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ORAL SUPPLEMENTATION EFFECT OF IRON AND ITS COMPLEX FORM WITH QUERCETIN ON OXIDANT STATUS AND ON REDISTRIBUTION OF ESSENTIAL METALS IN ORGANS OF STREPTOZOTOCIN DIABETIC RATS

Farid Berroukeche [1, ✉], ***Nassima Mokhtari-Soulimane*** [1], ***Asmahan Imessaoudene*** [1], ***Ahmed Sabri Cherrak*** [1], ***Pascale Ronot*** [2,3], ***Anne Boos*** [2,3], ***Amina Belhandouz*** [4], ***Hafida Merzouk*** [1], ***Mourad Elhabiri*** [5]

[1] Laboratory of Physiology, Pathophysiology and Biochemistry of Nutrition, Department of Biology, Faculty of Natural and Life Sciences, Earth and Universe, Tlemcen University, Algeria.
[2] IPHC, Becquerel Street, Strasbourg University, France.
[3] CNRS, UMR7178, Strasbourg, France.
[4] Faculty of Medecine, Bechar University, Algeria.
[5] Laboratory of Physico-Chemistry Bioinorganic, UMR 7509 of CNRS, ECPM, Louis Pasteur University, 25 Becquerel street, Bat R5-Level 1, 67200 Strasbourg, France.

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Abstract

Background and aims: *Quercetin, is a polyphenolic antioxidant compound. It is able to form complex with metal ions such as iron and exerts a broad range of biological activities like improving metabolic disorders. This research aims at investigating the effect of oral supplementation of iron (2.5mg Fe/Kg/day) and its complex form (molar ratio 1:5; 2.5mg/25mg/Kg/day) with quercetin (25mg/Kg/day) on lipid metabolism, oxidant status and trace elements contents in organs of Wistar diabetic rats (45 mg/kg/rat.ip of streptozotocin) during eight weeks of experimentation.* ***Material and method:*** *To achieve this, liver and adipose tissue enzymes activities, $NO^{\bullet}$, $O_2^{-\bullet}$, TBARs, carbonyl protein levels in plasma were analysed. Metals (Cu, Fe, Mg, Zn) analysis of organs were determined by inductively coupled plasma atomic emission spectroscopy.* ***Results:*** *Iron supplemented alone induced a noticeable disorder in lipid, lipoprotein, lipases and oxidant status. Yet, it caused an imbalance in the redistribution of metals in the organs of diabetic and non diabetic rats. Iron-quercetin complex was shown as less harmful and more beneficial than iron supplemented alone.* ***Conclusions:*** *This complex could reverse oxidative stress and iron deficiency mostly caused by the diabetic disease but at the same time it induces an imbalance in redistribution of other essential metals.*

key words: *Iron-quercetin complex, diabetes, oxidative stress, lipoproteins, trace elements.*

✉ Street 95, N° 01 Emir AEK Street, Maghnia, Tlemcen, Algéria. Tel: 00 213 662 617 077; Fax: 00 213 43212145
corresponding author e-mail: f.berroukeche@gmail.com

Background and aims

Diabetes mellitus (DM) is a chronic hyperglycemia and the major cause of deadly complication and disabling in the world. The global statistics of 2017 estimate a 451 million people with diabetes between age of 18 and 99 years [1]. Most of these patients are not insulin-dependent (type 2 diabetes). Those bodies are still able to produce insulin. Chronic hyperglycemia in DM is associated with several disorders such as oxidative stress and dyslipidemia/hyperlipidemia [2]; it can also compromise the body's balance of trace elements and minerals. In clinical research, copper (Cu), iron (Fe), magnesium (Mg) and zinc (Zn) have been widely used to evaluate the pathogenetic risk factors of the development of arteriosclerosis and hypertension along the DM disease process [3]. The aforementioned metals are highly interdependent and work mutually. However, the deficiency or the overload of one metal may influence the homeostasis of others; this interaction is made clearly in the utilization of Fe that is affected by the status of further nutrients such as selenium (Se) or Cu [4]. The latter plays a pivotal role with Zn in the oxidant/antioxidant status of DM's pathogenesis [5], like the incrimination of Mg deficiency in hyperlipidemia [4].

Treatment manages some of its serious sequelae but generally does not restore the normal glycemic levels or avoid all disastrous complications. Plants received a noticeable attention as a valuable source of bioactive substances including antioxidants, hypoglycemic and hypolipidemic agents [6]. In fact, quercetin (3,3´,4´,5,7-pentahydroxyflavone) is a polyphenolic compound that has been found in a large variety of vegetables and fruits, it exhibits a broad range of health benefits (pleiotropic effects). It is a potent antioxidant agent that prevents oxidant injuries and cell death by numerous interrelated mechanisms such as the inhibition of pro-oxidant enzymes, the up-regulation of genes expression encoding enzymes involved in drug metabolism and antioxidant defense [7].

Iron is a pro-oxidant and parradox essential element. In one hand, it is able to enhance insulin resistance in excess amounts. On the other hand, chronic iron deficiency in anemia can induce the development of cardiovascular diseases in both diabetic and non-diabetic persons [8]. Furthermore, the ability of flavonoids to chelate pro-oxidant metal such as $Fe^{2+/3+}$ is essential for intrinsic antioxidant activities. It can also prevent the toxicity induced by pro-oxidant metal ions through their tight binding [9], otherwise it may progressively deliver iron after complex metabolism. However, the role of organo-metallic complex in diabetic complications has not been investigated.

The aim of this work is to evaluate the influence of iron supplementation in its complex form with quercetin on oxidative markers and on the redistribution of metals (Cu, Fe, Mg and Zn) in the internal organs (liver, kidney and muscle) of diabetic rats compared with iron given alone.

