control group): received saline only and served as control for all other groups. Group II (iron overload): received six doses (three doses per week) of 100 mg/kg b.w. **(2)** of ferric hydroxide polymaltose complex by IP-injections. Group III (iron overload+rutin): received six doses (three doses per week) of 100 mg/kg b.w. of ferric hydroxide polymaltose by IP-injections, followed by daily oral administration of rutin at a dose level of 50 mg/Kg b.w. until the sacrificed day.

2.4. Sampling:

Blood samples and liver tissue specimens were collected from the three animals groups, three times along the duration of experiment at three, four and five weeks from the onset of rutin administration.

1- Blood samples:

Blood samples were collected by ocular vein puncture in dry, clean and screw capped tubes. Sera were centrifuged at 2500 r.p.m for 15 minutes. The clear sera were separated and received in dry sterile sample tubes, then kept in a deep freeze at -20°C

until be used for subsequent biochemical analysis.

2- Liver tissues:

At the end of each experimental period, rats were sacrificed by cervical decapitation. The liver specimen was quickly removed and weighted, then perfused with cold saline to exclude the blood cells and then blotted on filter paper; and stored at -20°C. Briefly, half of liver tissues were cut, weighed and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates. The homogenates were centrifuged at 5,000 r.p.m for 15 minutes at 4°C then the supernatant was used for the subsequent biochemical analysis. The other half of livers were weighed and putted into glass flask, then 5 volumes of mixed acid (4(nitric acid): 1(perchloric acid)) were added, heated until large amount of white vapors could be seen. The volumes of the digested samples were adjusted to 10 ml with double distilled water. The obtained solutions were used to analyze iron contents.

2.5. Biochemical analysis:

Serum iron and TIBC, ferritin, albumin, total Protein, total cholesterol, triacylglycerols levels, as well as transaminasis (AST and ALT) activities, were determined according to the methods described by 18, 19, 20, 21, 22, 23 and 21, respectively. Moreover, iron in the liver, L-MDA, GSH, NO and total nucleic acid levels as well as GPx, CAT and SOD activities were determined according to the methods described by 24, 25, 26, 27, 28, 29, 30, 31, respectively.

2.6. Statistical analysis:

The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan's multiple test. All analyses were performed using the statistical package for social science (SPSS, 13.0 software, 2009). Data are presented as (mean ± S.E.). S.E = Standard error. Values of $P<0.05$ were considered to be significant.

3. RESULTS

The results presented in Tables (1 and 2) revealed that, iron overload resulted in significant increases in serum iron, TIBC, Tf, TS% and ferritin levels as well as AST and ALT activities. It also increased liver iron, L-MDA, and NO levels. Meanwhile, IOL decreased serum UIBC, total protein, albumin, total cholesterol and triacylglycerols levels. Also it decreased liver GSH and TNA levels, as well as Gpx, CAT and SOD activities compared with the control rats. Rutin administration to IOL-rats significantly decreased serum iron, TIBC, Tf, TS%, ferritin levels, as well as AST and ALT activities. It also resulted in significant decreases liver iron, L-MDA and NO levels. Moreover, it significantly increased serum UIBC, total protein, albumin and total cholesterol levels, as well as liver GSH level, CAT and SOD activities compared with the IOL rats.

4. DISCUSSION

Iron overload in rats is an excellent model to study the in vivo lipid peroxidation in which excess iron induced oxidative stress by increasing lipid peroxide levels in liver and serum (33). Subsequently, MDA and 8-isoprostane adducts that were formed, significantly contributed to liver damage that is assessed by AST and ALT levels in the iron-supplemented rats (34). IOL enhances liver injury, and accelerates the process of fibrosis (6). This tissue injury can be relieved by the administration of an appropriate chelating agent which can combine with the iron and increase its rate of excretion (2), as flavonoids, that have both chelating and free radical scavenging properties (35) thus, rutin treatment may be a very useful medicine (33). Serum and

Table 1: Effect of rutin administration on some biochemical parameters levels in the blood of iron load rats.

