

# Quercetin Prevents Body Weight Loss Due to the Using of Superparamagnetic Iron Oxide Nanoparticles in Rat

## Abstract

**Background:** Superparamagnetic iron oxide nanoparticles (SPION) have been largely considered for numerous applications in biomedicine such as magnetic resonance imaging, hyperthermia, cell tracking, anticancer treatment, and targeted delivery of drugs or genes. However, they may have side effects such body weight loss. Quercetin (QT), a strong antioxidant and free radical scavenger and a natural flavonoid, has a wide range of biological and therapeutic effects. In this study, the effect of QT on prevention of weight loss due to the using of SPION has been investigated. **Materials and Methods:** SPION and QT-SPION were administered orally at 50 and 100 mg/kg for 7 days. Then, the body weight was measured at the beginning and the end of the study. **Results:** Rats fed with 50 and 100 mg/kg SPION showed a significant weight loss, whereas those that fed with 50 mg/kg QT-SPION did not. A weight loss was observed in rats treated with 100 mg/kg of QT-SPION. **Conclusions:** The results of this study showed that quercetin could prevent weight loss due to the SPION.

**Keywords:** Nanoparticles, quercetin, superparamagnetic iron oxide, weight loss

## Introduction

The quercetin (3,3',4',5,7-pentahydroxyflavone) as a flavonoid is biologically active compound that is found in eatable fruits, vegetables, and medicinal plants such as onions, cabbage, and apples in quantities of 15–30 mg kg<sup>-1</sup> of fresh produce.<sup>[1-4]</sup> Quercetin (QT) has antitumoral,<sup>[5,6]</sup> antiallergic,<sup>[7]</sup> antioxidant effects,<sup>[8]</sup> vasorelaxation,<sup>[9]</sup> anti-ischemic,<sup>[10,11]</sup> antiviral,<sup>[12]</sup> and anti-inflammatory activity.<sup>[13,14]</sup> Furthermore, it has some other beneficial effects in metabolic disorders<sup>[15]</sup> and protection of neurons.<sup>[16-20]</sup>

Furthermore, QT is considered as an ergogenic supplement.<sup>[15,21,22]</sup> QT has the ability to increase aerobic exercise performance and oxidative metabolism.<sup>[22]</sup> Furthermore, adiposity reduction of QT has been studied.<sup>[23]</sup> Different studies show various effects of quercetin on weight gain. A study shows a reduction in weight gain in fat rats fed QT,<sup>[24,25]</sup> whereas another study results showed no effect of QT on weight gain in thin rats.<sup>[26-28]</sup>

Abovementioned beneficial effects of QT make it a potential therapeutic alternative for various disorders. However, because of

the low water solubility, short biological half-life, low oral bioavailability, and poor body bioavailability of QT, it is necessary to use efficient drug delivery systems to improve QT effectiveness.<sup>[29-31]</sup> Therefore, a delivery system such as superparamagnetic iron oxide nanoparticles (SPION) could overcome these limitations. SPION have generated a lot of interests in biomedical applications such as magnetic resonance imaging, magnetic separation, targeted drug and gene delivery, cell tracking, tissue engineering, bioseparation, and magnetic hyperthermia.<sup>[32]</sup> However, biocompatibility and biosafety of SPION are needed to be considered. As long as we search literature, the effect of SPION on weight loss is not broadly investigated. Consequently, the aim of the current study was to evaluate the effect of SPION and QT conjugated with SPION on weight gain in rats.

## Materials and Methods

All the reagents were analytical grade and used without further purification and in all experiments, deionized water was used. The chemicals used in this work were purchased from Fluka, Merck and Sigma–Aldrich chemical companies.

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## Synthesis of quercetin conjugated with superparamagnetic iron oxide nanoparticles

QT conjugated Fe<sub>3</sub>O<sub>4</sub> nanoparticles (NPs) were prepared based on the existing literature with some modifications.<sup>[33]</sup> First, dextran-coated Fe<sub>3</sub>O<sub>4</sub> NPs were synthesized through a chemical coprecipitation method. Briefly, a mixture including of FeCl<sub>3</sub> anhydrous, FeCl<sub>2</sub>, and dextran dissolved in deionized (DI) water was put into a three-neck flask equipped with a mechanical stirrer. After the mixture was stirred and mixed completely, ammonia solution was dropped into the mixture with vigorous stirring under argon protection, until the pH of the solution reached 9. This solution was kept at 90°C for 2 h with constant stirring, and then the resultant precipitate was collected with the help of a strong external magnet. The supernatant was washed several times with DI water and ethanol and dried in oven at 70°C overnight.

QT conjugated magnetite NPs (QT-SPION) were prepared by addition of QT to dextran-coated Fe<sub>3</sub>O<sub>4</sub> NPs. The FT-IR spectroscopy and X-ray diffraction (XRD) were recorded. The morphological features were obtained on a Hitachi S-4700 field emission-scanning electron microscope, equipped with an energy dispersive X-ray analysis detector.