Material and method

Complex Synthesis

Quercetin (quercetin dihydrate, 97%) and iron sulfate ($FeSO_4.7H_2O$) were purchased from Alfa Aesar. Fe(II)-quercetin complex (molar ratio 1:5; 2.5 mg/25 mg) was synthesized according to published procedures [10]. In this work, the exact dosages of both quercetin and iron (25 mg/kg.b.w./rat and 2.5 mg/kg.b.w./rat) were used respectively; the complex was synthesized one day out of two days according to the evolution of rats' weight through all the experiment.

Animals

Male Albino Wistar rats (Pasteur's institute, Algeria) weighing 200-280 g were housed in spacious stainless steel cages and maintained under appropriate standard laboratory conditions (12:12 ± 1h light-dark cycle), temperature (20 ± 2°C) and relative humidity (50 ± 15%). For two weeks, animals had a free access to food (standard diet for rats ONAB), tap water ad libitum. They were also acclimatized to standard husbandry conditions to eliminate the effect of stress before the initiation of the experiment. Procedures involving animals was conducted in accordance with the national guidelines for the care and use of laboratory animals. All the experimental protocols were approved by the Regional Ethical Committee.

Experimental design

Animals were housed five per cage and fasted overnight. DM was experimentally induced by a single intraperitoneal (ip) injection of STZ (45 mg/kg.b.w/rat; Sigma Chemical) in freshly prepared 0.1 M cold citrate buffer, *p*H 4.5 and control groups received just ip injection of citrate buffer. However, STZ-treated rats received 5% (w/v) of sucrose instead of water for 24 h after DM induction to reduce death risk caused by hypoglycemic shock.

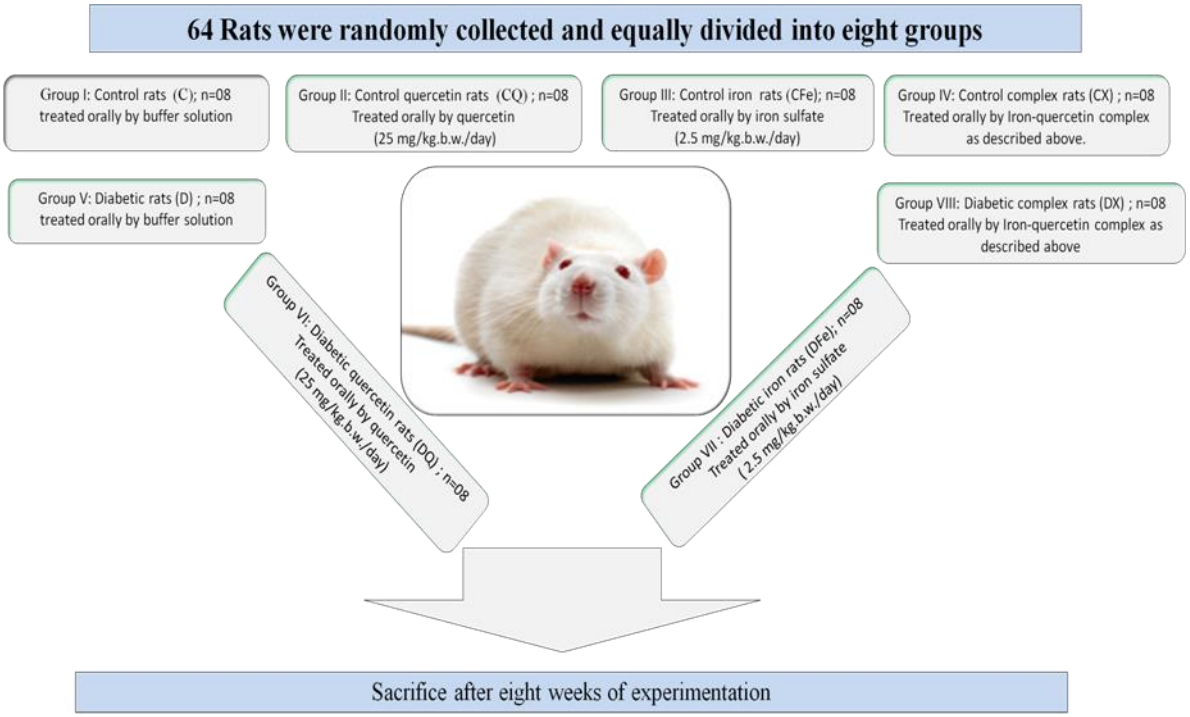

Figure 1. *Experimental protocol*.

After three days of STZ injection, the Wistar's blood glucose was estimated by an autoanalyzer Blood Glucose Meter (On Call Plus, USA). Only animals with high serum glucose (higher than 127 mg/dl) were considered diabetic rats, then they were selected for the current experiments. Rats were randomly collected and equally divided into eight groups (eight Wistar rats in each group), they received oral gavage treatments as described in Figure 1.

Homogenate organs and blood samples' preparation

After eight weeks of experiment, the animals were sacrificed after making them in a fasted situation. Liver and abdominal white adipose tissues were excised and immediately immersed

in ice-cooled 0.1 M phosphate buffered saline (PBS, *p*H 7.4), then, they were blotted and weighed quickly. An aliquot of each tissue was homogenized with an Ultraturrax homogenizer (Bioblock Scientific, Illkirch, France) in 10 volumes of ice-cold 10 mmol/l (PBS) containing 1.15% KCl. The homogenate was centrifuged at 6000 g, 4°C during 15 min. The supernatant fractions were removed and used for tissue triglycerides (TG) and lipase (LPL, HSL) assays. Afterwards, blood was collected; serum and plasma were separated and used for biochemical and oxidative stress analysis.

