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Effect of iron oxide nanoparticles and quercetin on rat body weight and brain iron content.

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A B S T R A C T

Iron oxide nanoparticles (IONPs), because of their superparamagnetic characteristics, are of considerable interest in nanotechnology related fields including biomedical, environmental, industrial applications. however, the knowledge of IONPs toxicity is very limited especially in vivo studies. Quercetin (QT); a natural flavonoid, strong antioxidant , free radical scavenger and iron chelator, has multiple therapeutic effects. The present study aimed to explore role of IONPs and QT in rat body weight and possible protective effect of QT in chelating iron preventing fenton reaction. Twenty five male adult rats were allocated equally into five groups: control, IONPs (50 mg/kg b.w i.p. 3 times/week), QT (25 mg/kg b.w. daily per os)+IONPs, QT (50 mg/kg b.w. daily per os) + IONPs, QT (100 mg/kg b.w. daily per os) + IONPs. Rat body weights were assessed weekly till end of the study. After the 30 days brain tissues were collected for measurement of iron level. IONPs induced a non-significant difference in all weeks except at day of sacrifice there was a significant increment in body weight. Compared to IONPs group, QT 25, 50 and 100 mg/kg b.w.+IONPs induced a non-significant difference in body weight in all weeks. IONPs induced a significant increment in Fe level. Compared to IONPs group, QT 25, 50 and 100 mg/kg+IONPs induced a non-significant reduction in Fe level. The results of this study showed role of IONPs and quercetin in body weight gain and suggested protective role of quercetin in chelating Fe preventing fenton reaction occurred from its increase by IONPs.

Keywords: Iron oxide nanoparticls, Quercetin, Body weight, Fe level, Fenton reaction.

1. Introduction

Iron oxide nanoparticles (IONPs) have many biomedical applications including, drug targeting, gene delivery, cell labeling, a contrast agent in magnetic resonance imaging and hyperthermia therapy (Mazdeh et al., 2016). Moreover, iron nanoparticle is used as food additive in iron-fortified drink and cereals for human consumption (fidler et al., 2004), also they have various industrial applications including wastewater treatment, gas sensing, semiconductors, sorbents, lubrications, pigments, coatings (Ramimoghadam et al., 2014).

Although IONPs have considerable potential benefits , there is a necessity for ascertaining any cellular damage linked with these nanoparticles (Singh et al., 2010). There are various routes of entry including ingestion, inhalation and dermal penetration, therefore, the impact of IONPs on health automatically creates a hot subject of research (Lewinski et al., 2008). IONPS introduced to blood stream binding to plasma proteins (Feng et al., 2018) distributed into different body organs including liver, spleen, kidney, lung, heart and able to penentrate blood brain barrier (BBB) reaching brain tissue (Gaharwar et al., 2019). Because of the high level of a metabolic rate, low endogenous scavenger levels, and extensive net¬works of neurons, the brain is more susceptible to damage than many other tissues (Radi et al., 2014).

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Since IONPs have a large content of iron, they can potentially damage the cells (Geppert et al., 2011). Iron-depen¬dent formation of ROS by the Fenton reaction is considered because of many NPs (Petters et al., 2014). Additionally, these con¬ditions may be augmented because of releasing irons from deposited IONPs, as iron stimulates IONPs causing a variety of tissue responses from ROS generation to cell death (Naqvi et al., 2010). low concentration of ROS has valuable role in signaling pathways, on the other hand, the high concentration could deleteriously affect the reduced glutathione (GSH) and the cellular antioxidant response (Blanco et al., 2018). ROS can motivate cellular injuries by altering lipids, proteins, and DNA or leading to produce secondary ROS and lastly cell death (Palmieri and Sblendorio, 2007). Moreover, IONPs might induce mitochondrial damage even if they are not localized into it (Zhu et al., 2010).

