ROLE OF QUERCETIN AND DEFEROXAMINE IN MITIGATING THE EFFECTS OF IRON OVERLOAD ON SOME SERUM CRITERIA IN RABBITS (PART-II)

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Article History: Received: 12.12.2022 **Revised:** 29.01.2023 **Accepted:** 15.03.2023

Abstract

The current study aimed to assess the ability of oral quercetin to treat iron overload and compare it with deferoxamine (DFO). Four groups (each of seven) of adult male New Zealand rabbits with six months of age used in the current study were treated as follows for 28 days: Group C: Animals were injected intraperitoneally (I/P) with normal saline every 72 hours + normal saline orally each day (negative control). T1, T2, and T3 groups were I/P injected with iron dextran (100 mg/kg) once every 72 hours. Additionally, T2 group animals were treated with quercetin (350 mg/kg) once per day for 28 days, and T3 group animals were injected with DFO (125 mg/kg) intramuscularly once per day for 28 days. After 24 hours since the last administration, animals were anesthetized and sacrificed, and blood samples were taken directly from the heart to obtain blood serum. The result showed a significant decrease in serum iron, total iron binding capacity (TIBC), and transferrin saturation percentage in the T2 and T3 groups compared with the T1 group, while, hemoglobin didn't show any significant difference between the T1, T2, and T3 groups. T2 and T3 also showed a significant decrease in malonaldehyde (MDA) and a significant increase in glutathione peroxidase (GPX) concentrations compared with the T1 group. Conclusion: Quercetin has a stronger effect as an iron chelating agent due to its antioxidant properties than deferoxamine in iron-overloaded rabbits. These results suggest that quercetin could be effective in the treatment of iron overload in the clinic.*Key words*: Quercetin, Deferoxamine, Iron overload, Rabbits, ferritin, HB, TIBC

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DOI: 10.31838/ecb/2023.12.s2.230

1. Introduction

Iron overload problem has great interest in the hematological diseases field (Fernández-Real and Manco, 2014). It is one of the important body elements that maintains normal physiological functions such as DNA synthesis, oxygen and electron transport, and enzymes reactions, iron absorbed from the intestines does not have any physiological route of excretion (Fleming and Ponka, 2012).

Iron overload produces reactive oxygen species (ROS) and contributes to oxidative stress (OS) (Gholampour et al., 2017; Hamed, and Al-Qayim, 2019). When storge proteins become saturated with iron, free iron accumulates in the tissue and plasma, this includes labile plasma iron and non-transferable. bound iron, also cells take free iron to form labile iron pools. (Hsu *et al.*, 2022). Iron overload is a serious clinical disease since it can lead to the dysfunction of several organs, such as the liver, heart, joints, skin, and endocrine glands (Camiolo, 2019; Dos Santos et al., 2022). Excess free iron in the plasma can be produce lipid peroxidation (LPO), which leads to cellular dysfunctions, and several chronic diseases, such as hematological diseases and cancer. Additionally, iron overload is a marker of ferroptosis that leading to LPO accumulation at lethal levels (Hasan and Mahmoud, 2018; Wu e t al., 2021).

Iron chelation therapies are a life-saving treatment for disorders caused by an iron overload (AbdElkader and Aly, 2015; Reddy et al., 2022). Due to its extensive clinical history and inexpensive cost, the iron chelator deferoxamine (DFO) remains a first-line treatment choice for reducing iron overload in the thalassemia patients (Musallam et al., 2013). But it has several side effects (Velasquez and Wray, 2022). Flavonoids may have antioxidant properties, iron chelation, and be less toxic due to their unique structures, so the flavonoids become an important topic as iron chelators treatment (Khudair and Naji, 2012; Al-Jumaily and Al-Isawi, 2014). Quercetin is an example of a natural compound; it is found in different fruits and vegetables such as apples, green tea, onions, cauliflower, nuts, berries, broccoli, and cabbage (Al-Momen et al, 2020; Di Petrillo et al., 2022). Quercetin has many functions, especially antioxidant properties (AL-Awady and AL-Zamely, 2016; Qi *et al.*, 2017). Also, it acts as chelating metals and ROS scavengers, so it can mitigate cell damage caused by lipid peroxidation (LPO) (Aziz and khudair, 2021). The mechanism by which flavonoids treated the iron overload depends on its antioxidant properties and iron chelation (Al-Okialy ,2012; Chen et al., 2020). However, a study found that, in addition to the antioxidant mechanism of quercetin, it has a double effect on reactions of the hemoglobin (Hb) redox, aggravating Hb–H2O2 induced protein oxidation with low concentrations while protecting with high concentrations, which provides new information about the pharmacological implications of it in diseases of iron overload (Lu et al., 2013).

