Original Article

The Role of Deferoxamine and Quercetin on the Short Term Chronic Toxicity of Iron on the Pituitary, Ovaries and Uterus of Prepubertal Albino Rats

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ABSTRACT

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Iron is an important metal for Hypothalamic-pituitary-gonadal axis. Deferoxamine used as a potent antioxidant plus its chelating function. Quercetin is an effective antioxidant and also reported to have estrogenic activities. The aim of this study was to evaluate the role of deferoxamine and quercetin in the treatment of toxicity induced by short term chronic administration of iron in prepubertal female rats. One hundred and ten rats were divided into 5 groups: Group I : control (40 rats). Group II:(10 rats) injected IP daily with deferoxamine (DFO) (125mg /kg) for 4 weeks. Group III:(10 rats) treated with quercetin (Q) (2g/kg daily) orally for 4 weeks. Group IV:(10 rats) received deferoxamine + quercetin for 4 weeks. Group V: (40 rats) subdivided into 4 sub-groups, Va: injected IP daily with Iron dextran (300 mg/kg) for 4 weeks then rats were sacrificed and submitted to biochemical and histopathological examination. Vb: injected intraperitoneally daily with iron dextran for 4 weeks, then received daily intraperitoneal deferoxamine for another 4 weeks. Vc: injected intraperitoneally daily with iron dextran for 4 weeks, then received daily quercetin orally by for another 4 weeks. Vd: injected intraperitoneally daily with iron dextran for 4 weeks, then received daily intraperitoneal deferoxamine + quercetin orally for another 4 weeks. The results showed significant increase in serum iron concentration, ferritin and NTBI and TBARS with significant decrease in serum (LH),(FSH) and estradiol hormones and serum (TAC) values in iron group when compared with other groups accompanied by histopathological changes in pituitary, ovaries and uterus, with marked iron deposits detected by Prussian blue stain and increased expression of caspase 3 . Treatment with deferoxamine or quercetin showed improvement in these parameters which nearly were equally effective. Combined treatment with deferoxamine and quercetin was better than single ones.
Key words: Iron, Deferoxamine, Quercetin, Prussian blue, caspace 3, pituitary, ovary, uterus.

I. INTRODUCTION

Iron is an essential metal of life that plays important physiological roles in mammals, such as production of red blood cells, transport of gases, production of energy, nucleic acid synthesis and the cell division process (Ganz, 2013), being found in functional form in hemoglobin, myoglobin, cytochrome enzymes with iron Sulphur complexes (Brewer et al., 2014). Although an optimum level of iron is always maintained by the cells to balance between essentiality and toxicity, in some situations it is disrupted, resulting in iron overload leading to the oxidative stress induced disorders (Shazia et al., 2012). Chronic iron overload was able to contribute to the development of endocrine disorders, such as diabetes mellitus, hypothyroidism and gonadal dysfunction (Musumeci et al., 2014). In mammalian hypogonadism, a reduction of

pituitary gonadotropin hormones levels, impairment in sexual hormone metabolism, estrogen receptor (ER) function, gonadal morpho-physiology and others dysfunctions may result from harmful effect of chronic iron deposit on the hypothalamic-pituitary-gonadal (HPG) axis, which may explain the impairment of reproductive function in any iron overload model (Roussou et al., 2013). Deferoxamine had long term data to support its use as a chelating agent in chronic iron overload in both adult and children (Borgna-Pignatti et al., 2004). Different studies had also focused on the antioxidant capacity of different chelation treatment modalities (Kushner et al., 2001). Flavonoids are phenolic compounds widely distributed in plants. It has been found that most of them are effective antioxidants (Liu et al., 2010). They have a strong affinity to iron ions. Flavonoids supplementation also increased the excretion of iron through feces (Kim et al., 2008). Quercetin had also been reported to have estrogenic activities by activating both estrogen receptors alpha (ERα) and beta (ERβ) (Maggiolini et al., 2001). So, the aim of this study was to evaluate the role of deferoxamine and quercetin in the treatment of toxicity and oxidative stress induced by short term chronic administration of iron in prepubertal female rats.

II. MATERIALS & METHODS

1- Chemicals:

1- Iron dextran (fercayl® Amp) 100 mg / 2 mL was obtained from Sunny medical group.

2- Deferoxamine (DFO) (desferal® vial) 500 mg was obtained from Novartis pharma.

3- Quercetin (Q) yellow powder was obtained from Sigma Aldrich in Germany imported by Cairo chemical company.