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) (QT) is an energetic biological flavonoid (Najafabadi et al., 2018a) present in fruits such as apples, berries, cherries and red grapes, vegetables such as onions and broccoli, in addition to many seeds, buckwheat, nuts, flowers, barks, green tea, olive oil (David et al.,2016). Animals are unable to synthesize the flavones nucleus, therefore, flavonoids are found exclusively in the plant kingdom (Baghel et al., 2016). QT is lipophilic and able to penetrate blood brain barrier (BBB) (Ferri et al., 2015). QT has plentiful valuable effects comprising anti-inflammatory (Periasamy et al., 2016), antioxidant (Xu et al., 2016), anti-apoptosis (Ben Salem et al., 2016), anti-mutagenic (Barcelos et al., 2011), anti-ischemic (Akdemir et al., 2016), and anti-viral (Wu et al., 2015) effects, in addition to promoting mitochondrial biogenesis (Sharma et al., 2015). furthermore, QT is considered as ergogenic supplement (Kressler et al., 2011) so it has ability to increase body weight. QT's antioxidant role is chiefly definited by its enhanced reduced glutathione (GSH) and antioxidant enzyme function via scavenging of ROS (El-Far et al., 2020). QT is able to chelate iron inhibiting fenton reaction (Lesjak and Srai, 2019). Neuronal protection of quercetin is counted in both in vitro and in vivo investigations (Lee et al., 2016, Ossola et al., 2009). Consequently, QT has a potential therapeutic for multiple neurodegenerative diseases and neuronal injuries (Bagad and Khan, 2015). As it protects brain cells from the oxidative stress which damages tissue leading to Alzheimer's disease and other neurological conditions (Lakhanpal and Rai, 2007), lipid peroxidation and apoptosis (Dong et al., 2014).Also, modulation of signal transduction cascades or effects on gene expression are means of neuroprotection of quercetin (Dajas et al., 2013, Kelsey et al., 2010).

This study is aimed at inspecting role of IONPs and QT in rat body weight through measuring them and the possible protective effect of QT in chelating iron preventing fenton reaction through determination of brain iron levels in tissues and using Prussian blue staining

2. Materials and Methods

2.1. Reagents and chemicals

Iron (III) oxide nanopowder were obtained from Sigma- Aldrich chemical St, Louis, Mo, USA. The particles were solubilized in deionized water before use. QT in powdered form was obtained from Sigma-Aldrich (Louis, Mo, USA). QT was dissolved in Dimethyl sulfoxide (DMSO) and distilled water at a ratio of 1:4 respectively prior to use. DMSO ≥99.6% was purchased from Sigma- Aldrich chemical Louis, Mo, USA and deionized water from Center for Graduate Studies and Research at Alexandria University. Tissue iron kit was obtained from MyBioSource, Inc. (San Diego, CA, USA).

2.2 Animals, housing conditions, and experimental protocol

Twenty five apparently healthy adult male albino rats weighing (150 ± 30 B.W), were purchased from Animal breeding unit, Medical Research Institute of Alexandria University, Egypt. The animals were kept in metal cages under environmental controlled conditions with optimum temperature (23±2), humidity (55±5), and dark/light cycle (12h) and free access to rat chow and drinking water. The international ethical guidelines for the care and use of laboratory animals were performed to handle the animals and the experimental procedures were approved by the Experimental Animal Use and Ethics Committee at the Faculty of Veterinary Medicine, Alexandria University, Egypt. All animals were housed for two weeks before experiment for acclimatization. The rats were randomly assigned to five groups (5 rats each); control received normal basal diet and water adlibitum, IONPs group intraperitoneally injected with IONPs 50 mg/kg b.w. three times a week (Sundarraj et al, 2017), IONPs+QT 25 mg group was administrated with the same dose of IONPs and gavaged with QT 25 mg QT/kg b.w. daily (Elsoadaa et al, 2018), IONPs+QT 50 mg group was administrated with the same dose of IONPs and gavaged with QT 50 mg QT/kg b.w. daily (Najafabadi et al, 2018), and IONPs+QT 100 mg group was administrated with the same dose of IONPs and gavaged with QT 100 mg QT/kg b.w. daily (Kazemipour et al, 2018).All treatments were continued for 30 days. At the end of experiment, the rats were fasted for 12 hrs and anesthetized using ketamine/xylazine (100 mg/kg, 10 mg/kg i.p.) then euthanized and the brain were immediately dissected, rinsed with chilled normal saline 0.9% and divided into two parts; the first one was used for estimation of iron level through tissue iron kit (weighed and stored at -80 oC), and the second part was used for histopathological examination (Prussain blue staining) (fixed in neutral buffered formalin10%).

2.3. Body weight measurement

Individual rat body weight was evaluated at the time of purchase, then weekly obtained till the end of experiment.

2.4. Estimation of iron content

The iron contents of the brain tissues were determined by tissue iron kit according to Nakaya et al., (2013) in brief, brain tissues were minced,weighed and homogenised (10%, w/v), in ice‑cold phosphate-buffered saline (0.01 M, PH 7.4) in a Potter‑Elvehjem type homogeniser. The homogenates were then centrifuged at 10,000 x g for 10 min at 4˚C, to pellet the cell debris. iron content was measured as microgram (µg) iron per tissue weight at by OD value at 520 nm in the spectrophotometer.

2.5. statistical analysis:

All data were analysed with the statistical software SAS for Windows Version 9.10 (SAS Institute Inc., Cary, NC, USA). The mean value and standard error were calculated for each variable. A probability value less than or equal to 0.05 was considered statistically significant. First, data was assessed for normality with Shapiro-Wilk test. Normally distributed iron values measured once were compared among groups with one-way analysis of variance (ANOVA) and Bonferroni post-hoc test. Body weight was analyzed with two-way ANOVA (week and group effects, and their interaction), and Bonferroni correction for multiple comparisons.