Lered for various time intervals.

### Animals and diets

Male Wistar rats, weighing 180–200 g, were purchased from Royan Institute (Isfahan, Iran). The animal experiments were approved by the Animal Ethics of Shiraz University and University of Isfahan. The animals were randomly divided into groups of 6 rats and were housed 3 per cage under standard conditions at a temperature of 25°C ± 2°C and relative humidity of 50%–60% and a 12-h light/dark cycle (lights on at 07:00 a.m.) with free access to tap water and diet for 1 week before the experiment. Any problem was detected during the experimental procedures.

### Bodyweight measurements

Healthy male Wistar rats ( $n = 5$ /group) were given of deionized water (DI) (sham group) or treated with 50 and 100 mg/kg Fe<sub>3</sub>O<sub>4</sub> NPs and 50 and 100 mg/kg QT-Fe<sub>3</sub>O<sub>4</sub>. A group of rats did not receive any drug or DI and served as control. Individual rat body weight was evaluated at the time of purchase, at the time of initiation of the experiment, and before sampling. Percent of the body weight changes were calculated by the following formula:

Percent of the body weight changes = % of day one body weight-day seven body weight/day one body weight

### Statistical analysis

The results were reported as mean ± standard error mean for the six independent experiments. Student's *t*-test was used to estimate the difference between weight in day one and day seven (GraphPad Prism software version 5,

San Diego, CA, USA).  $P < 0.05$  was considered to be statistically significant.

## Results

The Fourier transform-infrared spectroscopy spectrum of the synthesized QT-Fe<sub>3</sub>O<sub>4</sub> (data not shown) showed broad absorption bands at 3386 cm<sup>-1</sup>, which were assigned to the stretching vibrations of hydroxyl groups and other characteristic bands that confirmed the successful conjugation of QT on the Fe<sub>3</sub>O<sub>4</sub> NPs. The powder XRD pattern of the synthesized magnetic NPs was close to the pattern for crystalline magnetite Fe<sub>3</sub>O<sub>4</sub>. Field-emission scanning electron microscopy (SEM) (data not shown) results showed that the NPs are spherical and have diameters in the range of 30–50 nm. The presence of the iron and oxygen was also confirmed by the energy dispersive X-ray detector coupled to the SEM.

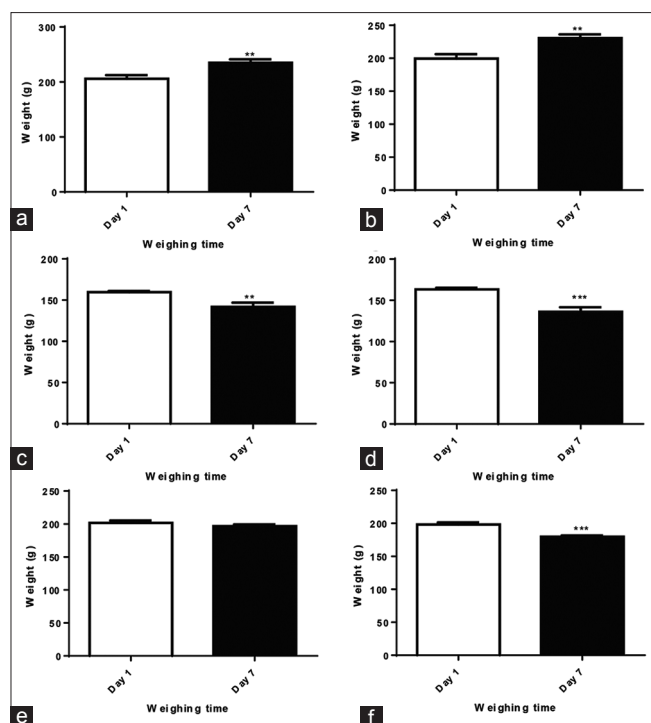
To investigate potential functional toxicity, the body weight of male Wistar rats was measured at the initial of experiment and before scarifying the animals. None of the rats was found dead during of the experiment. Analysis of body weight change indicated a significant reduction in body weight of rats treated with 50 mg/kg or 100 mg/kg of Fe<sub>3</sub>O<sub>4</sub> NPs [Figure 1c and d]. The body weight loss was higher in rats treated with 100 mg/kg of Fe<sub>3</sub>O<sub>4</sub> NPs in comparison to the rats received 50 mg/kg of Fe<sub>3</sub>O<sub>4</sub> NPs [Figure 1c and d]. About 100 mg/kg/day QT-Fe<sub>3</sub>O<sub>4</sub> NPs group lost weight significantly on days 7 when compared to the day one. The 50 mg/kg/day QT-Fe<sub>3</sub>O<sub>4</sub> NPs group show an increased in their weights [Figure 1]. In 50 mg/kg/day QT-Fe<sub>3</sub>O<sub>4</sub> NPs group, body weight neither decreased nor increased significantly.