4- Reagents and commercial kits serum iron concentrations, non-transferrin bound iron (NTBI), ferritin, luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol (E2), thiobarbituric acid reactive substances (TBARS) and total antioxidant capacity (TAC)

were purchased from Biodiagnostic Co. 29 El-Tahrer Street- Dokki- Giza, Eygpt.

2- Animals:

The experiment was performed in accordance with the guide for the care and use of laboratory animals (Institute of laboratory animals resources, 1996). The rats were obtained from the animal house of faculty of medicine, Zagazig University. The period of the experimental study was extended to 8 weeks. One hundred and ten (110) prepubertal female albino rats, their age about two weeks weighed (80-100 gm) were divided into five groups:-

Group I (control group): Consisted of 40 rats which equally and randomly subdivided into: **Negative control (Ia):** received only regular diet and tap water to measure the basic parameters. **Positive control (Ib):** received normal saline 0.9% NaCl (solvent of quercetin & iron) orally. Ten (10) rats from each group were sacrificed after 4 weeks the remaining rats were sacrificed after 8 weeks.

Group II (deferoxamine (DFO) treated group): (10 rats) injected intraperitoneally daily with deferoxamine (125mg /kg body weight) (Kontoghiorghes et al., 2003) for 4 weeks.

Group III (quercetin (Q) treated group):(10 rats) treated with quercetin (Q) (2g/kg b.w. daily) (Zhang et al., 2006) dissolved in saline orally by gavage for 4 weeks.

Group IV (deferoxamine (DFO)+ quercetin (Q) treated group):(10 rats) injected intraperitoneally daily with deferoxamine along with quercetin orally by gavage for 4 weeks in the same doses mentioned above.

Group V: Consisted of 40 rats equally and randomly subdivided into 4 sub-groups:

Sub-group Va (iron treated group): The rats were injected intraperitoneally (IP) daily with Iron dextran (300 mg/kg) (1/10 of LD50) for 4 weeks (Berdouka et al., 2013) then rats were sacrificed and submitted to biochemical and histopathological examination.

Sub-group Vb (iron & deferoxamine (DFO) treated group): The rats injected

intraperitoneally daily with iron dextran (300 mg/kg) for 4 weeks, then received daily intraperitoneal deferoxamine (125mg /kg body weight) 24 hrs after the last dose of iron dextran for another 4 weeks.

Sub-group Vc (iron& quercetin (Q) treated group): The rats were injected intraperitoneally daily with iron dextran (300 mg/kg) for 4 weeks, then received daily quercetin (Q) orally by gavage (2g/kg b.w.) for another 4 weeks.

Sub-group Vd (iron& deferoxamine (DFO) + quercetin (Q) treated group): The rats were injected intraperitoneally daily with iron dextran (300 mg/kg) for 4 weeks, then received daily intraperitoneal deferoxamine (125mg /kg body weight) in combination with quercetin (Q) (2g/kg b.w. daily) orally by gavage for another 4 weeks.

(B) Methods:
I-Biochemical parameters
A- Iron parameters:-
1- Serum Iron (ug/dL) (Tietz, 1976):
Transferrin-bound iron is measured spectrophotometrically at 560 nm. The absorbance measured at this wavelength is proportional to serum iron concentration

2- Serum Ferritin (Addison et al., 1972):
This assay employs the quantitative sandwich enzyme immunoassay technique (ELISA coated microliter strips).

3- Serum non transferrin bound iron (NTBI) (Hershko et al., 1978):
This approach includes two main steps; in the first step iron is chelated with the help of chelator which is separated from the biological fluid. In the second step chelated iron is subjected to column chromatographies which estimate it. Catechol disulphonic acid was used to capture unbound iron (NTPI) to transferrin in aliquots of serum filtered through DEAE-sephadex A50 columns.

B- Hormones:-
1- Estradiol (E2) (Gore-Langton& Armstrong, 1988):

ELISA is intended for the quantitative determination of Estradiol (E2) concentration in serum and plasma, its concentration in pg/mL.

2- Serum follicle-stimulating hormone (FSH) (Seth et al., 1989):
The microliters plate provided in this kit has been pre-coated with an antibody specific to FSH.

3- Serum luteinizing hormone (LH) (Cumming et al., 1985):
The concentration of LH in the samples is then determined by comparing the O.D. of the samples to the standard curve.

C-Oxidative stress:-
1- Serum thiobarbituric acid reactive substance (TBARS):
Serum (TBARS) was assayed calorimetrically according to the method of Ohkawa., et al (1979).