Animal groups	Control			Iron overloaded			Rutin treated		
	3 Weeks	4 Weeks	5 Weeks	3 Weeks	4 Weeks	5 Weeks	3 Weeks	4 Weeks	5 Weeks
Iron (µg/dl)	138.50 ±7.03^{b}	144.50 ±3.66^{c}	161.00 ±7.82^{b}	230.75 ±3.52^{a}	232.80 ±8.22^{a}	260.75 ±11.84^{a}	158.00 ±8.96^{b}	176.80 ±5.28^{b}	185.50 ±4.52^{b}
TIBC (µg/dl)	211.50 ±5.30^{c}	221.25 ±4.46^{d}	243.50 ±10.99^{b}	289.25 ±2.75^{a}	300.00 ±7.34^{a}	336.50 ±14.86^{a}	239.75 ±4.17^{b}	272.00 ±6.92^{b}	271.50 ±4.29^{b}
UIBC (µg/dl)	75.00 ±2.27^{a}	76.75 ±0.95^{b}	82.00 ±3.44^{ab}	54.75 ±0.85^{b}	58.75± 2.81^{c}	73.75 ±2.25^{b}	81.75 ±7.43^{a}	96.00 ±0.71^{a}	86.25 ±2.10^{a}
Transferrin (g/L)	1.50 ±0.04^{c}	1.55 ±0.03^{a}	1.70 ±0.08^{b}	2.04 ±0.04^{a}	2.10± 0.04^{a}	2.37 ±0.10^{a}	1.70 ±0.04^{b}	1.90 ±0.02^{a}	1.91 ±0.02^{b}
TS (%)	65.25 ±1.70^{bc}	65.25 ±0.25^{b}	66.50 ±0.65^{b}	79.75 ±1.32^{a}	79.25± 1.25^{a}	77.50 ±0.29^{a}	63.25 ±5.44^{c}	64.75 ±0.85^{b}	69.50 ±1.50^{b}
Ferritin (ng/ml)	2.07 ±0.08^{b}	2.25 ±0.03^{b}	3.33 ±0.04^{b}	2.47 ±0.05^{a}	2.60± 0.04^{a}	3.75 ±0.07^{a}	2.35 ±0.03^{a}	2.45 ±0.03^{a}	3.33 ±0.04^{b}
AST (U/l)	78.60 ±6.07^{d}	94.00 ±1.58^{c}	72.40 ±6.17^{c}	152.60 ±5.56^{a}	149.80 ±4.93^{a}	148.00 ±6.63^{a}	112.60 ±7.69^{c}	117.80 ±5.18^{b}	132.00 ±1.41^{b}
ALT (U/l)	14.80 ±1.28^{c}	15.60 ±0.75^{c}	16.60 ±1.21^{b}	25.60 ±1.08^{a}	34.40 ±2.11^{a}	31.80 ±2.35^{a}	19.60 ±1.54^{b}	22.80 ±1.88^{b}	19.20 ±1.16^{b}
Cholesterol (mg/dl)	110.25 ±5.65^{a}	90.76 ±7.44^{ab}	89.25 ±7.98^{b}	68.75 ±9.43^{b}	78.75 ±4.23^{b}	77.00 ±2.38^{b}	114.00 ±2.12^{a}	103.76 ±2.32^{a}	109.00 ±7.36^{a}
Triacylglycerols (mg/dl)	94.00 ±5.80^{a}	78.00 ±3.87^{a}	82.00 ±5.67^{a}	74.50 ±4.17^{b}	66.75 ±4.77^{a}	71.75 ±1.32^{a}	92.25 ±5.60^{a}	78.50 ±2.40^{a}	81.25 ±2.46^{a}
Albumin (g/dl)	3.02 ±0.17^{b}	3.44 ±0.18^{a}	3.84 ±0.39^{a}	2.04 ±0.13^{c}	2.72± 0.08^{b}	1.98 ±0.10^{b}	3.98 ±0.32^{a}	3.88 ±0.19^{a}	4.40 ±0.20^{a}
Total protein (g/dl)	6.36 ±0.31^{b}	6.90 ±0.44^{ab}	7.00 ±0.64^{a}	4.58 ±0.25^{c}	5.92 ±0.25^{b}	3.44 ±0.22^{b}	6.90 ±0.25^{b}	7.62 ±0.50^{a}	7.02 ±0.37^{a}

Data are presented as (mean ± S.E.). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at (P<0.05).

Table 2: Effect of rutin administration on some biochemical parameters levels in the liver of iron load rats.

