Attrition of Iron-Induced Biochemical Injury in Mice Kidney by a Citrus Bioflavonoid, Hesperidin

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Abstract

Iron is a trace element that plays a crucial role in various metabolic processes in all eukaryotic cells. Its ability to donate and accept electron makes it an important molecule of the redox pathways. The accumulation of iron is considered detrimental to health as iron overload leads to oxidative stress and triggers several diseases. The ability of 250 mg/kg body weight hesperidin to reduce the iron-induced biochemical injury in the kidney of mice treated with 5000, 10,000 and 20000 ppm FeCl3 for 30 days in drinking water was investigated. The levels of glutathione, glutathione-s-transferase, catalase, superoxide dismutase and lipid peroxidation were examined in the kidney of iron treated mice after 30 days post-iron treatment. The chronic administration of different doses of iron for 30 days caused a significant rise in the lipid peroxidation, whereas the activities of glutathione-s-transferase, catalase, and superoxide dismutase and the amount of glutathione declined indicating increased oxidative stress. However, administration of hesperidin for 5 consecutive days before iron overload augmented the glutathione-s-transferase, catalase, and superoxide dismutase activities and glutathione concentration, accompanied by an attrition in the lipid peroxidation. Our study demonstrates that pre-treatment of mice with hesperidin for five days reduced the iron induced biochemical stress as revealed by an elevation in the glutathione-s-transferase, catalase, and superoxide dismutase activities and glutathione contents followed by a decline in the lipid peroxidation in the mice kidney.

Keywords: Mice; kidney; Antioxidants; Glutathione; Catalase; lipid peroxidation; lactate dehydrogenase

Introduction

Iron is essential for the survival of all organisms. Iron is abundant in the biosphere and it is extremely important in carrying out various metabolic processes in all living organisms for their survival. Iron is primarily found in two forms: the ferrous (Fe++) and the ferric (Fe³⁺) and this transition from one form to another puts it in a unique position of donating or accepting electrons depending on the situation in the cell. Iron plays a crucial role in a number of metabolic processes including nitrogen fixation, oxygen transport, electron transfer, and DNA synthesis [1]. “Free” iron generates hydroxyl radicals from superoxide and hydrogen peroxide via Fenton reaction inducing oxidative stress in the cells [2]. The hydroxyl free radicals produced due to participation of iron are highly reactive. They interact with lipids, proteins and DNA causing severe damage to these macromolecules and subsequently damage vital tissues and organs [3]. The cells have inherent capacity to protect itself from oxidative stress-induced by iron, where it is oxidized to Fe³⁺, which tightly combines with transferrin and is kept in a non-toxic redox-inert state [4]. Two-thirds of serum transferrin is present as apotransferrin and will quickly capture the free iron that may be released from the cell whenever it is required [5].

Iron is a vital micronutrient and it is indispensable part of several biological phenomena including mammalian cell growth, DNA and heme synthesis, cytochrome p450 enzyme activity, hypoxic response reactions, oxygen transport, and cell proliferation [6]. Iron serves as a prosthetic group in different enzymes eg. iron-sulphur and heme proteins of the respiratory chain, as well as ribonucleotide reductase. These enzymes are involved in the rate-limiting step in DNA synthesis [7,8]. Iron also acts as a common prosthetic group composed of protoporphyrin IX and a Fe²⁺ ion. Presence of iron is a double-edged sword, it is extremely indispensable to carry various important metabolic processes. Whereas the deficiency and overload of iron are the result of defective metabolism and are associated with several diseases [9]. Mismanagement of iron in the cell is the principal cause of underlying pathogenic events in oxidative stress that is harbinger of several diseases [10,11].