3. Results

3.1. Effect of different doses of quercetin on body weight in rats exposed to iron oxide nanoparticles.

The data in Table (1) anapproved that compared with control values, IONPs induced a non-significant difference in all weeks except at day of sacrifice there was a significant ($P<0.05$) increment (183 ± 4.64) in body weight. Compared to IONPs group, Co-supplementation of QT 25, 50 and 100 mg/kg b.w. induced a non-significant difference in body weight in all weeks.

The data demonstrated in Table (2) show that in comparison to control values, IONPs induced a significant ($P<0.05$) increment in Fe (20.8 ± 1.05). Compared to IONPs group, Co-administration of QT 25, 50 and 100 mg/kg b.w. induced a non-significant reduction in Fe. Moreover, it was noticeable that QT 50/kg b.w. mg was better than QT 25 mg/kg b.w. and QT 100 mg/kg b.w was the best one.

4. Discussion

Iron oxide nanoparticles (IONPs) have recently appealed widespread concern due to their superparamagnetic physicochemical properties and potential biomedical applications including brain imaging, brain-targeted drug or gene delivery, catalytic materials, magnetic data storage devices (Wu et al., 2013) and industrial applications including, wastewater treatment adsorbents, pigments, coatings, gas sensors, ion exchangers (Hasany et al., 2013). Quercetin (QT) has highly potent antioxidant and cytoprotective effects in preventing endothelial apoptosis causes by oxidants (Dong et al., 2014), chelating metal ions, scavenging oxygen radicals protecting from lipid peroxidation therefore, terminating the radical chain reaction (Erden Inal et al., 2001). Accordingly, The present study aimed to explore role of IONPs and QT in rat body weight and possible protective effect of QT in chelating iron preventing fenton reaction.

Briefly, IONPs penetrate into the cells passing through receptor-mediated endocytosis settling into the lysosomes, organelles featured by the presence of an acidic medium, where the metabolization of the nanoparticles takes place and free iron ions are released into the cell (Yarjanli et al., 2017). The free Fe^{3+} ions react with hydrogen peroxide (H_2O_2) and determine the generation of reactive oxygen species (ROS) in a process known as Fenton reaction (Wu et al., 2014). An elevated ROS concentration initiates a cascade of events (release of iron ions into the cytosol by prompting an increased permeability of the outer mitochondrial membrane and destructive effects on lysosomal membrane, lipid peroxidation, damaged proteins, break of DNA chains and degradation of bases, mutations, deletions or translocations at nuclear level) that has as endpoint cell death. The pathologies that are associated with this type of cellular damage are aging, cancer and neurodegenerative diseases (Moacă et al., 2018). Another explanation of inducing cell death by the iron ions is the apoptotic pathway via mitochondria, as follows: a high amount of iron ions into the mitochondria determine the opening of the mitochondrial transition pore, release of $Ca^{2+}$and cytochrome c and instigation of apoptotic cascade (Farshbaf and Ghaedi, 2017).

In our study, body weights of rats significantly increased after intraperitoneal administration of IONPs. These results was in harmony with Askri et al., (2018) and Szalay et al., (2012) who reported that IONPs administration increased significantly the body weight of rats. An explanation of this increase may be that reactive oxygen species generated by IONPs can inhibit the normal mitochondrial metabolic function and prevent the mitochondria from producing energy in the form of ATP by oxidative phosphorylation. The lower levels of ATP combined to the reduced efficacy of the tricarboxylic acid (TCA) cycle as an outcome to inhibition of enzymes such as aconitase which are sensitive to oxidative stress would cause the liver to divert metabolites to lipogenesis so this oxidative stress increases lipogenesis at the expense of energy production and induce weight gain (Mailloux et al., 2007). Co-supplementation of IONPs-treated rats with QT maintained this increase at all different three doses especially QT 100 mg/kg b.w. in which there was more increase in body weight than rats administrated with IONPs only. These results were in concordance with those obtained by Najafabadi et al., (2018b) who found that there was increase in body weight after QT supplementation. This might be explained by that QT is considered as an ergogenic supplement (Davis et al., 2009) and has the ability to increase aerobic exercise performance and oxidative metabolism so it increase chance for more increase in body weight and adiposity (Kressler et al., 2011).