Biochemical analyses

Serum total cholesterol (TC) and tissue triglycerides (TG) were measured using colorimetric enzymatic kits (Sigma, St. Louis, MO). Serum lipoproteins like very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) fractions were separated using Burstein et al. method [11]. Different precipitate fractions were obtained from successive centrifugation with 0.1 ml phosphotungstic acid (30.3 mmol/L) and $MgCl_2$ (100 mmol/L) reagent mixture. VLDL, LDL and HDL- cholesterol contents were determined by enzymatic methods (Sigma). Hormone Sensitive Lipase (HSL; EC 3.1.1.3) activity was estimated spectrophotometrically using the esterase hydrolysis reaction of *p*-nitrophenylbutyrate (PNPB). The latter was hydrolyzed to *p*-nitrophenol and butyric acid in the presence of lipase. The release of *p*-nitrophenol is shown in the appearance of yellowish color detected at 400 nm. One enzymatic unit is the amount of enzyme able to release one µM of *p*-nitrophenol per minute per mg of protein. Liver and adipose tissue lipoprotein lipase (LPL) activities were measured in the supernatants containing heparin-releasable lipases that were previously reported in details by enzymatic methods. A lipase unit is the amount of enzyme that allows the release of one micromole of fatty acid per minute [12].

Oxidant markers analyses

Plasma carbonyl proteins (markers of protein oxidation) were estimated spectrophotometrically by the 2,4-dinitrophenylhydrazine (DPPH) reaction according to Levine et al. [13]. Thiobarbituric Acid Reactive Substances (TBARs) concentration was considered as the marker of lipid peroxidation. It was estimated in plasma and organs homogenate according to Draper and Hadley method [14] using a hot acid treatment and thiobarbituric acid (TBA). After incubation and centrifugation at 4000 rpm for 10 min, the supernatant contains the TBARs which were measured spectrophotometrically at 532 nm. Plasma superoxide anion was monitored according to the method described in literature [15]. This method was based on the reduction of the nitro-blue tetrazolium (NBT) to monoformazon in the presence of superoxide radicals. The obtained yellow color was measured spectrophotometrically at 560 nm. According to Guevara et al., nitric oxide ($NO^{\bullet}$) was measured using the colorimetric reaction of Griess. $NO^{\bullet}$ formation was assayed indirectly by determining the concentrations of nitrites (NO_2^-) and nitrates (NO_3^-) which constitute the oxidative degradation products of $NO^{\bullet}$. Briefly, plasma was deproteinized with methanol: diethylether; 3:1 mixture *v/v*. The nitrate was reduced to nitrite by cadmium, and then the concentration of nitrite was monitored directly at 492 nm [16].

The Analysis of total metal contents in liver, kidney and muscle

To analyze (Fe, Cu, Zn, and Mg) in organs, a portion fragment of each tissue were dried to a constant weight [17]. About 0.25 g of dried

organs was introduced in a Teflon reactor with a mixture of 3 mL of nitric acid (68%) of quality Traceselect (Fluka) and 1 mL of water Ultrapure. Then, the reactor was closed and placed on the HVT50, Anton Paar, rotor. Decomposition of the organic matrix in the tissue samples was performed using a microwave oven. The mineralizer microwave is programmed to a rise in T ° to 220 ° C in 10 minutes and a hold of 30 minutes. The final volume of mineral deposit obtained is determined by weighing. The samples were then diluted by a factor of 15 with deionized water. The diluted digests were analyzed for concentrations of Cu, Fe, Mg and Zn on a Varian Inductively Coupled Plasma Optical Emission Spectrometer 720-ES. Calibration of the instrument was established using certified standards (CPI International, USA). Two emission wavelengths were chosen for each element to control if there is no spectral interference or matrix effect: 223.009 nm and 224.700 nm for Cu, 238.204 nm and 259.940 nm for Fe, 279.553 nm and 280.270 nm for Mg, 206.200 nm and 213.857 nm for Zn (Table 1).

Table 1. ICP-AES operating conditions.

Instrument	Varian ICP-OES 720 ES
Spectrometer	Image Mapping CCD detector
RF-Generator	40 MHz
Output power	1.2 kW
Argon flow	Coolant: 15 L min^{-1} Auxiliary: 1.5 L min^{-1} Nebuliser: 0.75 L min^{-1}
Peristaltic pump	1.0 $mL.min^{-1}$
Nebuliser	Pneumatic (glass concentric)
Spray chamber	Glass cyclonic
Plasma viewing	Axial
Replicates for each analysis run	3
Simple uptake delay	30s

Statistical analysis

Results were expressed as means ± standard deviation (SD). They were tested for normal distribution using the Shapiro-Wilk test. Significant differences among the groups were analyzed statistically by one-way variance analysis (ANOVA). The significance level was set at $P < 0.05$. These calculations were performed using STATISTICA version 4.1 (STATSOFT, Tulsa, OK).

Results

The effects of treatments on serum lipids and lipoproteins metabolisms

Table 2 depicts the measurement of rats' lipids characteristics in each group. Iron gavage induced a high amount of TC, VLDLc and LDLc in both control and diabetic groups (CFe, DFe) compared to (C, D) groups. In contrast, the aforementioned parameters in diabetic rats (DX) were significantly ($P < 0.05$) decreased by iron-quercetin complex. The latter and quercetin alone were observed to enhance HDLc levels in both control (CQ, CX) and diabetic animals, (DQ, DX). Yet, free iron supplementation decreased HDLc concentration in both groups of rats (CFe, DFe) with respect to control (Table 2).

The effects of treatments on lipase activities (LPL, HSL) and TG levels in liver and adipose tissue

Iron significantly increased LPL activities of liver and adipose tissue in both control and diabetic rats (CFe, DFe). However, using the same treatment leads to reduce HSL activity only in diabetic rats (DFe). Besides, iron-

quercetin complex treatment decreased LPL activity in diabetic rats' liver and adipose tissue (DX). Quercetin and iron-quercetin complex administration had a weak effect on hepatic lipase and HSL activities. However, they decreased adipose LPL's activity in control animals (CX) compared to untreated rats (C) (Table 3).