Iron pool in the body is tightly regulated due to existence of negative feedback mechanism [12]. However, the excess iron in the body is referred to as iron overload. The gene mutations are responsible for most of the iron overload in populations of northern Europe that leads in an amino acid substitution at position 282 of the HFE protein. The C282Y substitution is rare outside the white ethnicity although other mutations do exist [13]. Iron overload is rare in Asian populations and if it is, it may be due to abnormalities in non-HFE iron-related molecules [14]. Iron overload in the body is highly toxic as it may be associated with serious health conditions including aging, autoimmune nephrotic syndromes, diabetes, cataractogenesis, cardiovascular diseases, degenerative retinal damage, gastrointestinal tract disorders, heavy metal toxicity, Parkinson’s disease, ischemia reflow states, Alzheimer’s disease, bronchopulmonary dysphasia, kidney damage, macular degeneration, stroke and cancer [15-24]. Therefore, it is imperative to reduce iron-induced oxidative stress by use of natural products. The dietary supplements may be of great value in arresting or treating iron-induced toxicity and subsequently the iron-induced disorders.

Plants synthesize a number of secondary metabolites, which are not essential for their survival but play other important roles during the life of plants. The citrus plants synthesize hesperidin as secondary metabolite, which is present in the fruit juices and rinds of these plants [25]. Hesperidin, a bioflavonoid protects plant against fungal and bacterial invasions [26]. The highest amount of hesperidin is found in

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sweet oranges (Citrus sinensis) and tangelos and orange juice with pulp has higher amount of hesperidin than the orange juice that is without pulp [27]. Usually one litre of orange juice contains 470-761 mg of hesperidin [28]. The ingestion of hesperidin leads to its conversion into the aglycone hesperitin in the colon by intestinal bacteria, which is degraded or absorbed [29]. Hesperidin is active against inflammation, oxidative stress, free radical generation and ulceration. It also blocks selected cytochrome p450 enzymes resulting in drug interactions [30,31]. Hesperidin increases capillary (blood vessels) permeability and is useful in absorption of vitamin C [32]. It has been indicted in hypertension in humans and it protects against haemorrhages, infections and heals ruptured capillaries and connective tissues [33]. The anti-inflammatory, analgesic, anti-cancerous, anti-inflammatory, antimicrobial anti-hypotensive and vasodilating activities of hesperidin have been reported in different study systems [34-38]. The daily intake of 500 mL orange juice consecutively for four weeks has been found to modulate the activation of 3422 genes in humans. However, only 1819 genes got activated when hesperidin was given in a similar fashion to the humans [39]. Hesperidin protected against oxidative stress, neurotoxicity, Herpes simplex virus infection, apoptosis, atherogenesis, inflammatory bowel disease, arthritis, platelet and erythrocyte aggregation and infection [40-50]. Hesperidin scavenged free various radicals in vitro and increased the wound healing of irradiated wounds. Oral administration of hesperidin was found to be non-toxic up to a dose of 2 g/kg body weight in mice [51,52]. Earlier sub chronic administration of 5% hesperidin for 13 weeks has been reported to be non-toxic in mice [53]. Kidney is an important organ of the body, which plays a major role in detoxification therefore, the present study was undertaken to obtain an insight into the effect of hesperidin on the iron-induced biochemical injury the mice kidney.

**Materials and Methods**

**Chemicals**

Ferric chloride, di-sodium hydrogen phosphate (Na,HPO₄), hydrogen peroxide (H₂O₂), di-potassium hydrogen phosphate (K,HPO₄), potassium di-hydrogen phosphate (KH₂PO₄) and carboxymethylcellulose (CMC) were procured from MERC, India Ltd., Mumbai, whereas hesperidin, 1-Chloro-2,4-Dinitrobenzene (CDNB), phenazine methosulphate, nitroblue-tetrazolium, sodium pyruvate, cumene hydroperoxide, thiobarbituric acid, 5′-5′-dithiobis[2-nitrobenzoic acid, 5′-thiobenzoic acid, mM tert-butylhydroperoxide and nicotinamide adenine dinucleotide (NADH) were supplied by the Sigma Aldrich Chemical Company, Kolkata, India.