So, the current study aimed to investigate the ability to use oral quercetin as a natural substance to mitigate iron overload in adult rabbits and compare it with DFO

2. Materials and Methods

Experimental design:

Twenty-eight adult male New Zealand rabbits were used in the current study, six months old and weighed 1.7 - 2 kg. They were obtained from the animal's house at the Veterinary Medicine College/University of Baghdad / Iraq. The animals were reared under controlled conditions at 22°–25°C. and divided randomly into four groups (each of seven) and treated for 28 days as follows: Group C: Animals were injected intraperitoneally (I/P) with normal saline every 72 hours + normal saline orally each day (negative control). T1, T2, and T3 groups were I/P injected with iron dextran (100 mg/kg) once every 72 hours (Khudiar and Naji, 2012). Additionally, T2 group, animals were treated with quercetin (350 mg/kg) orally once per day, and T3 group animals were injected with DFO (125 mg/kg) intramuscularly (I/M) once per day (El-Sheikh et al., 2018).

Blood Samples Collection:

Before and during the experiment, the animals were weighed weekly because quercetin and DFO were given according to weight. Then animals were anaesthetized by mix Ketamine + xylazin (9mg/kg/b. w,10mg/kg/B. W) respectively/IP and sacrificed. About 5 ml of blood specimens were collected directly using heart puncture (Parasuraman et al., 2010), then put in gel tubes and centrifuged to isolate the serum, which was kept at -18. Other blood samples were collected and placed in an EDTA tube containing anticoagulant for hemoglobin analysis.

Serum Criteria:

Serum iron, total iron binding capacity (TIBC), and ferritin assays in blood serum were estimated using enzymatic kits (biosystem/Spain) and the spectrophotometer device.

Transferrin saturation percentage (%) = serum iron concentration / TIBC×100. (Eleftheriadis *et al.*, 2010).

Hemoglobin assays in blood were done using a blood analyzer machine (Genix, USA). Furthermore, malonaldehyde (MDA) and glutathione peroxidase (GPX) were determined by

using enzymatic kits (Biosolar, China), (Northwest, USA) respectively and the spectrophotometer device.

Statistical analysis:
The data were analyzed statistically using the computer program SPSS version 24. The values expressed as mean± SE. Statistical analysis performed basis on one way ANOVA with lest significant difference (LSD) at P0.05 was used to compare between groups (Baarda et al., 2019).

3. Results

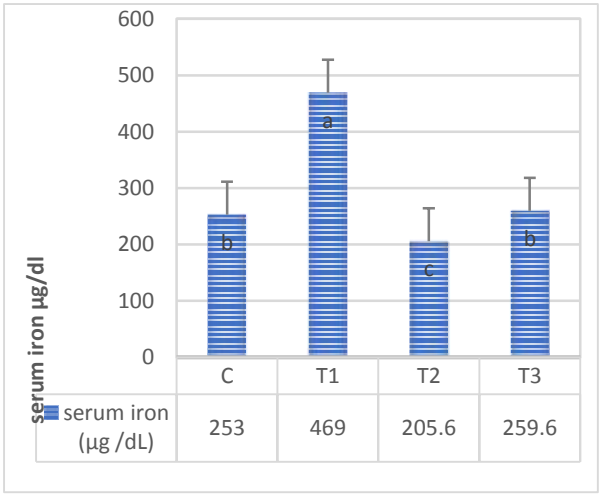
Serum Iron:
The results in the figure (1-A) revealed a significant (p≤0.05) increasing in T1 group (469±9.06) compared with other groups, and T2 group (205.6±2.15) showed a significant decrease compared with other groups, while control and T3 groups (253±1.83), (259.6±1.72) respectively not showed any significant difference between them.

