



## Determination of the Effective Dose of Quercetin in Iron Overload Rabbits

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### Abstract

The current study aimed to assess the effective dose of quercetin against iron overload in the adult rabbits by studying the dose response curve through using five successive doses of quercetin on serum iron and total iron binding capacity (TIBC) in iron overload rabbits. Thirty-six of adult male rabbits were used in current study and randomly divided into the six groups and handled as follows for 28 and 42 days: Control group: rabbits were intraperitoneally (IP) injected with normal saline every 72 hours + normal saline orally each day while rabbits in the T1, T2, T3, T4 and T5 groups were administered orally quercetin at doses 100,200,300,400 and 500 mg/kg B.W daily respectively and all animals were IP injected with iron dextran at a dose of 100 mg/kg every 72 hours. Blood samples were collected after 28 and 42 days from ear vein. The results showed a significant increase in serum iron of T1 and T2 groups, while T3, T4, and T5 groups did not show any significant difference when compared with the control group, while TIBC in T4 and T5 did not show any significant difference when compared with the control group after 28 and 42 days of quercetin treatment. Conclusion: As a result, obtained after 28 days of the experiment was used to calculate the ED of quercetin on iron overload which is 350 mg/kg.

**Key words:** Quercetin, Iron overload, Effective dose, Rabbits

### Introduction:

Iron is the most abundant metal on earth and an important component of biological systems, where it is required for cell proliferation, energy production, oxygen transportation, and the destruction of pathogens (1). Iron overload produces reactive oxygen species (ROS) and contributes to the generation of oxidative stress and produces lipid peroxidation (LPO), which leads to cellular dysfunctions, and several chronic diseases, such as hematological diseases and cancer (2) and (3). alter oxidant-anti-oxidant status (4). Iron toxicity comes from non-transferrin-bound iron (NTBI), which appears in the blood when transferrin saturation is above 45%. NTBI are a species of iron with a parenchymal target that is unregulatable and possibly harmful (5, 6). Since iron overload can cause the dysfunction of many organs, including the liver, heart, joints, skin, and endocrine glands, it results in a serious clinical condition (7). Additionally, iron overload is a marker of ferroptosis that

regulates cell death, leading to LPO accumulation at lethal levels (8, 9).

Iron chelation therapy is a life-saving treatment for disorders caused by an iron overload (10). 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 (11, 12). Quercetin is an example of naturally occurring flavonoids; it is found in different fruits and vegetables (13). A recent study showed the antioxidant properties of quercetin are crucial in the treatment and prevention of different diseases such as osteoporosis, tumors, and cardiovascular diseases (14). By mitigating cell damage caused by oxidative stress via chelating metals, scavenging ROS, and protecting against LPO (15, 16). The 3-hydroxyl and 4-carbonyl groups of quercetin, are the favored sites for iron chelation (17). Additionally, the mechanism by which flavonoids treated the iron overload depends on its antioxidant properties and iron chelation (18, 19). 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-H<sub>2</sub>O<sub>2</sub> 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 (20).

Therefore, the current study aimed to assess the effective dose of quercetin against iron overload in the adult rabbits by studying the dose response curve through using five successive doses of quercetin on serum iron and total iron binding capacity (TIBC) in iron overload rabbits.