Table 2. Total cholesterol and lipoproteins profile characteristics of studied rats.

	TC (mg/dL)	VLDLc (mg/dL)	LDLc (mg/dL)	HDLc (mg/dL)
Control rats				
C	172.74±17.72^{c}	32.02 ±0.49^{d}	53.87± 1.56^{e}	80.74 ±0.97^{c}
CQ	159.54±12.08^{c}	25.22± 1.48^{e}	42.23 ±2.24^{f}	92.22± 1.3^{b}
CFe	208.22±12.03^{b}	52.72 ±1.84^{c}	88.7 ±2.49^{b}	66.05± 0.79^{e}
CX	160.41±10.49^{c}	24.53± 1.51^{e}	41.04± 1.85^{f}	96.78 ±1.47^{a}
Diabetic rats				
D	190.13±15.63^{b}	56.95 ±1.76^{c}	69.45± 2.07^{c}	77.68 ± 1.57^{d}
DQ	176.41± 9.87^{c}	51.92 ±1.39^{c}	57.55±2.25^{e}	89.17± 1.56^{b}
DFe	234.38 ±4.42^{a}	65.18± 1.3^{b}	96,47± 1.77^{a}	70.62± 1.11^{e}
DX	181.37±11.81^{c}	76.62± 1.91^{a}	89.07± 1.8^{b}	87.26± 1.69^{b}
P(ANOVA)	0.006	0.005	0.004	0.001

Values are presented as means ± standard deviations (SD). Values with different superscript letters (a, b, c, d…) are significantly different according toTukey test at $P < 0.05$.

Table 3. Lipase activities of liver and adipose tissue in studied rats.

	Liver LPL (nmol/min/mg)	Adipose tissue LPL (nmol/min/mg)	Adipose tissue HSL (µmol/min/mg)
Control rats			
C	5.75± 0.45 ^{d}	18± 1.1 ^{d}	38.24 ±2.4^{b}
CQ	5.95± 0.46 ^{d}	11± 1.27 ^{e}	41.58± 2.41^{b}
CFe	11.75± 0.33 ^{c}	28.83± 2.12 ^{b}	40± 1.87 ^{b}
CX	6.17± 0.4 ^{d}	11± 1.32 ^{e}	41.58± 2.11^{b}
Diabetic rats			
D	13.26± 0.39 ^{c}	31.17 ±1.37 ^{b}	43 ±1.58 ^{b}
DQ	16.13± 0.58 ^{b}	23.33± 1.57 ^{c}	42.5± 1.55 ^{b}
DFe	22.93± 0.84 ^{a}	40,98± 1.58 ^{a}	46.18± 1.72 ^{a}
DX	7.46± 0.21^{d}	25.12± 2.03 ^{c}	40.42± 1.21^{b}
P(ANOVA)	0.0001	0.0001	0.031

Values are presented as means ± standard deviations (SD). Values with different superscript letters (a, b, c, d…) are significantly different according to Tukey test at $P < 0.05$.

Furthermore, iron supplementation worked remarkably to increase the liver TG levels in each group of control and diabetic rats. Liver TG amount was shown to be reduced significantly by iron-quercetin complex on diabetic rats (DX). Regardless of gavage used (D, DQ, DFe, DX), no noticeable change was observed in adipose TG level in all diabetic groups (Figure 2).

Plasma oxidative stress markers evaluation

In the following study, plasma $NO^{\bullet}$, $O_2^{-\bullet}$, TBARs and protein carbonyl levels were measured as biomarkers of the oxidative stress occurring in different rats groups. STZ injection was clearly observed to elevate plasma $NO^{\bullet}$, $O_2^{-\bullet}$, TBARs and protein carbonyl levels in untreated diabetic rats (D). The same observations were noted with iron supplementation but only with $O_2^{-\bullet}$, TBARs parameters in diabetic rats (DFe) compared with control diabetic group (D). Conversely, oral administration of iron-quercetin complex worked remarkably to reduce $NO^{\bullet}$, $O^{2-\bullet}$, TBARs and protein carbonyl concentrations in diabetic

animals (DX) in comparison with the effect of quercetin supplemented alone. The same results were observed only with $O_2^{-\bullet}$ and carbonyl protein of control groups (CX).

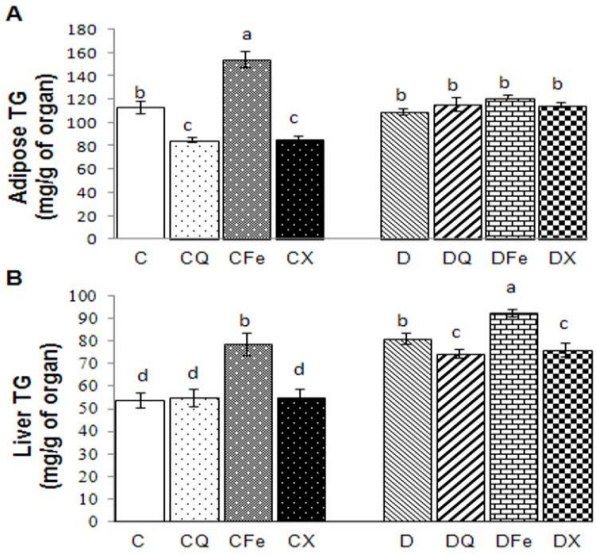

Figure 2. *Triglycerides (TG) levels in adipose and liver tissue in normal and diabetic rats during eight weeks of study. (A) Adipose tissue triglyceride levels; and (B) liver tissue triglyceride levels. Values are presented as means ± standard deviations (SD). Values with different superscript letters (a, b, c, d…) are significantly different according to Tukey test at P < 0.05.*