Total Iron Binding Capacity (TIBC):
The results in the figure (1-B) revealed a significant (p≤0.05) increasing in the C and T1 group (244±2.96) (241.8±2.49) respectively, compared with other experimental groups, while T2 group (205.8±3.14) showed a significant (p≤ 0.05) decrease compared with T3 group (220.8±2.08).

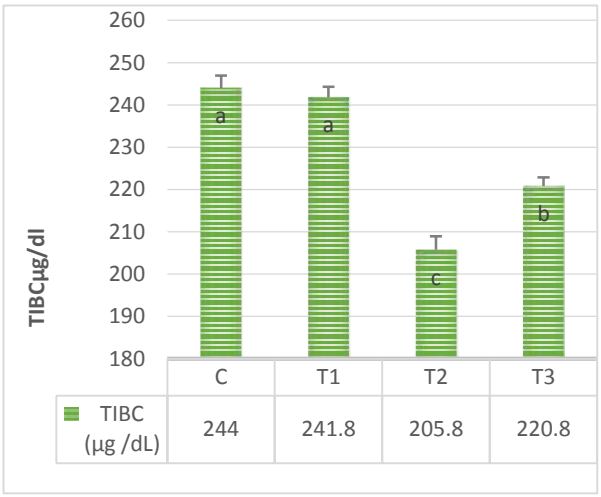
Transferrin Saturation Percentage:
The results in the figure (1-C) revealed a significant (p≤0.05) increasing in the T1 group (193.6±5.54) compared with other groups, C group (73.6±1.28) showed a significant (p≤ 0.05) decrease compared with other groups, while T3 group showed (117.2±1.49) a significant (p≤ 0.05) increase compared with T2 group (99.8±2.47).

Ferritin:
The results in the figure (1-D) revealed a significant (p≤0.05) increasing in the T1 group (61.32±0.32) compared with other experimental groups. C group (24.37±0.30) showed a significant (p≤ 0.05) decrease compared with other groups, while there was a significant (p≤ 0.05) increase in T3 group (34.06±0.20) compared with T2 group (32.37±0.29).

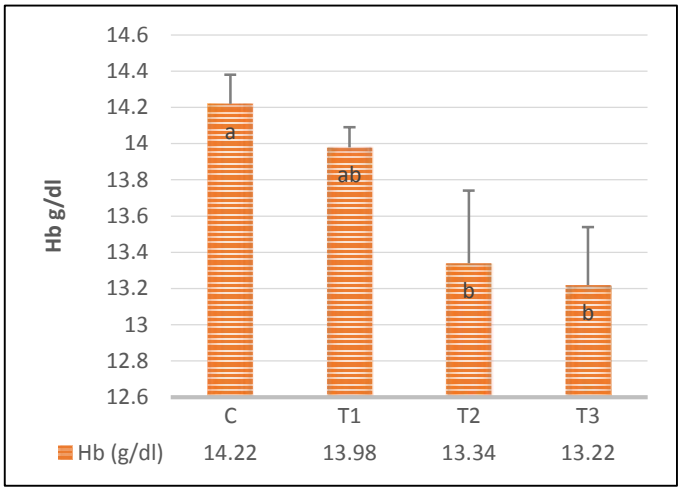
Hemoglobin:
The results in the figure (1-E) revealed a significant (p≤0.05) increasing in the C group (14.22±0.16) compared with T2 and T3 groups (13.34±0.40), (13.22±0.32) respectively, while there was no significant difference among T1, T2 and T3 groups (13.98±0.11), (13.34±0.40) and (13.22±0.32) respectively.