### Methodology:

#### Animals and experimental design:

Thirty-six adult male rabbits were used in the current study; six months old and weighed between (1.7 - 2 kg). They were obtained from the animal's house at the Veterinary Medicine College/University of Baghdad. The animals were reared under controlled conditions at 22°–25°C. Animals were equally divided randomly into six groups and treated for 28 and 42 days as follows: Control: rabbits were intraperitoneally (IP) injected with normal saline every 72 hours + normal saline orally each day whereas the T1, T2, T3, T4 and T5 rabbits were administrated quercetin with the purity of 95% (Brightol, China) at 100, 200, 300, 400 and 500 (mg/kg.B.W) daily orally. All animal were injected with iron dextran (O.L.KAR-AgroZooVet, Ukraine) IP at dose of 100 (mg/kg B.W) every 72 hours (11). Before and during the experiment, the animals were weighed weekly because quercetin was given according to weight. At 28 and 42 days, blood samples were collected from the ear vein, then put in gel tubes and centrifuged to isolate the serum, which was kept at -18 till analysis of serum iron and total iron binding capacity (TIBC) using the spectrophotometer technique according to the company's instructions (Biosystem/Spain). 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  $P \leq 0.05$  was used to compare between groups (21).

#### Ethical approval

The Ethical committee at the College of Veterinary Medicine, University of Baghdad approved the current study procedures.

#### Result:

The effect of different doses of quercetin on mean values of serum iron concentration of male rabbits after 28 days was showed in figure (1-A) a significant ( $p \leq 0.05$ ) increasing in T1 and T2 groups ( $267.2 \pm 7.5$ ), ( $261.4 \pm 13.3$ ) respectively compared with other groups, while the C, T3, T4 and T5 groups showed no significant difference ( $p \leq 0.05$ ) among them with mean values were ( $192.8 \pm 12.9$ ), ( $199.8 \pm 22.5$ ), ( $217 \pm 9.7$ ) and ( $214 \pm 7.5$ ) respectively.

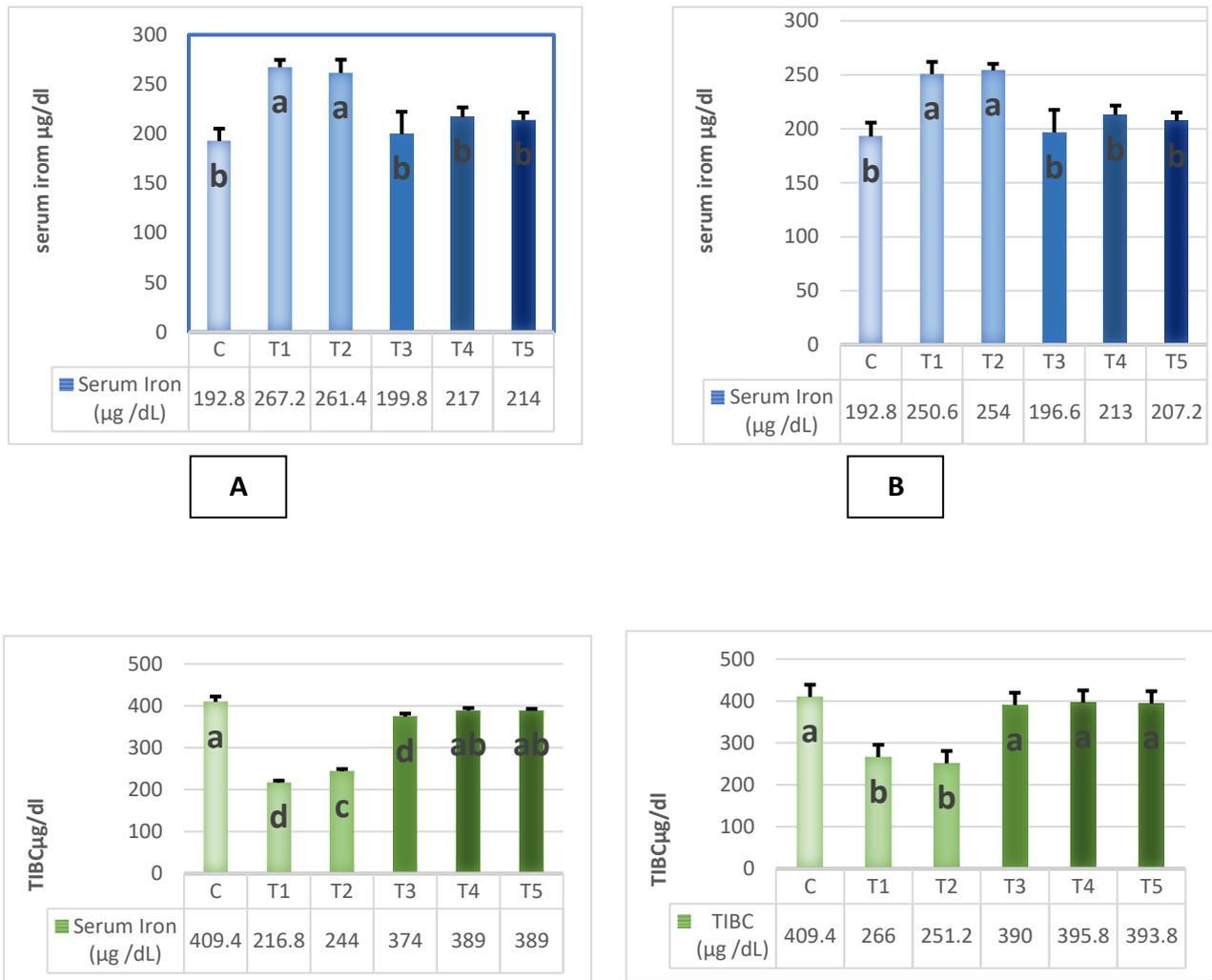
The effect of administration of quercetin for 42 days with an escalating five successive doses on serum iron in iron overload rabbits are clarified in the figure (1-B) showed a significant ( $p \leq 0.05$ ) increasing found in T1 and T2 groups ( $250.6 \pm 11.4$ ) and ( $254 \pm 6.2$ ) respectively compared with other groups. Whereas non-significant difference between the C, T3, T4, and T5 groups were observed with the mean values ( $192.8 \pm 12.9$ ), ( $196.6 \pm 20.9$ ), ( $213 \pm 8.5$ ), ( $207.2 \pm 185.45$ ) respectively.