2- Total antioxidant capacity (TAC):
Serum TAC was assayed calorimetrically according to the method of Koracevic and Koracevic, (2001).

II- Histopathological Study
A- Hematoxylin and Eosin: to verify the histological details, by following the method of (Bancroft& Gamble, 2002).

B- Iron - Prussian Blue Reaction - Mallory's Method (Carson, 1990)**:** to demonstrate ferric iron in tissue sections.

C- Immunohistochemistry (caspase 3):
Caspase 3 immunohistochemistry was performed by following the method of Gown and Willingham (2002).

Image analysis and quantitative morphometric measurements:
Image analysis and quantitative morphometric measurements were done by a method described by (Mustafa, 2015). The degree of immune reaction is indicated by optical density value; the darker it is, the larger the value is (Cheng and Zhang, 2014). Image J analysis of prepared histology microscopic slides provides researchers with a rapid, cheap assessment tool when compared with advanced/ ultrastructure methodology. Image J analysis can structure

into numerical data and easily measured (Williams et al., 2015).

III- Statistical analysis

Data for all groups were expressed as mean± standard deviation (X±SD). The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 20.0 (SPSS Inc, 2007). Statistically significant difference was determined by one-way analysis of variance (ANOVA), followed by the LSD test for multiple comparisons between different groups. The test results were considered significant when p-value <0.05, p-value <0.01 and <0.001 were considered highly significant.

III. RESULTS

I- Biochemical results:

The biochemical findings of –ve control and +ve control (4, 8 weeks of treatment), quercetin (Q), deferoxamine (DFO) and quercetin + deferoxamine (Q+ DFO) groups (4 weeks of treatment) were within normal values and revealed no significance difference (P> 0.05) as regard serum (iron, ferritin, non-transferrin bound iron (NTBI), thiobarbituric acid reactive substance (TBARS), total antioxidant capacity (TAC), estradiol (E2), FSH and LH all over the period of the study by ANOVA. Therefore, –ve control group was used as control group for comparison with other treated groups (table 1).

As regard treated groups iron, iron + deferoxamine (DFO), iron + quercetin(Q) and iron + deferoxamine (DFO) + quercetin (Q):-

A- Serum iron, ferritin and NTBI:

The results of the present study showed that administration of iron for 4 weeks in iron treated group led to a highly significant increase (P <0.001) in the mean values of serum iron, ferritin and NTBI compared with other groups, treatment with either DFO or Q led to a highly significant decrease in serum iron, ferritin and NTBI levels (P< 0.001) compared with iron treated group. Combined treatment with both DFO and Q led to a significant decrease in serum iron, ferritin and

NTBI levels (P< 0.001) compared with single treatment and there was non-significant difference in serum iron, ferritin and NTBI levels (P> 0.05) compared to –ve control group. There was a non-significant difference in serum iron, ferritin and NTBI levels (P> 0.05) in (Fe + DFO) group when compared with (Fe +Q) group (table 2).

B- Serum TBARS and TAC: -

The mean values of serum TBARS and TAC in iron treated group showed a highly significant increase in serum TBARS and a highly significant decrease in serum TAC levels (P <0.001) when compared with other groups. Treatment with either DFO or Q revealed a significant decrease in serum TBARS and a significant increase TAC levels (P<0.001) in (Fe + DFO) and (Fe + Q) groups compared with iron group. There was a significant decrease in serum TBARS and a significant increase TAC levels (P<0.001) in (Fe + DFO + Q) treated group when compared with (Fe + DFO) and (Fe + Q) groups. There was a non-significant difference in serum TBARS and TAC levels (P>0.05) in (Fe + DFO) group when compared with (Fe + Q) group. There was a non-significant difference in serum TBARS and TAC levels (P> 0.05) in (Fe + DFO + Q) treated group when compared with (-ve) control group (table 3).

C- Serum estradiol, FSH and LH:-

Comparison revealed a highly significant decrease in serum estradiol, FSH and LH levels (P<0.001) in (Fe), (Fe + DFO) and (Fe + Q) groups when compared with (-ve) control group but there was a non-significant difference in serum estradiol, FSH and LH levels (P>0.05) in (Fe + DFO + Q) treated group when compared with (-ve) control group. There was a significant decrease in serum estradiol, FSH and LH levels (P<0.001) in iron treated group when compared with (Fe+ DFO), (Fe + Q) and (Fe+ DFO+ Q) groups. There was a significant increase in serum estradiol, FSH and LH levels (P<0.001) in (Fe + DFO + Q) treated group when compared with (Fe + DFO) and (Fe + Q) groups. There was a non-significant difference in serum estradiol, FSH and LH levels (P>

0.05) in (Fe + DFO) group when compared with (Fe + Q) group (table 4).