**Animal care and handling**

The animal care and handling were performed in accordance with the guidelines issued by the World Health Organization, Geneva, Switzerland and the INSA (Indian National Science Academy, New Delhi, India). Generally, six to eight weeks old inbred male and female Swiss albino mice (1:1) weighing 25 to 30 g were requisitioned from the Sigma Aldrich Chemical Company, Kolkata, India. The animals were provided with standard food and water ad libitum. Five animals were housed in a polypropylene cage containing wood powder (procured locally) as bedding throughout the experiment. The Animal Ethics Committee of Mizoram University, Aizawl, India had approved the study.

**Preparation of Drug and Mode of Administration**

The required amount of hesperidin was dissolved in distilled water containing 1% CMC and the animals were administered with 250 mg/kg body weight of hesperidin orally [52].

**Experimental**

The effect of hesperidin on iron-induced oxidative stress in the mouse kidney was studied by dividing the animals into the following groups:

- **Iron**: The animals of this group were administered with 0, 5000, 10,000 and 20,000 ppm of ferric chloride in ordinary drinking water daily for 30 days consecutively.

- **Hesperidin+Iron**: This group of animals was orally administered with 250 mg/body weight of hesperidin intraperitoneally for five days before administering the animals with 0, 5000, 10,000 and 20,000 ppm of ferric chloride in ordinary drinking water daily up to 30 days.

Thirty days after the administration of ferric chloride, the animals from both the groups were killed by cervical dislocation. The mice were dissected and perfused with ice cold saline transcardially. The kidney from both group of animals was removed blot dried. A total of 80 mice were used to complete this experiment.

**Preparation of Homogenate**

The kidney was weighed and 10 % homogenate was prepared in a phosphate buffer saline using an electrical homogenizer (REMI, Mumbai, India). Various biochemical parameters were estimated in the kidney homogenate after 30 days of iron treatment.

**Total Proteins**: The total proteins were estimated by the Lowry's method with minor modification [54].

**Glutathione**: The glutathione (GSH) was assayed as described earlier with minor modifications [55]. In brief, the proteins were precipitated with 0.5 mL ice cold 10% 5-sulosalicylic acid and the tubes were incubated on ice for 10 min, centrifuged (Sorvall Instruments RC5C, DuPont, Minnesota, USA) for 15 min at 15,000 rpm at 4°C. The protein free supernatant was collected and mixed immediately with 0.5 mL of NADPH (4 mg of reduced form was dissolved in 100 mL of 0.5% NaHCO₃), 0.5 mL of glutathione reductase (6 units/mL in 0.1 M phosphate buffer, pH 7.0) and 1 mL of 0.6 M DTNB (prepared in 0.2 M phosphate buffer of pH 8). The formation of TNB was read against the blank in a UV-Visible double beam spectrophotometer (Shimadzu Corporation, Tokyo, Japan) at 412 nm. A sample without GSH was used as a blank. The GSH concentration has been expressed as μmol/mg protein. Standard curve was plotted using different concentrations of GSH.

**Glutathione-S-Transferase**: The glutathione-S-transferase (GST) activity was estimated as described by Habig and Pabst [56]. The tissue homogenate was mixed with 0.1 M potassium phosphate buffer, CDNB and 10 mM GSH, and kept in water bath for 10 min at 37°C. The absorbance was recorded at 1 min intervals against distilled water, which was used as a blank at 340 nm using a UV-VIS spectrophotometer. The GST activity has been expressed as nmol/mg of protein.

**Catalase**: The catalytic reduction of hydrogen peroxide was used as a measure of catalase activity [57]. The hydrogen peroxide was mixed with tissue homogenate and incubated at 37°C. The hydrogen peroxide decomposition was monitored at 0.5 s, 10 s intervals up to 30 s and the absorbance was read against the phosphate buffer blank at 240 nm using a UV-VIS spectrophotometer. The average difference in absorbance in 30 s was calculated.
**Superoxide Dismutase:** The activity of superoxide dismutase (SOD) was determined as described earlier [58]. Briefly, the tissue homogenate was mixed with phenazine methosulphate, nitroblue tetrazolium and NADH. The whole mixture was incubated at 30°C for 90 sec. The reaction was terminated by the addition of acetic acid and n-butanol. The blank was prepared in a similar fashion without the sample and the reaction was stopped by adding acetic acid and n-butanol. The sample absorbance was read against the blank at 560 nm in a UV-VIS spectrophotometer.