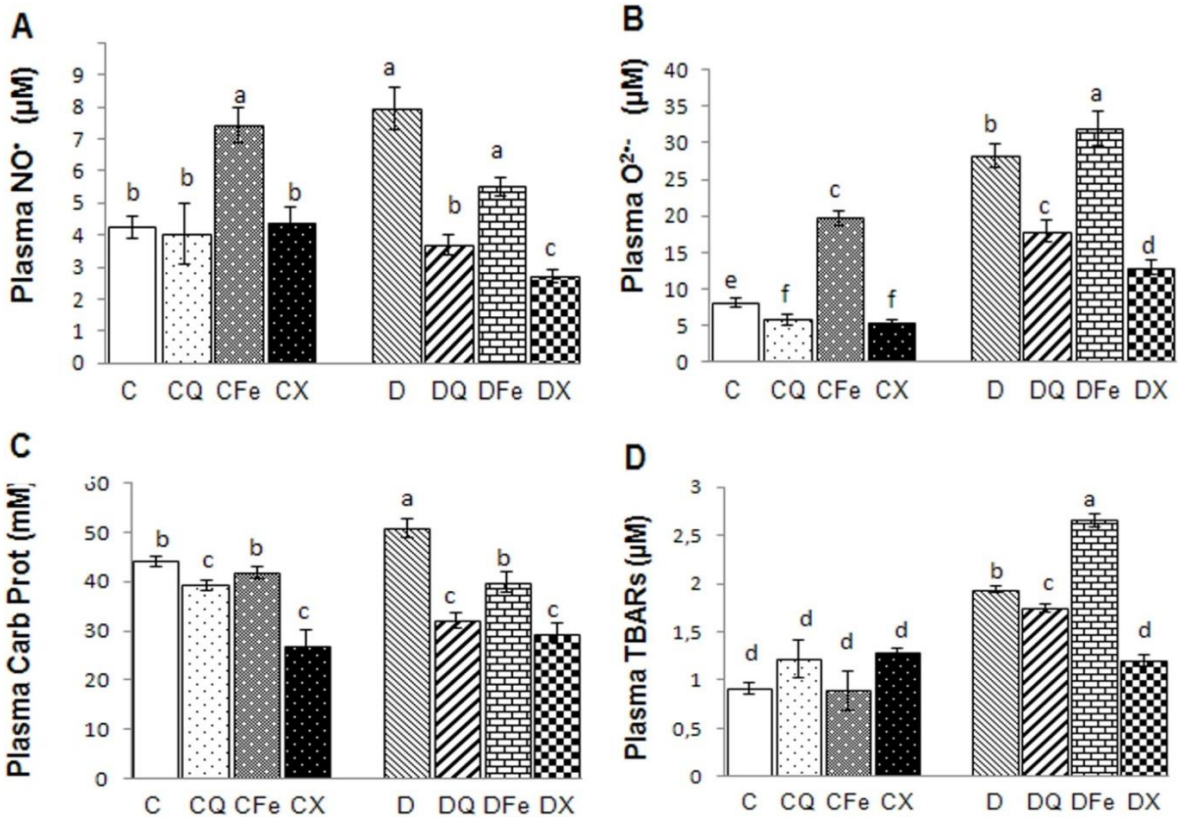

Figure 3. *Plasma oxidant markers levels in normal and diabetic rats during eight weeks of study. (A) Plasma NO• level; (B). Plasma $O_2^{-\bullet}$ level; (C) plasma Carb Prot (carbonyl protein) level; (D) plasma TBARs level. Values are presented as means ± standard deviations (SD). Values with different superscript letters (a, b, c, d…) are significantly different according to Tukey test at P < 0.05.*

Additionally, oral quercetin treatment was observed also to decrease $NO^{\bullet}$, $O_2^{-\bullet}$, TBARs and protein carbonyl to a lesser extent only in diabetic rat groups (DQ) (Figure 3).

Trace and major elements (Cu, Fe, Mg, Zn) analysis in liver, kidney and muscle tissue

Tables 4-6, respectively, depicted the overall average element levels in liver, kidney and muscles samples in diabetic and non-diabetic animals. The metals levels in control (C) and diabetic (D) rats were closely dependent on the type of tissues and the nature of elements. Interestingly, this study revealed that Cu, Fe and Mg contents in liver were significantly decreased by quercetin and Fe gavage in control rats (CQ, CFe). Furthermore, iron-quercetin complex administration (CX) reduced Cu, Mg and Zn liver concentrations while at the same time it increased iron levels in the same group (CX).

In diabetic groups, STZ injection reduces Cu, Fe, Mg and Zn liver levels compared to control rats (C) while quercetin treatment (DQ) restored the aforementioned elements in the same organ. Furthermore, iron-quercetin complex treatment was observed to be more effective to enhance Cu and Zn liver levels (DX) than iron treatment alone (DFe) in comparison with diabetic group (D) (Table 4).

Table 4. Liver tissue concentrations (mg/Kg of dry tissue) of trace and major elements.

Liver	[Cu] µg/g	[Fe] µg/g	[Mg] µg/g	[Zn] µg/g
Control rats				
C	18.8±0.8 ^{a}	399.29±3.95 ^{c}	765.3±4.43 ^{a}	108.95±1.92 ^{b}
CQ	13.8±0.88 ^{d}	233.36±1.94 ^{g}	566.21±6.22 ^{e}	103.3±0.57 ^{b}
CFe	14.04±0.46 ^{d}	357.29±2.05 ^{d}	629.32±6.18 ^{d}	105.47±0.63 ^{b}
CX	13.25±0.29 ^{d}	447.77±3.08 ^{a}	724.79±5.54 ^{b}	98.55±1.56 ^{c}
Diabetic rats				
D	13.65±0.31 ^{d}	322.75±4.1 ^{e}	736.39±12.48 ^{b}	78.07±1.63 ^{d}
DQ	15.23±1.21 ^{b}	431.7±6.02 ^{b}	765.92±9.94 ^{a}	96.74±1.01 ^{c}
DFe	14.44±0.67 ^{c}	362.44±3.19 ^{d}	709.68±2.45 ^{c}	99.6±1.5 ^{c}
DX	16.5±0.48 ^{b}	293.17±1.7 ^{f}	729.8±3.81 ^{b}	162.32±8.54 ^{a}
P (ANOVA)	<0.0001	<0.0001	<0.0001	<0.0001

Table 5. Kidney tissue concentrations (mg/Kg of dry tissue) of trace and major elements.