In the current study, the percentage of body weight changes were also assessed [Table 1]. The results showed a 16% weight gain and 11% weight loss. These results are in line with the abovementioned assessments.

## Discussion

The results of physicochemical characterization of QT-Fe<sub>3</sub>O<sub>4</sub> NPs are in agreement with the previously reported study.<sup>[33]</sup> The results strongly evidence that the QT was successfully conjugated to the magnetite NPs coated with dextran. An *in vivo* study showed that Fe<sub>3</sub>O<sub>4</sub> NPs are relatively safe as they do not accumulate in the vital organs and are rapidly removed from the body.<sup>[34]</sup>

In the current study, rats showed body weight loss when treated with Fe<sub>3</sub>O<sub>4</sub>-NPs. All Fe<sub>3</sub>O<sub>4</sub>-NPs groups (50 and 100 mg/kg/day) showed weight loss. In a study (Hong 2008), to evaluate toxicity of Fe<sub>3</sub>O<sub>4</sub> ferrofluid on the rats, 1.5 mL of a solution containing 4 mg Fe/mL were injected intravenous for 1 month. No rats died and no changes in body weight have been observed. The results of this study revealed that the ferrofluid has no acute fatal toxicity and can be used as a biocompatible material



**Figure 1: Bodyweight change of Wistar rats at day 1 and 7 of the experiment.** (a) control group did not receive anything, (b) sham group received only deionized water, (c-f) rats in these groups treated with Fe<sub>3</sub>O<sub>4</sub> at a dose of (c) 50, (d) 100 mg/kg, and quercetin conjugated Fe<sub>3</sub>O<sub>4</sub> nanoparticles at a dose of (e) 50, (f) 100 mg/kg. All values reported are mean  $\pm$  standard error mean ( $n = 6$ ). (\* for  $P < 0.05$ , \*\* for  $P < 0.01$ , and \*\*\* for  $P < 0.001$ ). QT: Quercetin

**Table 1: Percent of body weight changes in rats treated with superparamagnetic iron oxide nanoparticles, quercetin alone and in conjugated with superparamagnetic iron oxide nanoparticles**

Group	Treatment	Bodyweight day one	Bodyweight day 7	Percentage of bodyweight changes
Control	-	205.8 $\pm$ 6.63	235.0 $\pm$ 6.05**	+14
Sham	DIW	199.5 $\pm$ 6.62	230.5 $\pm$ 5.65**	+15
NPs 50	50 mg/kg	159.5 $\pm$ 1.47	141.8 $\pm$ 5.15**	-11
NPs 100	100 mg/kg	163.3 $\pm$ 1.92	136.0 $\pm$ 5.59***	-16
QT-50	50 mg/kg	213.3 $\pm$ 8.02	247.5 $\pm$ 7.82*	+16
QT-100	100 mg/kg	213.3 $\pm$ 6.66	245.0 $\pm$ 6.70**	+14
QT-NPs 50	50 mg/kg	201.7 $\pm$ 3.57	196.8 $\pm$ 2.60	-2.5
QT-NPs 100	100 mg/kg	198.3 $\pm$ 3.07	179.7 $\pm$ 1.99***	-9

\* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ , All values reported are mean $\pm$ SEM ( $n=6$ ). DIW: Deionized water, QT: Quercetin; NPs: Nanoparticles, SEM: Standard error mean

suitable for biomedical applications. As it can be seen the amounts of Fe NPs used is one-tenth of our study. On the other hand, QT at least at a dose of 50 mg/kg could overcome weight loss.

In another study,<sup>[35]</sup> 1 and 5 mg/kg iron oxide NP were injected to rats for 14 days. A significant weight loss was observed in rats treated with 5 mg/kg of iron oxide

NPs. An explanation mechanism by iron oxide NP could be its oxidative stress.<sup>[36]</sup> Reactive oxygen species (ROS) are generated by iron oxide NPs that leads to production of the hydroxyl radicals and super hydroxide hydrogen. Consequently, this phenomenon could induce cell death (apoptosis).<sup>[37]</sup> On the other hand, iron oxide NPs could decrease superoxide dismutase and glutathione enzymes.<sup>[38]</sup> These enzymes have important roles in reducing of the ROS. QT has the antioxidant capacity, and thus, it could prevent the oxidative stress of the NPs.<sup>[39]</sup> Furthermore, it could clean up cell from ROS.<sup>[40]</sup> This capacity of QT is dose dependence.<sup>[41]</sup> This could explain why in our study rats treated with 100 mg/kg/day of QT did not show weight loss.

## Conclusions

Quercetin could inhibit body weight loss due to the application of the superparamagnetic iron oxide. However, the future studies should measure food consumption and continue measurements for a longer duration. In addition, the dissolution studies of Fe<sub>3</sub>O<sub>4</sub> NPs could identify if the Fe<sub>3</sub>O<sub>4</sub> ion or Fe<sub>3</sub>O<sub>4</sub>-NPs contribute to the body weight responses.

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## Conflicts of interest

There are no conflicts of interest.

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