**Lipid Peroxidation:** The induction of lipid peroxidation (LOO) was measured as various thiobarbituric acid reactive substances (TBARS) including malondialdehyde, lipid hydroperoxides and aldehydes in the tissue homogenate [59]. The homogenate was heated with thiobarbituric acid (0.8%), sodium dodecyl sulphate (0.1%) and acetic acid (20%) in a boiling water bath for 30 min to precipitate the lipoproteins. The resultant mixture was cooled, extracted with n-butanol-pyridine, and the absorbance of the butanol layer was recorded at 532 nm using UV-Visible double beam spectrophotometer. The resultant concentration of TBA reactive substances is expressed as nmol/mg protein obtained from a standard curve of tetraethoxypropane.

**Statistical Analysis**

The significance between the treatments was determined using the Student’s t test and one-way ANOVA with Tukey’s post-hoc test. A p value of <0.05 was considered statistically significant. The Solo 4 statistical package (BMDP Statistical Software Inc, Los Angeles, CA, USA) was used for statistical analyses.

**Results**

The results of all biochemical analyses are represented as mean ± standard error of the mean in Tables 1-5 and Figures 1-5.

**Glutathione (GSH)**

The estimation of glutathione in kidney showed a spontaneous level of 12.10 ± 0.18 µmoles/mg protein in control animals and administration of hesperidin alone did not change GSH contents significantly when compared to non-drug treated control (Table 1). The chronic administration of different doses of ferric chloride for thirty days resulted in a dose dependent and significant decline in the GSH concentration when compared to untreated control (Table 1 and Figure 1). The administration of mice with hesperidin before iron overload significantly raised the glutathione contents (Table 1 and Figure 1).

**Glutathione-S-transferase (GST)**

The spontaneous glutathione-s-transferase activity in the kidney of mice was estimated to be 4.55 ± 0.13 nmol/mg protein and treatment of mice with hesperidin increased this activity significantly when compared to non-drug treated control (Table 2). The administration

![Graph 1: Alteration in the glutathione concentration in the kidney of mouse treated with of 250 mg/kg body weight hesperidin before administration of different doses of iron for 30 days. Red bars: iron alone and Blue bars: Hesperidin+iron.]

| FeCl₃ (PPM) | Iron | Hesperidin + Iron |
|-------------|------|-------------------|
| 0           | 12.10 ± 0.18 | 11.64 ± 0.22 |
| 5000        | 10.61 ± 0.10** | 12.02 ± 0.22a |
| 10000       | 9.42 ± 0.12** | 10.84 ± 0.16c |
| 20000       | 8.27 ± 0.08** | 10.60 ± 0.13c |

Level of significance= **P<0.05; aP < 0.01; bP<0.001; cP < 0.0001 When Iron group compared with Hesperidin+Iron group; dP<0.001; eP<0.0001, Iron group compared to control (0). N=10

**Table 1:** Alteration in the glutathione concentration by 250 mg/kg body weight of hesperidin in the kidney of mouse treated with different doses of iron for 30 days.

![Graph 2: Alteration in the glutathione-s-transferase activity in the kidney of mouse treated with of 250 mg/kg body weight hesperidin before administration of different doses of iron for 30 days. Red bars: iron alone and Blue bars: Hesperidin+iron.]

| FeCl₃ (PPM) | Iron | Hesperidin + Iron |
|-------------|------|-------------------|
| 0           | 5.37 ± 0.04d | 5.37 ± 0.04d |
| 5000        | 3.12 ± 0.16d | 3.12 ± 0.16d |
| 10000       | 2.31 ± 0.12d | 2.31 ± 0.12d |
| 20000       | 2.04 ± 0.09d | 2.04 ± 0.09d |

Level of significance= **P<0.05; aP < 0.01; bP<0.001; cP < 0.0001 When Iron group compared with Hesperidin+Iron group; dP<0.001; eP<0.0001, Iron group compared to control (0). N=10

**Table 2:** Alteration in the glutathione-s-transferase activity by 250 mg/kg body weight of hesperidin in the kidney of mouse treated with different doses of iron for 30 days.
receiving 5000 ppm iron after hesperidin (p<0.0001) treatment (Figure 2). With increasing iron overload, the increase in GST activity by hesperidin was lesser (Table 2 and Figure 2).