II- Histopathological results:-

a) **Light microscopic examination**: -

1-The pituitary gland:-

Sections from the pituitary gland of negative, positive control group, DFO group, Q group and DFO + Q group showed normal pituitary tissue formed of normal sheets and groups of acidophil and basophil cells separated by thin connective tissue stroma and thin walled vascular spaces**.** Rats treated with iron for 4 weeks, showed atrophy of the cellular components surrounded by wide area of connective tissue stroma, fragmentation of the connective tissue stroma**.** Administration of DFO or Q in iron treated group for 4 weeks resulted in partial improvement in the histopathological changes of pituitary tissues. There was more improvement in the histopathological changes of the pituitary tissue with combined treatment of iron treated group with deferoxamine and quercetin as shown in Fig. (1).

2- The ovary:-

Sections from the ovaries of negative, positive control group, DFO group, Q group and DFO + Q group (after 4 weeks) showed normal ovarian tissues and normal signs of maturity in the form of primordial follicles, growing follicles and mature graafian follicles surround by dense ovarian stroma**.** Rats treated with iron for 4 weeks, showed absence of primordial follicles and variability as regard ovarian stroma may be dense or atrophic infilterated by extensive areas of hemorrhage. Administration of DFO or Q in iron treated group for 4 weeks revealed partial improvement in histopathological changes of the ovarian tissues. There was more improvement in the histopathological changes of ovarian tissues with combined treatment of iron treated group with deferoxamine and quercetin as shown in Fig. (2).

3-The uterus:-

Sections from uterus of negative, positive control group, DFO group, Q and DFO + Q group after 4 weeks showed normal uterine tissues in the form of normal endometrium; round proliferative endometrial glands lined by columnar epithelium and surrounded by sheets of spindle shaped stromal cells, normal myometrium formed of longitudinal and cross sectional smooth muscle fibers**.** Rats treated with iron for 4 weeks, showed atrophic endometrial glands and areas of hemorrhage in the myometrium**.** Administration of DFO or Q in iron treated group for 4 weeks revealed partial improvement in histopathological changes of the uterine tissues. There was more improvement in the histopathological changes of uterine tissues with combined treatment of iron treated group with deferoxamine and quercetin as shown in Fig. (3).

B) Special stain with Prussian blue:-

1- The pituitary gland

By special stain for iron (Prussian stain) the negative, positive control group, DFO, Q and DFO + Q treated groups showed no iron deposits in the pituitary tissue**.** Iron treated group showed marked iron deposits were irregularly distributed in pituitary cells and stroma**.** Iron + DFO group showed moderate irregular scattered iron pigments, iron + Q treated group showed mild scattered iron pigments and iron+ DFO+ Q group showed faint iron deposits Fig. (4).

2-The ovary:-

By special stain for iron (Prussian stain), the negative, positive control group, DFO, Q and DFO + Q treated groups showed no iron deposits in the follicles and ovarian stroma**.** Iron treated group showed marked iron deposits were irregularly distributed in the ovarian tissue. Iron + DFO group showed moderate irregular scattered iron pigments, iron + Q treated group showed mild scattered iron pigments and Iron + DFO+ Q group showed faint iron deposits Fig. (5).

3-The uterus:-

By special stain for iron (Prussian blue), the negative control, positive control group, DFO, Q and DFO + Q treated groups showed no iron

pigments in endometrial glands and uterine stroma**.** Iron treated group showed marked iron deposits were irregularly distributed in the uterine stroma and myometrium**.** Iron+ DFO group and iron+ Q group showed mild irregular scattered iron pigments, while iron+ DFO + Q group showed scanty localized iron pigments around well-formed proliferated endometrial glands Fig (6).

d) Immune- histochemical examination:
Intensity of the staining was scored as negative reaction, positive (marked, moderate and mild) reaction according to intensity of positive staining of cells

1-The pituitary gland:-
The Immune-histochemical examination of the pituitary gland of negative control, positive control, DFO, Q and DFO + Q treated groups showed negative immunoreactivity for caspase 3. Iron treated group showed marked immunoreactivity for caspase 3 was irregularly distributed in the pituitary tissues. Iron + DFO group showed moderate immunoreactivity for caspase 3, iron + Q treated group showed mild immunostaining for caspase 3, iron+ DFO + Q group showed scanty immunoreactivity for caspase 3 Fig (7).