Animal groups	Control			Iron overloaded			Rutin treated		
	3 Weeks	4 Weeks	5 Weeks	3 Weeks	4 Weeks	5 Weeks	3 Weeks	4 Weeks	5 Weeks
Total iron (µg/dl)	290.00 ±17.32^{c}	323.33 ±14.53^{c}	353.33 ±26.03^{c}	1266.67 ±46.67^{a}	1313.33 ±17.64^{a}	1373.33 ±12.02^{a}	1140.00 ±45.83^{ab}	1130.00 ±35.12^{b}	1204.33 ±83.96^{ab}
MDA (nmol/g)	131.60 ±5.07^{b}	139.80 ±6.99^{b}	136.00 ±8.33^{c}	189.60 ±18.38^{a}	197.80 ±9.34^{a}	182.00 ±5.72^{a}	128.80 ±7.18^{b}	132.40 ±11.02^{b}	154.40 ±9.20^{bc}
GSH (nmol/g)	87.60 ±3.75^{a}	82.60 ±1.50^{a}	80.00 ±0.89^{a}	76.00 ±1.23^{b}	75.40 ±0.93^{b}	73.60 ±0.93^{b}	79.00 ±0.71^{b}	78.60 ±0.93^{b}	77.80 ±1.24^{a}
GP_X (U/g)	0.49 ±0.02^{b}	0.54 ±0.01^{a}	0.17 ±0.02^{a}	0.58 ±0.01^{a}	0.44± 0.02^{bc}	0.23 ±0.02^{a}	0.56± 0.02^{a}	0.48 ±0.01^{b}	0.18 ±0.02^{a}
CAT (U/g)	14.78 ±0.33^{a}	14.94 ±0.23^{a}	14.84 ±0.29^{a}	12.16 ±0.37^{b}	12.46 ±0.39^{b}	12.72 ±0.29^{b}	15.50 ±0.13^{a}	14.20 ±0.18^{a}	14.22 ±0.20^{a}
SOD (U/g)	304.00 ±19.65^{a}	270.00 ±7.07^{a}	278.00 ±18.55^{a}	174.40 ±10.27^{c}	174.00 ±16.31^{b}	145.00 ±9.22^{c}	270.00 ±25.50^{b}	260.00 ±10.49^{a}	230.00 ±14.83^{b}
NO (µmol/g)	0.159 ±0.002^{b}	0.164 ±0.002^{b}	0.153± 0.003^{b}	0.193 ±0.004^{a}	0.225± 0.02^{a}	0.176 ±0.004^{a}	0.154 ±0.002^{b}	0.159 ±0.002^{b}	0.131 ±0.001^{d}
TNA (µg/g)	0.43 ±0.04^{a}	0.37 ±0.03^{a}	0.45 ±0.04^{a}	0.32 ±0.04^{a}	0.25 ±0.03^{b}	0.33 ±0.03^{a}	0.37 ±0.06^{a}	0.36 ±0.03^{ab}	0.43 ±0.04^{a}

Data are presented as (mean ± S.E.). S.E = Standard error. Mean values with different superscript letters in the same row are significantly different at (P<0.05).