**Catalase**

The spontaneous catalase activity in the mouse kidney has been found to be 86.09 ± 1.33 nmol/mg protein and administration of hesperidin alone did not alter this activity significantly when compared to non-drug treated control. The chronic administration of ferric chloride for 30 days reduced the catalase activity in a dose dependent manner (Figure 3). This decline was significantly higher than the non-iron treated control. The maximum decline of 3.25 folds in catalase activity was observed for 10,000 and 20,000 ppm hesperidin and iron treated animals (Table 3).

**Superoxide dismutase (SOD)**

The spontaneous superoxide dismutase activity in the kidney of mouse was measured as 5.15 ± 0.23 nmol/mg protein and the administration of hesperidin alone did not alter this activity significantly when compared to non-drug treated control. The oral administration of different doses of ferric chloride for 30 days in drinking water reduced the SOD activity significantly (Table 4). The SOD activity depleted in iron dose dependent manner and a maximum decline was observed in the SOD activity in the kidney of animals that received 20,000 ppm iron overload (Table 4 and Figure 3). Hesperidin treatment before iron overload elevated the catalase activity significantly (Table 3 and Figure 3). This rise in the catalase activity was approximately 2-fold for 10,000 and 20,000 ppm hesperidin and iron treated animals (Table 3).

**Lipid peroxidation (LOO)**

The spontaneous lipid peroxidation in the mice kidneys was estimated to be 5.24 ± 0.19 nmol/mg proteins (Table 5 and Figure 5). The oral feeding of different doses of ferric chloride for 30 days raised the lipid peroxidation in a dose dependent but significant manner when compared to untreated control (Table 5 and Figure 5). The iron overload increased the lipid peroxidation approximately by 2, 3 and 4 folds in the mice kidneys receiving 5000, 10,000 and 20,000 ppm of ferric chloride for 30 days (Table 5). The administration of hesperidin alone did not increase the level of lipid peroxidation which was already similar to the spontaneous level (5.57 ± 0.07 nmol/mg protein). Treatment of mice with hesperidin for five days before oral administration of different doses of ferric chloride in drinking water for 30 days significantly reduced lipid peroxidation when compared to iron loaded group (Table 5 and Figure 5). A maximum reduction of 1.5 folds was recorded for 5000 ppm iron overload, whereas it was 1.4 folds for 10,000 and 20,000 ppm iron overload, respectively (Table 5).

**Discussion**

Large amounts of ingested iron can cause excessive levels of iron in the blood [60]. High blood levels of free ferrous iron react with hydrogen peroxide to produce hydroxyl free radicals, which are highly reactive and can damage DNA, proteins, lipids, and other cellular components.
Iron toxicity occurs when there is free iron in the cell, which generally occurs when iron levels exceed the capacity of transferrin to bind the iron [62]. Damage to the cells of the gastrointestinal tract can also prevent them from regulating iron absorption leading to further increases in blood iron levels [60]. Iron typically damages cells in the heart, liver and elsewhere, which can cause significant adverse effects including coma, metabolic acidosis, shock, liver failure, coagulopathy, adult respiratory distress syndrome, long-term organ damage, and even death [63]. The human body does not have any mechanism to remove excess iron, which is stored as a complex with ferritin protein and in certain conditions it is also stored as hemosiderin, a degradation product of ferritin [64,65]. This leads to iron-induced oxidative stress that subsequently damages several organs including kidneys, where it induces cancer and [64]. Iron is known to cause kidney injury in humans [60,66,67]. Therefore, present study was undertaken to evaluate the role of hesperidin, a citrus bioflavonoid in protecting the mice kidney against the iron-induced biochemical injury.