Kidney	[Cu]mg/Kg	[Fe]mg/Kg	[Mg]mg/Kg	[Zn]mg/Kg
Control rats				
C	57.04±0.63 ^{b}	264.08±2.38 ^{b}	696.73±4.12 ^{d}	119.77±1.27 ^{b}
CQ	48.04±1.11 ^{d}	202.35±0.96 ^{d}	604.88±3.59 ^{f}	102.2±0.69 ^{e}
CFe	49.41±0.84 ^{d}	268.73±1.16 ^{b}	656.21±5.29 ^{e}	115.97±1.33 ^{c}
CX	39.22±0.71 ^{e}	263.35±2.69 ^{b}	745.18±4.88 ^{b}	118.44±1.52 ^{b}
Diabetic rats				
D	54.28±1.18 ^{c}	255.89±1.77 ^{c}	755.67±5.12 ^{a}	124.67±0.57 ^{a}
DQ	57.61±0.71 ^{b}	307.58±0.98 ^{a}	768.2±4.15 ^{a}	125.01±1.31 ^{a}
DFe	46.67±0.8 ^{d}	252.18±2.84 ^{c}	711.35±5.72 ^{c}	123.14±1.79 ^{a}
DX	99.7±0.42 ^{a}	307.63±3.05 ^{a}	695.63±4.54 ^{d}	105.48±1.28 ^{d}
P (ANOVA)	<0.0001	<0.0001	<0.0001	<0.0001

Values are presented as means ± standard deviations (SD). Values with different superscript letters (a, b, c, d…) are significantly different according to Tukey test at $P < 0.05$.

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In kidney tissue, each one of quercetin and iron administration was observed to decrease Cu, Mg and Zn levels in the control rats (CQ, CFe). Additionally, iron-quercetin complex reduced

significantly Cu levels and increased Mg concentrations in control group (CX) without altering Fe and Zn values compared with control group (C).

Moreover, iron supplementation reduced notably Cu and Mg contents in diabetic animals (DFe). Furthermore, complex gavage was observed to increase Cu and Fe levels and to reduce Mg and Zn kidney contents in diabetic group (DX) in comparison with untreated animals (D) (Table 5).

Muscle's Cu and Fe values were noticed as lower in (CQ, CFe, CX and DQ, DFe, DX) groups than those of the control (C) and diabetic (D) animals (Table 06). In addition to this, Mg levels were reduced in all groups (except in DQ and DFe) compared to control rats (C). Quercetin and Fe treatment enhanced remarkably Mg muscle level in diabetic groups (DQ, DFe). However, these levels were reduced by the complex treatment (DX) compared with the untreated diabetic rats (D).

In the same tissue, Zn level was significantly reduced in all groups compared with the control (C) rats. However, Zn level of DQ, DFe and CX rats were observed to be lower than (D) animals (Table 6).

Table 6. Muscle tissue concentrations (mg/Kg of dry tissue) of trace and major elements.

Muscle	[Cu]mg/g	[Fe]mg/g	[Mg]mg/g	[Zn]mg/g
Control rats				
C	5.02±0.36 ^{b}	130.64±0.53 ^{b}	929.63±3 ^{c}	59.17±0.42 ^{a}
CQ	3.2±0.22 ^{c}	83.65±0.6 ^{d}	898.58±4.57 ^{d}	56.38±0.51 ^{b}
CFe	4.41±0.95 ^{b}	90.08±0.55 ^{c}	717.81±4.34 ^{f}	53.8±0.54 ^{c}
CX	3.2±0.35^{c}	60.87±0.45 ^{g}	915.73±2.48 ^{c}	40.35±0.59 ^{e}
Diabetic rats				
D	7.7±0.51 ^{a}	168.76±1.4 ^{a}	770.91±7.01 ^{e}	53.18±0.78 ^{c}
DQ	5.33±0.49 ^{b}	65.91±1.38 ^{f}	1004.57±7.65 ^{a}	35.42±0.66 ^{f}
DFe	3.2±0.35 ^{c}	60.87±0.45 ^{g}	969.96±9.43 ^{b}	45.87±0.88 ^{d}
DX	4.4±0.34 ^{b}	77.83±0.6 ^{e}	731.28±9.41 ^{f}	35.26±0.84 ^{f}
P (ANOVA)	<0.0001	<0.0001	<0.0001	<0.0001

Values are presented as means ± standard deviations (SD). Values with different superscript letters (a, b, c, d…) are significantly different according to Tukey test at $P < 0.05$.

Discussion

STZ, the most potent diabetogenic agent, was frequently used to investigate the disturbances in glucose and lipid metabolisms under experimental diabetes conditions. This chemical compound is recognized to induce similar alterations to those seen in diabetic humans. Dyslipidemia is one of the key risk factors for cardiovascular disease (CVD) in DM which is responsible for a multiple array of metabolic abnormalities [18], including oxidative stress and trace elements status alteration [19].

A recent experimental study [10] reports that iron administration induces a significant increase in glucose level in control and diabetic Wistar rats. Besides, the complex treatment (CX) is observed to induce hyperglycemia only in control animals. However, this high glucose level is significantly reduced by quercetin gavage in diabetic group (DQ) compared with (D) [10]. There are various evidences that relate iron overload with the pathogenesis DM's complications. Moreover, iron overload

influences glucose metabolism ,insulin function and it is closely relevant to oxidative stress [20].