The data pertaining to the total iron binding capacity (TIBC) of the control and quercetin treated groups after 28 days were depicted in the figure (1-C), comparing to the control, T4 and T5 groups, rabbits received quercetin in the T1, T2 and T3 groups, revealed a significant ( $p \leq 0.05$ ) decreasing in serum TIBC after 28 days of. Besides, T4 ( $389 \pm 5.6$ ) and T5 ( $389 \pm 3.6$ ) groups showed non-significant difference ( $p \leq 0.05$ ) when compared with control group ( $409.4 \pm 12.6$ ).

Figure(1-D), demonstrate the mean values of TIBC concentration of control and other treated groups with successive doses of quercetin in iron over load rabbits by iron dextran after 42 days. There was a significant ( $p \leq 0.05$ ) decreasing in TIBC in T1 ( $266.2.9$ ) and T2 ( $251.2 \pm 5.9$ ) groups compared with



other groups. while TIBC concentration in T3, T4, T5 and control groups, not showed any significant difference when compared between them. The mean values were (390±4.3), (395.8±5.8), (393.8±3.5) and (409.4±12.6) for T3, T4, T5 and control respectively.



**Figure (1)** Effect of successive doses of quercetin on serum iron (A) and 42 days (B) and TIBC (C) at 28 days (C) and 42 days (D) in iron overload rabbits by iron dextran.