2 - The ovary:-
The immunehistochemical examination of the ovaries of negative control, positive control, DFO, Q and DFO + Q treated groups showed negative immunoreactivity for caspase 3. Iron treated group showed marked immunoreactivity for caspase 3 was irregularly distributed in the ovarian tissues. Iron + DFO group showed moderate immunostaining for caspase 3, iron + Q treated group showed mild immunostaining for caspase 3, iron + DFO + Q group showed scanty immunostaining for caspase 3 Fig (8).

3– The uterus:-
The Immune-histochemical examination of the uterus of negative control, positive control, DFO, Q and DFO + Q treated groups showed negative immunoreactivity for caspase 3. Iron treated group showed marked

immunoreactivity for caspase 3 was irregularly distributed in the uterine stroma and myometrium. Iron + DFO group showed moderate immunostaining for caspase 3, iron + Q treated group showed mild immunostaining for caspase 3, while iron + DFO + Q group showed scanty immunostaining for caspase 3 Fig (9).

Quantitative morphometric measurements of caspase3:
Statistical comparison of quantitative morphometric measurements of caspase3 (optical density) in the pituitary gland, ovary and uterus among -ve control, +ve control, Q, DFO, Q + DFO groups by using ANOVA test showed non-significant difference ($p > 0.05$) among these groups. So the negative control group was used as a control group to be compared with other treated groups.

There was a highly significant increase in the pituitary gland, ovarian and uterine tissue caspase 3 optical density ($P < 0.001$) among iron, (Fe + DFO), (Fe + Q), and (Fe+ DFO+ Q) groups. Results of comparison revealed a highly significant increase in mean values of caspase 3 optical density ($p < 0.001$) in the pituitary gland, ovary and uterus in (Fe), (Fe + DFO), (Fe + Q) groups when compared with (-ve) control group, but there was a non-significant increase in their levels ($P > 0.05$) in (Fe+ DFO+ Q) treated group when compared with (-ve) control group. There was a highly significant increase in mean values of caspase3 optical density ($P < 0.001$) in Iron treated group (Fe) when compared with (Fe + DFO), (Fe + Q), (Fe+ DFO+ Q) groups. There was a highly significant decrease in mean values of caspase3 optical density ($P < 0.001$) in (Fe + DFO + Q) treated group when compared with (Fe + DFO) and (Fe + Q) groups. There was a non-significant difference in mean values of caspase3 optical density ($P > 0.05$) in (Fe + DFO) group when compared with (Fe + Q) group

Table (1): Statistical comparison of serum (iron, ferritin, NTBI, TBARS, TAC, estradiol, FSH and LH) among different studied groups by ANOVA test: -

Parameters	-ve control		+ve control		Q No.=10	DFO No.=10	Q +DFO No.=10	F	p. value
	(4 w) No.=10	(8 w) No.=10	(4 w) No.=10	(8 w) No.=10					
	Mean ± SD								
Iron	157.3±2.1	156±1.8	156.6±2.5	154.9±0.9	156.3±3.2	159.5±1.2	158.1±3	2.503	0.056
Ferritin	105.03 ±11.7	103.9±9.8	101.7±13.3	100±12.9	102.7 ±4.9	101.8 ±6.8	101.6 ±5.1	0.251	0.908
NTBI%	5.7±1.1	5± 0.9	5.9±1.3	5.2± 0.8	5.5±0.9	5.6±0.7	4.9±0.7	1.273	0.295
TBARS	90.3±1.6	92.04±1.2	90.6±0.9	91± 0.4	89.7±0.4	89.9±0.4	89.6±0.4	2.287	0.075
TAC	1.6±0.2	1.2±0.6	1.59±0.1	1.4± 0.3	1.5±0.2	1.6±0.1	1.7±0.1	0.616	0.654
Estradiol	16.8±0.4	15.8±0.2	16.7±0.4	16± 0.2	16.74±0.6	16.6±0.5	17±0.7	0.88	0.483
FSH	2.2±0.4	1.9±0.6	2.1±0.6	2± 0.5	2.6±0.5	2.3±0.5	2.5±0.4	1.191	0.328
LH	0.85±0.1	0.86±0.3	0.88±0.1	0.85± 0.04	0.89±0.1	0.9±0.08	0.97±0.2	1.041	0.396

All values are expressed as Mean ± SD (Standard deviation)
No: number of rats in each group=10 rats.   P: > 0.05 non-significant.   P: < 0.05 significant.
NTBI: non transferrin bund iron.   TBARS: thiobarbituric acid reactive substance.   TAC: total antioxidant capacity.
FSH: follicle stimulating hormone.   LH: lutinizing hormone.   Q: quercetin.
DFO: deferoxamine   Q + DFO: quercetin+ deferoxamine.