Iron ions create main biological roles in multiple physiological processes comprising heme synthesis, oxygen transport, mitochondrial respiration, DNA synthesis, and in metabolic functions at central nervous system (nitric oxide metabolism, oxidative phosphorylation and myelin and neurotransmitter synthesis). Moreover, iron ascertained to be a necessary factor for an proper function of neurons as performing cofactor for tyrosine hydroxylase, an enzyme with a serious role in dopamine synthesis and the viability of neural cells. Unbalanced physiological functions and cytotoxic reactions can be occurred if the iron homeostasis or transport were dysregulated (Yarjanli et al., 2017).

In our study, iron level significantly increased after intraperitoneal administration of IONPs. Our results were in concordance with those obtained by Dhakshinamoorthy et al., (2017) who demonstrated that iron content found to be increased significantly in the brain tissue of IONPs treated groups compared to the control. Prussian blue staining for iron

accumulation was carried out in the brain regions and increased blue spots in the frontal cortex, hippocampus and cerebellum were found and confirmed the result. Also our results was in harmony with El-Sayed et al., (2019) who illustrated that significant rise of iron level in brain tissue. An explanation of this increase may be that The magnetic character of iron oxide nanoparticles offers some advantages including the capacity of this nanosized compounds to be driven to targeted sites by an external magnetic field even to tissues and organs that are difficult to reach in normal conditions (BBB and central nervous system) (Moacă et al., 2018). Also, Lauret et al., (2012), Mahmoudi et al., (2011) stated that magnetic nanoparticles could increase penetration of the BBB and The sensitivity of brain cells to these nanoparticles is higher than other organs cells. in addition, Imam et al., 2015 informed that a damage to the membrane of rat's brain endothelial cells caused by IONPs through generating ROS Consequently, IONPs are driven into endothelial cells or via destroying cellular membranes cross the BBB. Furthermore, increased iron level in the brain restrains occluding expression. According to the role of occluding, a protein of tight junction, in the BBB, decreasing its expression may interrupt the function of Blood and thus, more iron raised and more damage to the brain (Sripetchwandee et al., 2016). Another explanation may be that Increased iron levels in brain due to binding of iron to transferrin that triggers up regulation of iron receptors in the brain, as a consequence transporting iron across the BBB (Reddy et al., 2017).

Co-administration of IONPs-treated rats with QT decreased iron level in brain tissue. Our results were in concordance with those results obtained by Leopoldini et al., (2006), Zhang et al., (2011), Raza et al., (2016), Horniblow et al., (2017) and El-Sheikh et al., (2018) who revealed that QT could suppress iron level in brain tissue and might be explained by properity of iron chelation by QT. As QT structurally consists of three phenolic rings including A, B and C rings so it possesses three places available to chelate metals involving 3-hydroxy-4-keto group, 5-hydroxy-4-keto group and ortho-dihydroxyl (catechol) groups of B ring. Both keto and hydroxy groups of QT have ability to bind with metals forming metal complexes (Torreggiani et al., 2005, Ahmadi et al., 2011).

5. Conclusion

The present study revealed role of IONPs and QT in body weight gain and suggested protective role of QT in chelating Fe preventing fenton reaction occurred from its increase by IONPs.

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Table 1. Effect of different doses of QT on body weight in rats exposed to iron oxide nanoparticles.

	Group				
Week	Control	IONPs	IONP + QT 25 mg	IONP + QT 50 mg	IONP + QT 100 mg
1	181 ± 3.06[a]	167 ± 4.84[ab]	163 ± 3.09[b]	159 ± 2.48[b]	162 ± 4.60[b]
2	173 ± 2.81[a]	175 ± 4.37[a]	175 ± 3.83[a]	169 ± 4.96[a]	169 ± 5.26[a]
3	174 ± 3.79[a]	183 ± 3.96[a]	184 ± 3.95[a]	188 ± 5.27[a]	183 ± 5.97[a]
4	177 ± 3.18[b]	191 ± 4.33[ab]	199 ± 3.20[a]	197 ± 3.45[a]	191 ± 5.81[ab]
At sacrifice	166 ± 4.85[b]	183 ± 4.64[a]	178 ± 4.64[ab]	174 ± 3.67[ab]	185 ± 3.87[a]

IONP = Iron oxide nanoparticles. Doses are in milligrams per kg body weight.Values are means ± standard error of mean. Means without a common superscript letter differ significantly (P<0.05)

Table 2. Effect of different doses of QT on brain iron levels in rats exposed to iron oxide nanoparticles

Parameter	Group			
	Control	IONPs	IONPs + QT 25 mg	IONPs+ QT 50 mg	IONPs + QT 100 mg
Fe (µg/g tissue)	15.6 ± 0.71b	16.3 ± 1.30ab	20.8 ± 1.05a	16.0 ± 1.61ab	17.3 ± 1.28ab

IONP = Iron oxide nanoparticles. Doses are in milligrams per kg body weight.
Values are means ± standard error of mean.
Means without a common superscript letter differ significantly (P<0.05).