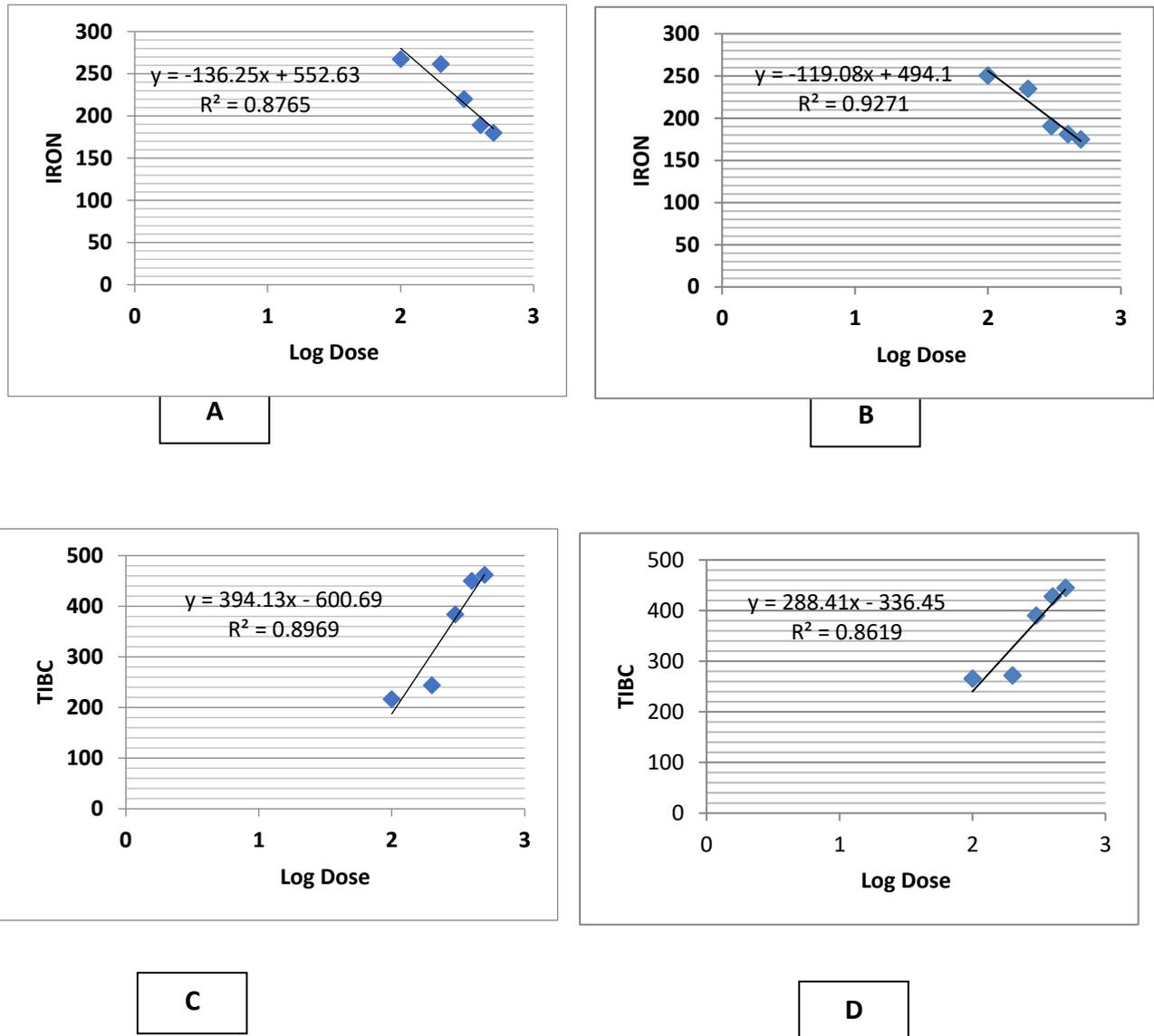
**a**

Values are expressed as mean ± SE. n=6

**Control:** rabbits were I/P injected with normal saline every 72 hour + normal saline orally each day.

**T1, T2, T3, T4 and T5:** rabbits were administrated quercetin 100,200,300,400 and 500 mg/kg B.W daily and all animal injected with iron dextran 100 mg/kg B.W every 72 hours

Different letters denote significant differences between groups.