liver iron, as well as serum TIBC, Tf, TS% and ferritin levels were significantly elevated in the iron-loaded rats, while serum UIBC was significantly decreased (Tables 1, 2). These results came in accordance with the data of Maísa Silva et al. (36) who reported that serum iron and TS% were 75% higher in rats with iron-dextran treatment when compared with the untreated control group. Also, Nahdi et al. (37) observed that, IOL elicited significant enhancement in serum iron with significant increase (>10-fold) in liver iron in rats. Moreover, Crisponi and Remelli (4) reported that, when the iron load increases, the iron binding capacity of serum Tf is exceeded and a NTBI fraction of plasma iron appears which generates free hydroxyl radicals and induces dangerous tissue damage. Additionaly, Theurl et al. (38) found that, liver ferritin levels were increased with prolonged iron challenge as iron initially accumulated in spleen macrophages with subsequent increase in macrophage ferroportin and ferritin expression. Thus, iron overload treatment suggesting a novel mechanistic link between dopaminergic GSH depletion and increased iron levels based on increased translational regulation of transferrin receptor 1(TfR1) (39), in which iron deposition and related damages in liver indicate a strong relation between alterations of cellular redox condtion/increase in ROS generation due to GSH depletion with altered iron homeostasis in hepatic cell that led to iron deposition (40). However, exess iron induced increase in hepcidin mRNA level that was not sufficient to prevent increased intestinal iron absorption and onset of IOL compatible with the observation that serum iron was very high in that condition, and TS was more than 100% that most certainly resulted in the presence of NTBI (37). In cases of iron overload, the natural storage and transport proteins such as ferritin and transferrin become saturated and overwhelmed, and then the iron spills over into other tissues and organs, and oxidative stress arises because of the catalytic activity of the metal ion on producing high reactive oxygen radicals, and finally leads to tissue injury (2). When rats were administerated with rutin serum and liver iron, and serum TIBC, Tf, TS% and ferritin levels were significantly decreased and serum UIBC was significantly increased than that of iron-loaded group (Tables 1, 2). Similarly, Gao et al. (41) found that, iron contents were significantly decreased in the liver of rutin and baicalin fed rat. Also, Gao et al. (42) reported that, oral adminstration of higher doses of rutin in mice can cause a decrease of serum iron, copper and zinc concentrations. In addition to Gao et al. (43) that recoreded, the iron contents, in the liver of rutin or baicalin containing diet (1%) fed rats were significantly decreased. Moreover, Zhang et al.(44) observed that, the increased NTBI in quercetin supplemented mice caused no further oxidation indicating that the increased serum non-heme iron may come from flavonoids chelated iron, and although serum ferritin level was still higher than that of normal mouse, it was significantly decreased compared with IOL-mouse. These results can be attributed to metal chelating effects of rutin, which are involved in the Fenton reaction (45) and can be responsible for the documented antioxidant capacity of flavonoids (46). These chelation effects of flavonoids are structure specific (43), suggests that these high reducing power and metal chelating activity mechanisms may play a key role in the inhibition of oxidative processes (47). However, although rutin form chelates with iron ions, it is hydrolyzed by the intestinal flora to its corresponding aglycone, quercetin (48), which is responsible for its in vivo antioxidant activity, therefore, radical scavenging activity of rutin may be more important than its metal chelating activity (49).

Iron overload resulted in significant increase in serum AST and ALT activities compared with the control rats. The obtained results are nearly similar to data reported by Asare et al. (34) who reported, in the iron-supplemented rats all of the indices of LPO, including AST and ALT, were increased

significantly (~5-fold) compared with the control. AST and ALT were used as sensitive indicators of liver damage (50), the increased activities of AST and ALT in IOL-rats can be attributed to the generation of ROS and oxidative damage by excess hepatic iron that may result in chronic necroinflammatory hepatic disease, which in turn generates more ROS and causes additional oxidative damage (51). Rutin administration in IOL-rats decreased serum transaminases activities (table 1). Similar results were reported by Mahmoud (50) who observed that, rutin administration significantly decreased the levels of AST and ALT activities in hyperammonemic rats, suggesting protection by preserving the structural integrity of the hepatocellular membrane against ammonium chloride. Rutin also scavenged free radicals and inhibiting LPO process (52), in which the iron-rutin complexes not only retained the antioxidant properties of rutin, but in many cases exhibited enhanced free radical-scavenging activity (53).

Iron overload resulted in significant decrease in serum total protein and albumin levels (table 1). The results agree well with those recorded by Asare et al. (34) that found, iron accumulation disrupts the cell redox balance and geneates chronic oxidative stress, which damages DNA, lipids and protein in hepatocytes leading to both necrosis and apoptosis. That can be explained by protein oxidation that give rise to alterations in both the backbone and side chains of the molecule, leading to the denaturation and loss of biological activities of various important proteins and cell death (44). The LPO releasing cytotoxic products as MDA (34) may impair cellular functions including nucleotide and protein synthesis (54). This suggestion was confirmed by Youdim et al. (55) who reported, ROS are capable of oxidizing cellular proteins, nucleic acids and lipids. Rutin administration to iron-overloaded rats induced significant increases in serum total protein and albumin concentrations compared with the IOL-rats. Similar results were reported by Kamalakannan and Prince (56) also observed that, oral administration of rutin to diabetic rats lead to significant increase in the plasma total protein and albumin concentrations when compared with the diabetic control. The antioxidant activity of rutin in Fenton reaction (57) may be explained as a mechanism of action preventing protein oxidation. The reduction of liver protein oxidation can be considered as a sign of protection under IOL due to the treatment with baicalin and quercetin in which the inhibitive effects of flavonoids on protein oxidation may come from the combination of both iron eliminating and free radical scavenging activities (44).