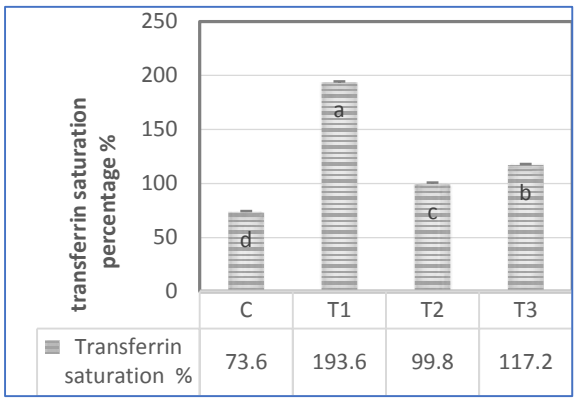
1-A



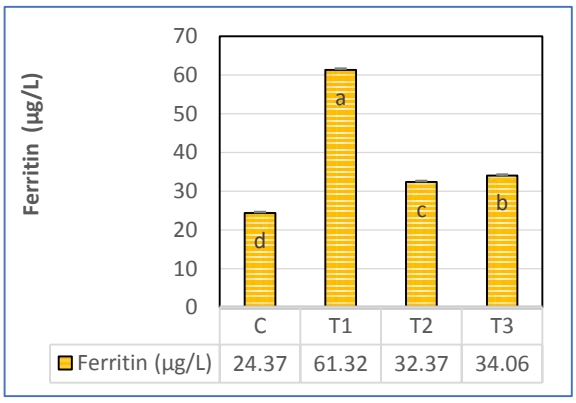
1-B



1-E



1-C



1-D

Figure (1) Effect of Quercetin and DFO on Serum Iron (A), Total iron binding capacity (TIBC) (B), Transferrin Saturation Percentage (C), Ferritin (D) and Hemoglobin (E) in Adult New Zealand Rabbits Exposed to Iron Overload by Iron Dextran for 28 days/ Iraq

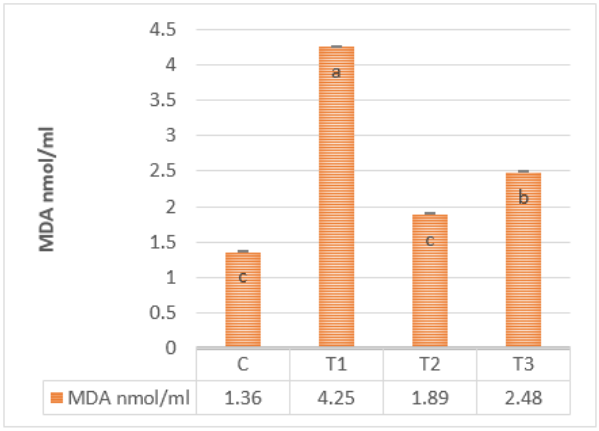
Values are expressed as mean± SE. n= 7.
Group C: Animals were injected intraperitoneally with normal saline every 72 hours + normal saline orally each day (negative control). T1, T2, and T3 groups were injected intraperitoneally with iron dextran (100 mg/kg) every 72 hours Additionally, T2 animals were treated with quercetin (350 mg/kg) orally every day, and T3 group intramuscularly (I/M) animals were injected with deferoxamine (DFO) (125 mg/kg) orally every day Different letters means significant differences ($p \leq 0.05$) between shaps.

Malonaldehyde (MDA):

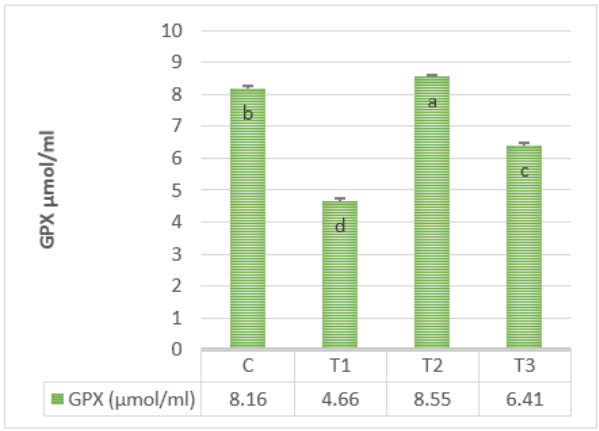
The results in the figure (2-A) revealed a significant ($p \leq 0.05$) increasing in the T1 group (4.25±0.01) compared with other groups, while C and T2 groups (1.36±0.01), (1.89±0.02) respectively showed a significant ($p \leq 0.05$) decrease in MDA levels compared with T3 group (2.48±0.02).