According to Aguirre et al., quercetin improves glycemic control by reducing the intestinal glucose absorption in the glucose transporters GLUT2 *in vitro* and by increasing the glucokinase activity or the prevention of the capital number of pancreatic β-cells islet from degeneration *in vivo* studies [21]. The concentrations of serum TC, TG, LDLc and HDLc are important indexes for the overall metabolic control in diabetic situation [18]. In this study, it is clearly observed that iron gavage increases serum TC, VLDLc and LDLc levels in control and diabetic (CFe, DFe) groups compared with control (C) and diabetic (D) rats, respectively.

In contrast, iron-quercetin complex treatment decreases significantly ($P < 0.05$) the aforementioned parameters in diabetic animals (DX). Also, quercetin and its complex form with iron administration are observed to increase HDLc levels in control and diabetic animals (CQ, CX, DQ, DX). Oral iron administration is shown to decrease HDLc levels in the corresponding groups of rats (CFe, DFe) compared with control group (C).

Hyperglycemia is closely associated with iron stores [20] and dyslipidemia. The latter, is characterized by increasing each of TC, TG [22], LDL, VLDL levels and decreasing of HDL concentrations [23]. Additionally, LDL, either oxidized and/or glycated, are present in plasma that affects the vasculature of diabetic patients [24].

In the same context, several flavonoid-containing plants have been proven to display antidiabetic and antihyperlipidemic potential that are effectively tested in animal models. These group types of polyphenols are observed to decrease TC, TG, VLDL and LDL levels accompanied with an increase of HDL serum in hyperlipidemic rats [25]. Recently, Pillai et al. have demonstrated that treating diabetic rats with vanadium-3-hydroxy flavone complex reinstates the altered lipoproteins through the enhancement of clearance of lipoproteins cholesterol and triglycerides [18]. On the one hand, LDL contains α-tocopherol which is an efficient lipophilic antioxidant that shields the lipoprotein molecules from the harmful effects of oxidation [26]. Likewise, quercetin works to regenerate α-tocopherol from its α-tocopheryl radical in LDL and to reduce lipid peroxidation [21]. On the other hand, Kaluza et al. reports that the disturbance of the trace elements status of Fe, Mg and Zn is intimately correlated to an increase of lipids peroxidation, TC, TG, LDLc concentrations and a decrease of HDLc [27].

Iron-quercetin complex seemingly regulates the lipid profile disturbances directly or indirectly through its antioxidant or its insulin-mimetic effects, or simply by the influence of metal complex on redistribution of other essential elements of diabetic animals.

Iron gavage has different effects on lipases tissues activities. It is significantly shown to increase liver and adipose tissue LPL activity in control and diabetic animals (CFe, DFe). Furthermore, oral iron treatment is shown to reduce HSL activity in diabetic rats (DFe). Likewise, iron-quercetin complex reduces notably liver and adipose LPL activity in diabetic rats (DX). LPL [28] and HSL play a pivotal role in lipids homeostasis [29]. Recently, Imessaoudene et al. have described that oral administration of the same dose of iron-quercetin complex (the same one used in this experiment) induces a significance alteration in hepatic and adipose tissue lipids associated with alterations in lipases activities such as the increase in hepatic and adipose lipase activities and the decrease in adipose hormone sensitive lipase activity in high fat diet obese rats [30]. These

results suggest that iron-quercetin complex treatment has a different effect *in vivo* according to each kind of animal model illness. The reported data also suggest that lipolytic activity of adipose cells variy according to the anatomic location of the fat depot from which the cells have been isolated [31]. Besides, it is stated that both of iron overload and iron deficiency are able to induce lipid peroxidation in diabetic and non diabetic persons. In addition, the impairment of compensative mechanisms in diabetic patients is caused by hyperglycemia or hyperlipidemia, and make the organs or tissues more susceptible to oxidative stress [8].

Iron administration is observed to enhanse significantly liver TG in control and diabetic rats and also adipose TG in control animals (CFe). Iron-quercetin complex treatment works to reduce significantly the liver TG levels. This organ is the main site for quercetin [32], lipid metabolisms and iron storage. Therefore, it is an important site of interactions between these metabolic pathways [33]. Gnoni et al. show that quercetin reduces synthesis and accumulation of lipids and/or TG in rat liver cells [32]. In fact, flavonoids prevent metabolic deregulation by restricting ectopic lipid accumulation and by stimulating the use of both fatty acids and glucose [34]. Iron-quercetin complex may increase and promote the synthesis of triglyceride-rich lipoproteins by the liver when it stimulates its transport and uptake by peripheral tissues. Consequently, a decrease in serum TG levels highlights the hypotriglyceridemia properties of the complex as it was observed in an earlier case study [10].

STZ injection is observed to rise the plasma NO•, O_2^{-}•, TBARs and protein carbonyl in untreated diabetic rats (D). However, iron-quercetin complex administration significantly reduces the aforementioned plasma oxidant parameters in the diabetic animals (DX). Reactive species are often suggested to play a key role in the pathophysiology of various diseases including DM [35]. Numerous studies suggest that quercetin prevents formation of TBARs [36], protein carbonyl [37], $O_2^{-\bullet}$, $NO^{\bullet}$ [21] and peroxynitrite $ONOO^{\bullet}$ [38]. The latter is a potent cytotoxic reactive nitrogen species subsequently reacts with proteins, lipids and DNA to induce tissue's damage [39]. Recent data reported by Liu and Guo state that metal-quercetin complexes exhibit a broad biological activities such as anti-oxidation, SOD like activity and increase the polyphenol bioavailability, thereby suggesting a new approach to deliver quercetin *in vivo* [40].