Serum total cholesterol and triacyglycerols levels were significantly decreased after three weeks only in the IOL-rats. These obtained results may be explained by Maísa Silva et al. (36) who reported, hepatic injury triggered by iron excess may increase the concentration of secondary serum metabolites, such as cholesterol, triacylglycerols and glucose, and also recorded that, treatment with iron dextran in male rats increased serum triacylglycerols level, but had no effect on the cholesterol evel. However, Turbino-Ribeiro et al. (58) reported that, absence of alteration in serum cholesterol in rabbits receiving iron dextran injections. Rutin administeration significantly increased serum conc. of triacyglycerols after three weeks only and total cholesterol allover the experimental period than that of the IOL-rats. Contradictory results were obtained by Park et al. (59) recorded that, supplementation of 0.1% rutin and tannic acid significantly lowered both plasma total cholesterol and triacylglycerols compared with control. These results may be explained by the lose of the amphiphilic properties of rutin, that be less capable of scavenging free radicals from the most lipophilic regions of the LDL particle (cholesterol esters and triacylglycerols) (47). In which the decrease in cholesterol level was due exclusively to the LDL and VLDL fraction (60).

Liver L-MDA concentrations were significantly elevated in the iron-loaded rats as compared with control rats (table 2). The obtained results are nearly similar to those Nahdi et al. (37) who reported that, IOL in rats was accompanied by enhancement in LPO with a significant increase in all tissue MDA concentration in iron supplemented group except the spleen, and added, there was a perfect correlation between MDA level and tissue iron content in liver, suggesting production of oxidative stress. LPO generated by ROS was measured in terms of MDA, a measure of free radical generation, is an end product of LPO (45). Focusing on the liver organ, the formation of liver microsomal MDA protein adducts during IOL in mice lead to the microsomal function impairment that may alter protein function and might lead to cellular injury and iron associated hepatotoxicity (61). When rats were administerated with rutin, hepatic L-MDA concentrations were significantly decreased than that of IOL-rats (Table 2). The obtained results agree with the data of Afanas'ev et al. (14) who showed that rutin administration significantly (4-fold) decreased the level of thiobarbituric reactive substances and inhibited LPO by 75% in microsomes of IOL rats. Also, Gao et al. (43) reported that, LPO level in the liver of the rutin fed group was significantly decreased in comparison to the control group. As iron overload led to enhancement of LPO (37), some flavonoids can be both antioxidants and iron chelators, as they can play a double role in reducing the rate of oxidation (62). The antioxidant activity of rutin may be also attributed to the free radical scavenging property, in which IOL in rats induces oxidative stress characterized by oxygen radical overproduction in liver microsomes, peritoneal macrophages and blood neutronphils (50). Iron overload resulted in significant decrease in liver GSH levels when compared with the control rats (table 2). Similarly, Pardo-Andreu et al. (63) who observed that, iron-dextran administration in liver tissue evidenced by a 34% decrease of its GSH content in iron loaded rats. GSH was often used as an estimation of the redox environment of the cell (64). Iron deposition and related damages in liver indicate a strong relation between alterations of cellular redox condtion/increase in ROS, e.g., redox status of iron that play a significant role in LPO (33) due to GSH depletion, suggesting a novel mechanistic link between dopaminergic GSH depletion and increased iron levels (39). Rutin administration to iron overloaded rats resulted in significant increase in liver GSH level compared with the IOL-group. Similarly, Arjumand et al. (45) reported that, rutin treatment in cisplatin administerated rats showed significant improvement in GSH concentration, suggesting its role in scavenging the free radicals generated by cisplatin-induced renal inflammation and apoptosis. The antioxidant imbalance was compensated by the prophylactic treatment of rutin as excessive LPO can cause increased GSH consumption (65). These inhibitory effects of rutin on in vivo free radical production in IOL-rats are probably explained by its ability to form inactive iron-rutin complexes (66). The obtained results revealed that, iron overload resulted in significant decrease in liver Gpx after three weeks, CAT and SOD activities when compared with the control group. Iron overload can destruct the balance between prooxidants and antioxidants, leading to severe loss of total antioxidant status level (2). This phenomenon can be seen in most iron overload animal models (67). Chronic iron administration induced adaptive responses involving stimulation of the antioxidant defenses (14) including catalase which is an iron-containing antioxidant enzyme that showed a significant decrease in liver after iron-dextran injection in mouse (2). Additionally, GPx significantly competes with CAT for H2O2 substrate (68). Moreover, SOD work in conjunction with CAT and GPX (69), preventing its interaction with iron and therefore formation of the highly toxic •OH, in which SOD and GPX are supportive enzyme system of the