Table (2): Statistical comparison of mean values of serum (iron, ferritin and NTBI) in different studied groups using ANOVA & LSD test: -

Studied groups	Iron	Ferritin	NTBI%
	Mean ±SD	Mean ±SD	Mean ±SD
-ve control	157.3±2.1	105.4 ±1.1	5.7±1.1
Fe	338.5±7.7	334.3 ±2.2	15.2±1.1
Fe + DFO	232.5±46.4	182.4±2.1	10.6±0.5
Fe + Q	230.7±50.7	180.8±0.8	10.7±0.6
Fe + DFO + Q	179.8±11.4	107±2.5	9.5±0.4
F	49.682	90.363	174.002
P .value	<0.001**	<0.001**	<0.001**
LSD	Control versus Fe (p≤ 0.001)** Control versus Fe + DFO (p≤0.001)** Control versus Fe + Q (p≤0.001)** Control versus Fe + DFO + Q (p= 0.116) Fe versus Fe + DFO (p≤ 0.001)** Fe versus Fe + Q (p≤ 0.001)** Fe versus Fe + DFO + Q (p≤ 0.001)** Fe+ DFO+ Q versus Fe+ DFO (p≤0.001)** Fe+ DFO + Q versus Fe+ Q (p≤ 0.001)** Fe+ DFO versus Fe+ Q (P= 0.895)	Control versus Fe (p≤ 0.001)** Control versus Fe + DFO (p≤ 0.001)** Control versus Fe + Q (p≤ 0.001)** Control versus Fe + DFO + Q (p= 0.197) Fe versus Fe + DFO (p≤ 0.001)** Fe versus Fe + Q (p≤ 0.001)** Fe versus Fe + DFO+ Q (p≤ 0.001)** Fe + DFO + Q versus Fe+ DFO (P≤ 0.001) Fe + DFO+ Q versus Fe+ Q (p≤ 0.001) Fe + DFO versus Fe+ Q (p=0.195)	Control versus Fe (p≤ 0.001)** Control versus Fe + DFO (p≤ 0.001)** Control versus Fe + Q (p≤ 0.001)** Control versus Fe + DFO+ Q (p= 0.56) Fe versus Fe + DFO (p≤ 0.001)** Fe versus Fe + Q (p≤ 0.001)** Fe versus Fe + DFO+ Q (p≤ 0.001)** Fe+ DFO+ Q versus Fe+ DFO (p<0.001)** Fe + DFO + Q versus Fe + Q (p≤ 0.001)** Fe + DFO versus Fe + Q (P=0.68)

All values are expressed as Mean± SD. **SD:** Standard deviation   P > 0.05 non-significant.   P < 0.05 significant *   P < 0.001 highly significant **

NTBI: non transferrin bound iron.   Fe: iron group.   Fe + Q: iron + quercetin.   Fe+ DFO: iron+ deferoxamine

Fe+ DFO +Q: iron+ deferoxamine+ quercetin.

Table (3): Statistical comparison of mean values of serum (TBARS and TAC) in different studied groups using ANOVA& LSD test:

Studied groups		TBARS	TAC
		Mean ±SD	**Mean ±SD**
-ve control		90.3± 1.6	1.6± 0.2
Fe		151.4±1.9	0.6± 0.1
Fe + DFO		98.2± 0.9	1.2± 0.3
Fe + Q		97.4± 0.9	1.3± 0.3
Fe + DFO + Q		89.6± 2	1.5± 0.2
One-way ANOVA	**F**	2824.153	24.7
	P	<0.001**	<0.001**
	LSD	Control versus Fe **(P≤ 0.001**)** Control versus Fe + DFO **(P ≤ 0.001**)** Control versus Fe + Q **(P ≤ 0.001**)** Control versus Fe + DFO + Q **(P= 0.277)** Fe versus Fe + DFO **(P≤ 0.001**)** Fe versus Fe + Q **(P≤ 0.001**)** Fe versus Fe + DFO+ Q **(P≤ 0.001**)** Fe + DFO + Q versus Fe+ DFO **(P≤0.001**)** Fe+ DFO+Q versus Fe+ Q **(p≤ 0.001**)** Fe + DFO versus Fe+ Q **(P= 0.234)**	Control versus Fe **(P≤ 0.001**)** Control versus Fe+ DFO **(P= 0.001*)** Control versus Fe+ Q **(P= 0.028*)** Control versus Fe+ DFO+ Q **(P = 0.778)** Fe versus Fe+ DFO **(P≤ 0.001**)** Fe versus Fe+ Q **(P≤ 0.001**)** Fe versus Fe+ DFO+ Q **(P ≤ 0.001**)** Fe + DFO + Q versus Fe + DFO **(P= 0.002*)** Fe + DFO + Q versus Fe + Q **(P= 0.005*)** Fe + DFO versus Fe + Q **(P= 0.172)**