Glutathione or γ-glutamylcysteinylglycine (GSH) is a tripeptide, which controls thiol redox reactions and maturation of extra-mitochondrial iron sulphur clusters [68]. GSH exists in two forms the reduced and oxidized [68]. Glutathione insulates cytosolic function in iron metabolism by varying its concentration during redox stresses [69]. Glutathione participates in a number of redox reactions, which are vital to the cell. It plays a crucial role to neutralize the endogenous and exogenous toxic injuries in the cells [70]. Cells have inbuilt mechanisms to tightly regulate the concentration of iron. However, dysregulation of these mechanisms and iron overload stimulates production of free radicals leading to increased oxidative stress [71]. The endogenous glutathione may be active in neutralizing the free radicals produced by excess iron as a result there has been a dose dependent decline in the glutathione concentration in the mice kidney exposed to different doses of iron overload for 30 days. Iron treatment has been reported to reduce glutathione concentration in vitro and in vivo [72-74]. Pre-treatment of mice with hesperidin attenuated the iron induced decline in the GSH concentration in mice kidney. Similarly, quercetin has been reported to protect against the iron induced depletion in the glutathione in rat kidney [72]. Naringin has been found to alleviate the iron induced glutathione concentration in vitro [73,74].

Glutathione-s-transferases are a group of ubiquitous detoxifying enzymes, which are present in all aerobic eukaryotes, which protect cells from the toxic insults by deactivating hydrophobic cytotoxic, genotoxic compounds and reactive oxygen species [75]. The administration of mice with different doses of iron for one month in drinking water caused a dose dependent reduction in the glutathione-s-transferase activity in the kidney. A similar effect has been observed in the sera of Caucasian patients receiving iron therapy for the treatment of β-thalassemia, where GSTT1 and GSTM1 genes were down regulated [76]. This indicates that iron overload is detrimental to glutathione-s-transferase activity due to its ability to induce oxidative stress in the cells. Hesperidin treatment for five days before iron overload increased the glutathione-s-transferase activity in mice kidney, when compared to iron treatment alone. The reports regarding the estimation of GST activity in hesperidin treated iron overloaded mouse kidney are unavailable. However, hesperidin has been reported to attenuate iron-induced decline in glutathione-s-transferase activity in rat liver earlier [77].

Catalase or oxidoreductase (EC: .1.11.1.6) is a tetrameric enzyme, found in all those organisms that utilize oxygen for respiration and energy production. Catalase detoxifies H2O2, a strong oxidizing agent, generated during respiration, which is the major cause of tissue pathogenesis. The catalase contains four porphyrin heme groups, which allow it to interacts with H2O2 and decomposes it into water and molecular oxygen [78,79]. The exposure of mice to different doses of iron for 30 days decreased the activity of catalase in the mouse kidney. Likewise, rats exposed to iron overload has shown a reduction in catalase activity in their kidneys and livers earlier [72,77]. The mice fed with iron overload diet for four months also showed a reduced catalase activity in the liver [80]. Similarly, iron overload has been reported to attenuate the catalase activity in vitro [73,74]. The hesperidin treatment arrested this decline in the catalase activity by elevating the activity of catalase in mice kidney significantly. Hesperidin treatment has been reported to elevated catalase activity in the iron overloaded liver earlier [77]. Quercetin has been reported to augment the activity of catalase in the kidney of iron overloaded rats [72]. A similar effect has been detected with naringin, a citrus bioflavonoid in HepG2 cells and isolated liver mitochondria in vitro [73,74].