first line cellular defense against oxidative injury (70). GPx decomposes peroxides (H2O2) to H2O (or alcohol) while simultaneously oxidizing GSH, as it is the major source of protection against low levels of oxidative stress (68). The increase of SOD/GPx ratio in iron treated cells compared to control cells indicated that iron-induced oxidative injury appeared, that might be indicative of ROS increase due to unefficient scavenging by enzymes (71).

Rutin administration to iron-loaded rats resulted in significant increases in liver antioxidant enzymes CAT and SOD activities compared with the IOL group (Table 2). These results are in conformity with the data reported by Park et al. (59) who recorded, dietary rutin and tannic acid have a significant effect on SOD and GPx in rats, as a concomitant increase in CAT and/or GPx activity is essential if a beneficial effect from the high SOD be expected. As the natural antioxidants, flavonoids intake may increase total antioxidant status level in living body, supplementation of baicalin or another flavonoid, rutin, could increase hepatic total antioxidant status level in rats (43) and mice (2). This effect may come from the chelation of free iron ion with stopping iron-catalyzed oxidative reaction (2). In addition to its role in scavenging the free radicals generated, rutin also possesses an antioxidant activity that could lead to increase the activity of the antioxidant enzymes SOD, CAT and GPx (45), that can prevent damage by detoxifying ROS, in which rutin protective action on LPO and the enhancing effect on cellular antioxidant defense contributing to the protection against oxidative damage (50).

Iron overload resulted in significant increase in liver NO levels when compared with the control. Similar results were recorded by Cornejo et al. (72) that reported, increased NO generation was evidenced in the liver under conditions of acute IOL. Also Cornejo et al. (73) confirmed that, chronic IOL leads to a substantial increase in rat liver NOS activity. NO is an inorganic reactive nitrogen species synthesized in liver by inducible nitric oxide synthase (iNOS) found in hepatocytes, Kupffer cells, and endothelial cells (74). The increase in rat liver NOS activity due to chronic IOL (73) is related to upregulation of iNOS expression (75). Rutin administration to iron loaded rats resulted in significant decreased liver NO levels compared with the IOL rats. The data obtained are in harmony with Shen et al. (76) that showed, rutin inhibit lipopolysaccharide-induced NO production. NO was proposed to act as a pro-oxidant at high conc. (77), or when it reacts with superoxide anion, forming the highly reactive peroxynitrite (78) that is suppressed by flavonoids by direct scavenging (79). Iron overload resulted in significant decreases in liver TNA levels when compared with the control group. Similarly, Youdim et al. (55) explored that, iron is a major generator of ROS that lead to damage of lipids, proteins carbohydrates, and nucleic acids. Likewise Díaz-Castro et al. (80) who reported that IOL in control and anaemic rats caused DNA damage in normal iron content or iron loaded diets. The decrease in total nuclic acid level can be attributed to •OH may damage the nucleotide bases themselves, resulting in oxidized base products such as 8-oxo-guanine and fragmented or ring-opened derivatives (81). Iron also is thought to be involved in β-cleavage of lipid hydroperoxides, producing biogenic aldehydes that interact with DNA to form exocyclic products (82) that trigger free radical-mediated chain reaction including LPO, DNA damage and protein oxidation (83). Rutin administration to iron loaded rats none significantly increased liver TNA concentration compared with the IOL-rats. The obtained results are of the same harmony with the data of Perron and Brumaghim (84) who observed correlations between polyphenol iron-chelating ability and lipophilicity on prevention of DNA damage in H2O2-treated cells. Rutin protect the stability of the genome (85). Oxidative damage to DNA, especially strand breaks, is highly dependent on the amount of iron

bound to DNA (80), in which polyphenols are one large class of antioxidants that were extensively examined for treatment and prevention of conditions associated with iron-generated ROS and oxidative stress (84), as iron chelation was responsible for prevention of nuclear DNA damage by quercetin (86). From the obtained results it could be concluded that the natural flavonoids, rutin inhibited the adverse effect of ferric ion induced oxidative stress by reducing protein and DNA oxidation, and inhibition of lipid peroxidation in liver tissue due to its marked hepatoprotective role in the experimental rats. Rutin treatment improved the cytoprotective enzymatic and non-enzymatic antioxidants and thus, might protect the cellular environments from iron-induced free radical damage. The results indicate that rutin may have potential effects in inhibiting the iron-induced oxidative stress in human. The protective effect of rutin on livers of iron overloaded rats may be due to its high antioxidant activity, including both its radical scavenging and iron chelation activities. Therefore, we recommended using rutin-enriched food regularly with additional research work for medicine manufacturing for protection against the bad complications of IOL-induced oxidative stress.