Glutathione peroxidase (GPX):

The results in the figure (2-B) revealed a significant ($p \leq 0.05$) increasing in the T2 group (8.55±0.07) compared with other groups. T1group (4.66±0.07) showed a significant ($p \leq 0.05$) decrease compared with control and T3 groups (8.16±0.1), (6.41±0.08) respectively, while C group (8.16±0.1) revealed a significant ($p \leq 0.05$) increase in compared with T3 group (6.41±0.08).



2-A



2-B

Figure (2) Effect of Quercetin and DFO on malonaldehyde (MDA) (A) and glutathione peroxidase (GPX) (B) in Adult New Zealand Rabbits Exposed to Iron Overload by Iron Dextran for 28 days/ Iraq

Values are expressed as mean± SE. n= 7.
Group C: Animals were injected intraperitoneally with normal saline every 72 hours + normal saline orally each day (negative control). T1, T2, and T3 groups were injected intraperitoneally with iron dextran (100 mg/kg) every 72 hours Additionally, T2 animals were treated with quercetin (350 mg/kg) orally every day, and T3 group intramuscularly (I/M) animals were injected with deferoxamine (DFO) (125 mg/kg) orally every day Different letters means significant differences ($p \leq 0.05$) between shaps.

4. Discussion

The current study aimed to investigate the quercetin effects as a chelate on iron overload and compare it with DFO. All of the animals that were injected with iron dextran showed an increase in serum iron levels, which was comparable to many other studies that used the same treatment protocol (Marques et al., 2019; Jensen et al., 2020). The current study found that quercetin effects on iron criteria were better than those of DFO.

Rising serum iron were a marker of iron poisoning, in the current study, the T1 group had a significant increase in iron levels, whereas in T2 group after quercetin administration, the iron level was reduced compared to the T1 group, these findings are consistent with previous studies that have shown quercetin to be an iron chelator by suppressing iron overload-induced toxicity (Horniblow et al., 2017; El-Sheikh et al., 2018; and El-Sayed et al., 2019). Many flavonoids have phenolic hydroxyl structures and multiple metal binding sites, so it has good oxygen reduction properties, as a result, flavonoids reduce iron accumulation in tissues by forming complexes with it for excretion, also, flavonoids impede ROS formation, and decrease LPO, that leads to the cells damage (Wang et al., 2021). Study has recently demonstrated that flavonoids play an essential role in iron management and are believed to be developing as medications for the iron homeostasis problems treatment (Chen et al., 2020).

The results showed that ferritin levels in the group treated with flavonoids were reduced compared with iron overload group. Thus, in iron overload cases, the increased amount of non-heme iron that is not bound to transferrin is responsible for free radicals' formation (Yas, 2020). In the T1 group, increased serum iron leads to an increase in ferritin production from the liver, to reduce free iron (Shalitin et al., 2005). Transferrin saturation percentage increased to bind free iron and avoid its toxic effects, decrease TIBC, is one of the iron overloads signs, the findings of current study show that quercetin significantly reduces ferritin levels in the T2 group, indicating that quercetin not only prevents ferritin from increasing but also decreases it, quercetin reduced iron overload by decreasing free serum iron and transferrin saturation while increasing transferrin (Hezaveh et al., 2019). TIBC decreased in theT2 and T3 groups, but it was more pronounced in the quercetin group, this result comes from decreased serum iron levels.

Quercetin significantly reduced hepatic iron, decreased ferritin levels, and increased iron excretion through the feces in iron overloaded mice induced by iron-dextran (Zhang et al., 2006; Zhang et al., 2011). Quercetin mitigates hepcidin expression suppression mediated by ethanol in mice (Tang et al., 2014). As well as decreased duodenal iron absorption and reduce iron efflux from the subsequent basolateral into the blood circulation (Lesjak et al., 2014). The flavonoid's ability to chelate iron may depend on time and dose. Moreover, flavonoids suffer a number of challenges, including low oral consumption rates and water insolubility (Wang et al.,2021). The

hemoglobin concentration didn't show any significantly difference between the C and T1 groups, The reason for this is that hemoglobin is only synthesized when the body requires it, such as in cases of hypoxia (Storz and Moriyama, 2008).