Superoxide dismutases are ubiquitously present in all organisms that use oxygen for their energy requirement. SOD acts as a signaling molecule, toxic agent or a harmless species [81]. All organisms that employ oxygen for respiration generate superoxide radical (O2•-) during respiration. Although O2•− per se is not highly reactive, it becomes reactive when it interacts with transition metal complexes of iron, copper and manganese leading to increased oxidative stress [82,83]. The SOD dismutates O2•− into less harmful product hydrogen peroxide and acts as a first line of defence against oxidative stress [82,83]. SOD also inactivates nitric oxide reducing oxidative stress. The iron overload in the mouse kidney has alleviated the SOD activity in a dose dependent
manner in the present study. Iron overload has been reported to deplete the SOD activity in rat kidney [72]. Likewise, iron overload has been reported to reduce the SOD activity in rat and mice liver earlier [77,80]. Hesperidin treatment for five consecutive days before iron overload led to a rise in the SOD activity in mice kidney in the present study. In an earlier study hesperidin has been reported to elevate SOD activity in iron overloaded rat liver [77]. Quercetin has been found to increase the SOD activity in the rat kidney [72]. Similarly, naringin treatment has been reported to elevate the SOD activity in iron overloaded HepG2 cells and isolated mice liver mitochondria earlier [73,74].

The inactivation of hydroxyl and hydroperoxyl radicals results in the formation of lipid peroxidation. Hydroxyl radicals are formed due to the redox cycling of Fe²⁺ via Fenton reaction, where iron reacts with H₂O₂ to produce hydroxyl radical. The Haber Weiss reaction regenerates Fe²⁺ when O₂⁻ reacts with Fe³⁺ [61,84]. The increased iron overload has been indicated in kidney cancer [64]. Therefore, it is pertinent to assay lipid peroxidation in the iron overloaded mice kidney. Iron overload has increased lipid peroxidation in the mouse kidney by 2 to 4 folds depending on the dose of iron. The rats receiving iron exhibited an increase in the lipid peroxidation in the rat kidney and liver [72,77,85]. Similarly, our earlier investigations have reported accelerated lipid peroxidation in vitro [73,74]. The lipid peroxidation showed an increase in mice liver fed iron diet for four months [80]. An increased lipid peroxidation was observed in the serum of iron overloaded patients [86]. The administration of hesperidin for five consecutive days retarded lipid peroxidation significantly in the kidney of iron overloaded mouse. A similar effect has been observed in iron overloaded rat liver treated with hesperidin earlier [77]. Quercetin has also been found to attenuate the iron-induced lipid peroxidation in the rat kidney in an earlier study [72]. Likewise, naringin has been reported to reduce lipid peroxidation in the HepG2 cells and isolated mice liver mitochondria in vitro [73,74].

The exact mechanism by which hesperidin reduced the iron induced oxidative stress is not clearly known. However, iron triggers the formation of free radicals via Fenton and Haber Weiss reactions during various metabolic activities [61,84] and presence of hesperidin before iron treatment might have blocked the generation of iron-induced free radicals resulting in the decline in iron-induced oxidative stress in the mice kidney. Our earlier study has shown that hesperidin inhibited the production of different free radicals in vitro [51]. The iron overload triggered the transcriptional activation of IKKβ resulting in the transcription of NF-κB, and TNF-α and also activation of COX-II, and prostaglandins that stimulate inflammation and are responsible for pathophysiology [87,88]. Hesperidin has been found to inhibit the stimulation of NF-κB and COX-II earlier [89,90], which may have led to the alleviation in the iron induced oxidant status in the kidney of hesperidin pre-treated group. The Nr2f activation has been reported to inhibit iron accumulation in the mouse liver the site of iron accumulation and metabolism [91]. Hesperidin has been reported to stimulate Nr2f in rat kidney, which may have reduced the accumulation of iron and reduced the iron-induced oxidative stress [92].

Conclusion

The present study demonstrates that hesperidin treatment reduced iron-induced biochemical injury in the mice kidney alleviating, lipid peroxidation and increasing the glutathione, glutathione-s-transferase, catalase and superoxide dismutase levels. This action of hesperidin may be mediated by free radical inhibition and suppression of NF-κB, COX-II, prostaglandins and TNF-α activation. The Nr2f activation by hesperidin may have also contributed to reduced iron-induced oxidative stress in the mouse kidney. Our study indicates the utility of hesperidin to reduce iron-induced oxidative stress in clinical situation.

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