**Figure (2) Effect of different successive doses of quercetin on serum iron concentration after 28 days (A) and 42 days (B) and TIBC after 28 days (C) and 42 days(D) in adult rabbits administrated iron dextran**

**Determination the effective dose (ED) of quercetin.**

Depending on the results shown in figure 1- A, B, C and D, maximal significant changes (increment and decrement) in the above parameters were recorded after 28 days of quercetin treatment in male rabbits. Accordingly, the results obtained after 28 days of the experiment were used for estimation of the ED of quercetin as follows:

The results in the Figure (2-A) and (2-B) after 28 and 42 days respectively, showed a negative relationship between serum iron concentration and five successive doses of quercetin.

Correlation coefficient was equal (0.8765) and (0.9271) after 28 and 42 days respectively. From the equations the estimated ED of quercetin that cause a marked



decrement in serum iron concentration was found to be equal to 350 (mg/kg). The results in the figure (2-C) and (2-D) after 28 and 42 days respectively, showed a positive relationship between serum TIBC concentration and five successive doses of quercetin. Correlation coefficient was equal (0.8969) and (0.8619) after 28 and 42 days respectively. From the equation the estimated ED of quercetin that cause a marked elevation in serum TIBC quercetin concentration was found to be equal to 350 (mg/kg). There for, depending on the ED of serum iron and TIBC figure (2) (A and C) the estimated ED of quercetin was equal to 350 (mg/kg) (22).

### Discussion:

In current study we chose the intraperitoneal model of iron dextran injection to exclude the iron chelation in the intestine before absorption. In the current study, the quercetin effect on iron overload for the first time has been investigated. According to the findings, 28- and 42-days treatment with quercetin could significantly decreased serum iron, and ameliorate TIBC.

Increase non-heme serum iron level consider one of the symbols of iron overload (23). Studies on iron overloaded mice induced by iron dextran showed that quercetin injected reduced serum ferritin and hepatic iron overload and increase the iron excretion through fecal (24). The mechanism by which flavonoids decrease non-haem iron bioavailability is not understood, however his believed that flavonoids can be chelate non-haem iron (25). Quercetin, forms a complex with  $Fe^{3+}$ , like other flavonoids with more stability than  $Fe^{2+}$  Even though when quercetin forms a complex with  $Fe^{2+}$ , it will be auto oxidase to  $Fe^{3+}$  (26). A second possibility is that quercetin could form a complex with free iron after influx into the cell.

Furthermore, quercetin has been demonstrated to act as a substrate for duodenal cytochrome b (Dcytb) by enhancing its reduction potential and supplying additional  $Fe^{2+}$  for cellular absorption by divalent metal transporter 1 (DMT1) (27).

Quercetin consists from A, B and C phenolic rings, with keto and hydroxy groups quercetin have ability to chelate metals and forming complexes after bind with it (28).

Researchers found that quercetin mitigated hepcidin expression suppression by ethanol-mediated (29, 30). As well, quercetin can decrease the iron absorption from intestinal and decreased iron efflux into the circulation (31). Human studies reported showed, in a clinical experiment, with silymarin intervention with 6 months, quercetin could a significantly reduce serum iron and ferritin in compared to the placebo group (32).

The flavonoid's ability to chelate iron may be affected by dose, concentration, and pH; all these should be noted in the studies (33). It is difficult to say if flavonoids have considerable benefits in this regard, it's also worth investigating if flavonoids, which chelate iron and prevent LPO, can play a role in ferroptosis. Moreover, flavonoids suffer a number of challenges, including low oral consumption rates and water insolubility (34, 35).

Analysis of current data showed that quercetin is effectively reduce serum iron. We conclude that quercetin supplement is a useful iron chelating and its considered one of the most promising materials in reducing iron overload.

**Conclusion:** As a results, obtained after 28 days of the experiment were used to calculate the ED of quercetin on iron overload which is 350 mg/kg

**Conflict of interest:** There is no conflict of interest in the present study as stated by the authors.

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