Our findings suggested that DFO treatment reduced serum iron, this is in agreement with studies, (Bellotti and Remelli, 2021; Takpradit et al., 2021). DFO binds to excess iron in cells and free plasma iron is then excreted in the urine or bile (Velasquez and Wray, 2022). DFO chelates free iron that transits between transferrin and ferritin, hemosiderin, and ferritin, but DFO cannot bind iron that is already bound to transferrin, hemoglobin, or cytochromes (Hershko et al., 2001).

DFO a significantly decrees ferritin level compared with iron group, this agreed with (Hajigholami *et al*., 2018). Serum ferritin amount in DFO group is very low compared with iron group.

The findings of current study showed the iron overload ability to create OS with antioxidant alterations, A significant increase was observed in MDA and decreasing in GPX concentrations in the T1 group when compared with other groups, in contrast the quercetin treated group revealed a significant decrease in MDA and increasing in GPX concentrations, which is consistent with the findings of other studies (Abdelkader and Aly, 2015; El-Sheikh et al., 2018). MDA is a good marker of ROS and formation of OS (Awadi et al., 2016; Alwan and Al-Okialy, 2018). Iron can induce OS through Fenton reaction, that able to damage cell macromolecules, which are involved in cells pathogenesis (Kremastinos et al., 2010; Kim and Leitch, 2021; and Ascar et al., 2022). In current study, the quercetin effects on decreasing MDA levels and ameliorating GPX were better than those of DFO.

In *vitro*, quercetin has been well-known to be an effective LPO inhibitor (Gheshlaghi-Ghadim et al., 2022). Quercetin can reduce ROS and inhibit OS in the T2 group directly by chelating iron and restricting iron's participation in redox reactions (Uzun and Kalender, 2013). and antioxidant properties, interferes with nitric oxide synthase and prevents irreversible cell membrane damage (Li et al., 2016a). Due to its antioxidant properties of flavonoid (AL-Okaily *et al*., 2012). Quercetin also prevents cardiac muscle damage by removing ROS caused by lipopolysaccharide induced endotoxemia, demonstrating that quercetin improved antioxidant defenses (Akkoyun et al., 2016; Abood and Al-Okialy, 2019). In the current study, DFO significantly reduced the MDA levels when compared with the control and iron groups, DFO administration reversed the MDA levels and DPX in iron overload rabbits, the findings of current study were agreement with (Dinc et al., 2013; Zhang et al., 2015). DFO has a protective effect against cardiotoxicity in lead (Pb) treated rats, possibly due to reducing MDA and increasing endogenous antioxidant capacity and GSH levels (Gazeri and Aminzadeh, 2020; Al-Okaily and Murad, 2021). The DFO ability to bind iron in linoleic acid suspension, explanation its mechanism as antioxidant by an electron donor and its ability to react with the OH or the $O2^{-}$ (Holden and Nair, 2019).

5. Conclusion

Quercetin alleviates the redox status of iron-overloaded rabbits, indicating that quercetin may prevent cell damage due to its ability to eliminate iron and scavenge free radicals in comparison to DFO. These results suggest that quercetin could be effective in the treatment of iron overload in the clinic.

Declarations

Data availability

Data derived from public domain resources.

Authors' contribution

The experiment was designed and the study was supported technically by Baraa Najim Al-Okaily. Muntasser Alawi Awad performed the experiment and analyzed and interpreted the data.

Acknowledgments

The authors would like to thank the University of Baghdad / Faculty of Veterinary Medicine for allowing us to conduct the current research and for all of the resources and assistance they gave.

Competing interests

There was no conflict of interest.

Ethical consideration

Ethical issues have been checked by the authors.

Funding

The work received no financial assistance

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