All values are expressed as Mean± SD. SD: Standard deviation   P > 0.05 non-significant.   P < 0.05 significant *   P < 0.001 highly
TBARS: thiobarbituric acid reactive substance.   TAC: total antioxidant capacity.
 Fe: iron group.   Fe + Q: iron + quercetin.   Fe+ DFO: iron+ deferoxamine
        DFO: deferoxamine   Fe+ DFO +Q: iron+ deferoxamine+ quercetin

Table (4): Statistical comparison of mean values of serum (estradiol, FSH and LH) in different studied groups using ANOVA & LSD test:-

Studied groups	Estradiol	FSH	LH
	Mean ±SD	**Mean ±SD**	**Mean ±SD**
-ve control	16.8±0.4	2.2±0.07	0.85±0.1
Fe	6±1.2	0.8±0.07	0.34±0.08
Fe + DFO	12.5±1.5	1.3±0.09	0.70±0.08
Fe + Q	11.7±1.7	1.4±0.08	0.64±0.1
Fe + DFO+ Q	16.9±1.5	2.0±0.7	0.74±0.1
F	113.071	15.328	32.898
P	<0.001**	<0.001**	<0.001**
LSD	Control versus Fe (p≤ 0.001**) Control versus Fe+ DFO (p≤ 0.001**) Control versus Fe+ Q (p ≤ 0.001**) Control versus Fe+ DFO+ Q (p= 0.895) Fe versus Fe+ DFO (p ≤ 0.001**) Fe versus Fe+ Q (p≤ 0.001**) Fe versus Fe+ DFO+ Q (p≤ 0.001**) Fe+ DFO+ Q versus Fe+ DFO (p≤0.001**) Fe+ DFO+Q versus Fe+ Q (p≤0.001**) Fe+ DFO versus Fe+ Q (p= 0.218)	Control versus Fe (p≤ 0.001**) Control versus Fe+ DFO (p≤ 0.001**) Control versus Fe+ Q (p= 0.001**) Control versus Fe+ DFO+ Q (p=0.341) Fe versus Fe+ DFO (p ≤ 0.036*) Fe versus Fe+ Q (p ≤ 0.007*) Fe versus Fe+ DFO+ Q (p ≤ 0.001**) Fe+ DFO + Q versus Fe+ DFO (p ≤ 0.002*). Fe+ DFO+ Q versus Fe+ Q (p ≤0.01*) Fe+ DFO versus Fe+ Q (p= 0.444).	Control versus Fe (p ≤ 0.001**) Control versus Fe+ DFO (p =0.041*) Control versus Fe+ Q (p ≤ 0.001**) Control versus Fe+ DFO+ Q (p=0.33) Fe versus Fe+ DFO (p ≤ 0.001**) Fe versus Fe+ Q (p ≤ 0.001**) Fe versus Fe+ DFO+Q (p ≤ 0.001**) Fe+ DFO + Q versus Fe+ DFO (p ≤0.001**) Fe+ DFO+ Q versus Fe+ Q (p ≤0.001**) Fe+ DFO versus Fe+ Q (p= 0. 23)