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الروتن يحسن الاجهاد التأكسدى الناتج من التحميل الزائد للحديد فى كبد الفئران

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الملخص العربي

يهدف هذا البحث إلى دراسة التأثير الكيميائى الحيوى **للروتن**، من مجموعة الفلافينويد، كمضاد اكسدة طبيعي على الإجهاد التأكسدى نتيجة التحميل الزائد للحديد المحدث في الفئران تجريبياً. وذلك لوقاية الفئران من التأثيرات الضاره الناتجه عن الإجهاد التأكسدى الناتج عن الحديد الزائد. وقد أجريت هذه التجربة على ستون من فئران التجارب تتراوح أعمارهم من 8–10 أسابيع وأوزانهم من 180–220 جم، وقسمت الفئران إلى ثلاث مجموعات على النحو التالى: المجموعة الأولى (المجموعة الضابطة): اشتملت على 20 فأراً ولم تعطى أية أدوية واستخدمت كمجموعة ضابطة للمجموعات الأخرى. المجموعة الثانية (المجموعة المحدث بها تحميل زائد للحديد تجريبياً): تكونت من 20 فأراً تم حقنهم في الغشاء البروتونى بمركب اكسيد الحديديك متعدد المالتوز بجرعة مقدراها (100 مللى جرام/كيلوجرام من الوزن) لمدة 14 يوم بمعدل جرعه واحده كل 48 ساعة، اى 6 جرعات فقط طوال مدة التجربه. المجموعة الثالثة (المجموعة المعالجة بمادة الروتن): تكونت من 20 فأراً تم تجريعهم باللروتن بجرعة مقدراها (50 مللى جرام/كيلوجرام) يوميا لمدة 5 أسابيع وبالتزامن مع حقن الفئران بالحديد كما بالمجموعة الثانية. وأظهرت النتائج وجود إعتلال خلايا الكبد كما هو موضح فى التغيير المعنوى فى معظم القياسات البيوكيميائية فى الدم ودلالات وظائف الكبد، كما تبين من الزيادة المعنوية فى مستوى الحديد فى دم وكبد الفئران ومستوى المعدلات الاتية: (TIBC, Tf, TS%, ferritin) و نشاط إنزيمات الكبد فى الدم، ومستوى الأكسدة الفوقية للدهون (MDA) وتركيز اكسيد النيتريك (NO) فى انسجة الكبد، بالإضافة إلى النقص المعنوى فى مستوى UIBC والألبيومين والبروتين الكلى فى الدم، و نشاط الإنزيمات المضادة للأكسدة (الكاتاليز وسوبرأوكسيد دِسميوتيز) وتركيز الجلوتاثيون المُختَزَل (GSH) و تركيز الحمض النووى الكلى فى أنسجة كبد الفئران فى المجموعة الثانية. وعلى العكس ظهر تحسن واضح فى نتائج المجموعة الثالثة المعالجه بالروتن وذلك لحماية الكبد من الاجهاد التأكسدى والحفاظ على نسب القياسات البيوكيميائية السابقة فى الدم والأنسجة لما يقارب النسب الطبيعية، ووجود زيادة واضحة فى الانزيمات المضادة للأكسدة فى كبد الفئرا، ويرجع ذلك إلى نشاطة القوى كمضاد أكسدة طبيعي فى التخلص من الشوارد الحره والحديد الحر الزائد. مما سبق نستنتج أن الروتن له تأثير وقائى واضح فى حماية الكبد من الإجهاد التأكسدى نتيجة التحميل الزائد للحديد، ولذلك ننصح بضرورة استخدامه كمادة طبيعية وقائية مضادة للأكسدة ومادة فعالة فى العقاقير الدوائية.

(مجلة بنها للعلوم الطبية البيطرية: عدد 25(2):84–98, ديسمبر 2013)