Memişoğulları et al., suggests a positive correlation between serum TG, TC, and MDA. They have explained that hyperlipidemia contributes to a significant elevation in serum MDA level while hyperlipidemia cannot explain the MDA increase alone [41]. Additionally, animal and human experimentation data confirm that DM have been linked to a compromised status of Cu, Mg, Se, Zn, [42] and Fe [43]. These results agree on earlier findings that demonstrate that the metal concentrations in STZ diabetic rats in some organs are influenced by iron or quercetin supplementation or simply by the synergistic interaction between them (*i.e.* iron-quercetin complex). This study exposes that STZ injection diminish Cu, Fe, Mg and Zn liver levels with respect to the control (C) group. Quercetin treatment enhances significantly Cu, Fe, Mg and Zn contents of liver in (DQ) group with respect to (D) animals. Similarly, the complex gavage which increases Cu and Zn levels and reduces Fe liver contents in (DX) group compared with (D) animals. In kidney tissue, STZ injection decreases Cu and Fe and increases Mg and Zn levels in (D) animals compared with the control group (C). Complex gavage is observed to increase significantly Cu

and Fe levels and to reduce Mg and Zn contents in kidneys diabetic group animals (DX) compared to untreated animals (D).

A significant increase of Cu and Fe has been noticed and a decrease of Mg and Zn muscle levels in diabetic group (D) compared with control rats (C). Besides, iron-quercetin complex treatment is clearly observed to decrease the micronutrient (Cu, Fe, Mg and Zn) muscle levels in the corresponding diabetic group (DX) with respect to the untreated (D) animals. The three considered organs (liver, kidney and muscle) with adipose tissue are known to play a crucial role in the pathogenesis and the progress of DM. The divalent cation transporter 1 (DCT1, also known as NRAMP2 or DCM1) is a metal ion transporter (Fe, Zn, and other bivalent ions) expressed in a variety of tissues of rats, including liver. Different ways of regulation of this divalent metal transporter in liver, kidney and muscle could explain the differences in trace element concentrations observed in these organs [44]. Minerals with chemical similarities such Fe/Zn [45] and Cu/Zn can potentially result in one element mimicking or interfering with another one in their chemical behavior or in the deleterious competition [3]. Furthermore, they can also compete for transport proteins or uptake mechanisms depending on the concentration of each metal. Thus, at standard (homeostatic) concentrations, the absorption of most metals is active or saturable, while at higher intakes, passive diffusion might take place [45]. For example, high levels of Mg are known to restrain the use and absorption of Ca, affecting glucose metabolism and reducing insulin secretion [3]. Johnson et al. have shown an increase in hepatic and renal levels of Cu, Zn in STZ diabetic rats. They have proposed that STZ rat injection generally leads to elevated organ concentrations of Cu, Fe, and Zn [46]. As a result these outcomes confirm the previous findings.

Zn and Fe are mainly stored in the liver and tightly bound to metallothionein and/or ferritin. The abundance of this latter may retain Zn in the iron-overloaded liver. Published reports suggest that Zn reduces Cu absorption by increasing the production of metallothionein in mucosal cells, which then bind Cu preferentially [44]. In addition, some of Mg, Zn and Cu minerals are excreted at higher rates in patients with DM than normal subjects, often leading to excessive urinary mineral wasting [42]. Controversially, another study on STZ diabetes rats shows that, regardless of this increased urinary mineral (Zn and Cu) loss, the contents of these metals in different tissues are increased [47]. So, these outcomes are in agreement with this experiment observations.

The imbalance between the elevated and reduced Cu, Fe, Mg and Zn concentrations are observed in different considered organs, also they could be explained by a selective influence of the hyperglycaemic-hyperinsulinaemic state on the ability of different organs to extract these metals. This supposition is confirmed by the study of Raz et al. who demonstrate that fetuses of diabetic rats have severely zinc-deficient, in spite of the high zinc concentrations in the maternal liver [47].

As final findings, the main factor that determines trace metal absorption and tissue storage is the hyperglycemia that is associated with either a lack or an excess of insulin. This latter is reported to move Mg from the extracellular space to the liver and muscle cells [48]. As mentioned in a docking investigation, insulin-mimic property of flavonoids [49] arises from their ability to bind with insulin binding sites within the receptor [50]. These earlier studies explain the most prominent effects of iron and its complex with quercetin on fluctuation of essential metal in organs' contents of diabetes rats.

Conclusions

In short, both of iron deficiency and iron-overload are implicated in oxidative stress and exacerbation of diabetes complications. Iron supplemented alone induces a deep disturbance in lipid, lipoprotein, lipases, and oxidant status; it causes an imbalance in redistribution of endogenous trace elements organs in both diabetic and non diabetic rats.

In contrast, oral administration of iron complex with quercetin is shown to be less harmful and more beneficial than iron supplemented alone. The complex is also shown to reduce serum cholesterol, liver TG and plasma pro-oxidant markers. However, plasma HDLc of diabetic rats is raised probably due to an increase in physiological response of serum VLDLc under the effects of complex treatment.

Additionally, high variability of elements levels is shown in given tissues of diabetic and non-diabetic rats treated with iron free or iron-quercetin complex. This fluctuation in organs metal contents are element-dependent, suggesting differences in the accumulation and the distribution of the elements in tissues of diabetic and non-diabetic rats. It is healthier to correct metals deficiencies shown in most pathology by natural nutrient present in food to avoid brutal disturbance in redistribution of other essential elements especially for metals sharing the same transporter protein (Fe, Mn, Zn…) if these patients do not suffer from bad absorption.

To sum up, the aforementioned effects *in vivo* of iron-quercetin complex shown in this study are not exhaustive. However, enzymes or hormone bio-mimics such as SOD, catalase activities or insulin mimics, respectively, can be proposed to be considered as mechanisms of iron-quercetin complex' function but these suggestions need further researches to prove them.

Conflict of interest. The authors have no conflicting financial interests.

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