All values are expressed as Mean± SD. SD: Standard deviation   P > 0.05 non-significant. P < 0.05 significant *   P < 0.001 highly
FSH: follicle stimulating hormone.   LH: lutinizing hormone.
Fe: iron group.   Fe + Q: iron + quercetin.   Fe+ DFO: iron+ deferoxamine.   Fe+ DFO +Q: iron+ deferoxamine+ quercetin.
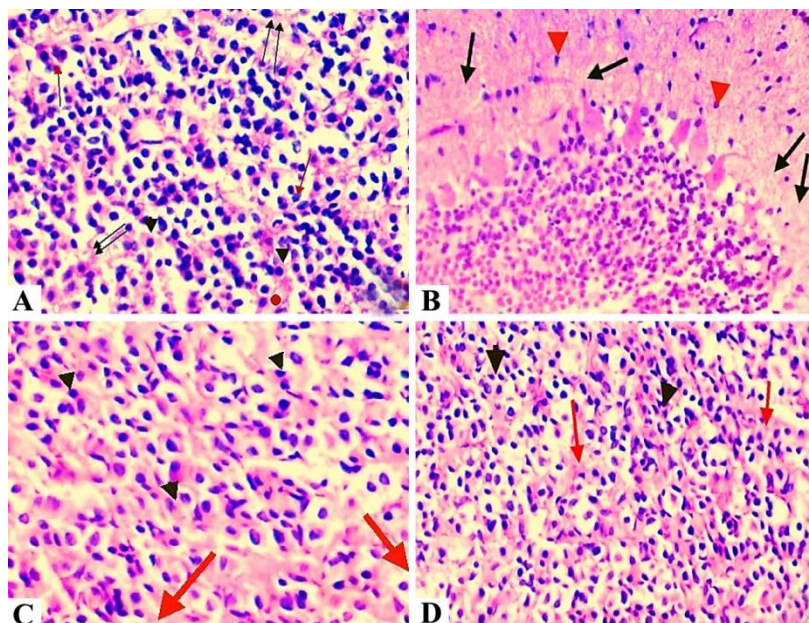
Fig(1): A photomicrograph of a section from the pituitary gland of prepubertal female albino rat from (A) negative control, deferoxamine, quercetin and deferoxamine + quercetin treated groups showing normal sheets and groups of acidophils (arrow head▼ ▼) and basophils (arrow↓) separated by thin connective tissue stroma (double arrow ↑↑) and thin walled vascular spaces (circle ●), (B) iron treated group showing atrophy of the cellular components (head arrow▼) surrounded by wide area of connective tissue stroma (black arrow↓), fragmentation of the connective tissue stroma and separation of the pituitary tissue (red arrow↓), (C) iron+ deferoxamine treated group and (D) iron+ deferoxamine + quercetin group showing regenerated pituitary cells (arrow head ▼ ▼) with mild fragmentation of the connective tissue stroma (arrow↓↓) (H&E X 400).
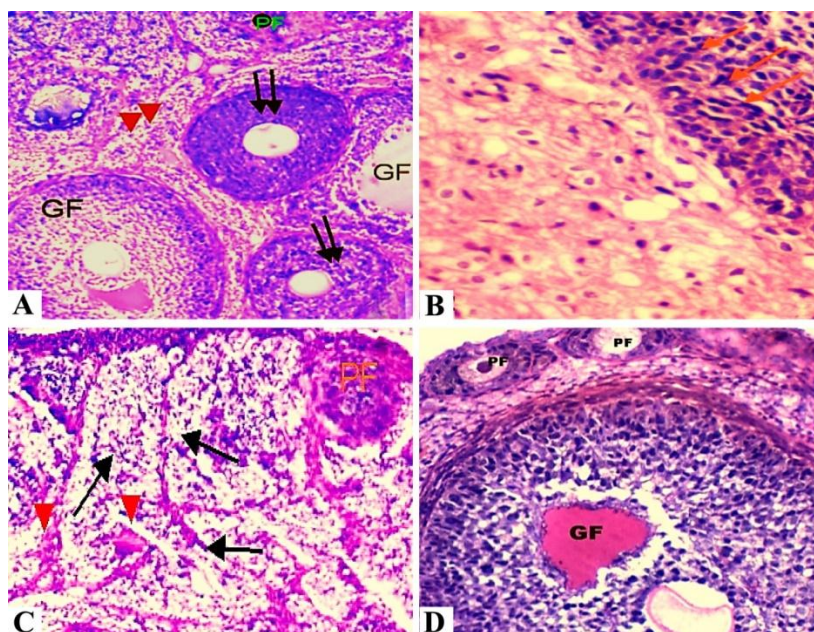
Fig (2): A photomicrograph of a section from ovary of prepubertal female albino rat from (A) negative control group, showing normal ovarian tissues; primordial follicles (PF), growing follicles (black arrow↓) and mature graafian follicles (GF) surrounded by dense ovarian stroma (red arrow↓), (B) iron treated group showing dense ovarian stroma (red arrows↓↓↓) , absence of primordial gollicles and, atrophic stroma infilterated by extensive areas of hemorrhage (black arrows↓↓↓) and absence of primordial gollicles, (C) iron+ quercetin treated group showing regenerated primordial follicle (PF), mild congestion (arrow head ▼) of light dense connective tissue stroma (arrow↓) and (D) iron+ deferoxamine + quercetin group showing well-formed primordial (PF), mature and graafian follicles (GF) embedded in connective tissue stroma ([H&E X400].
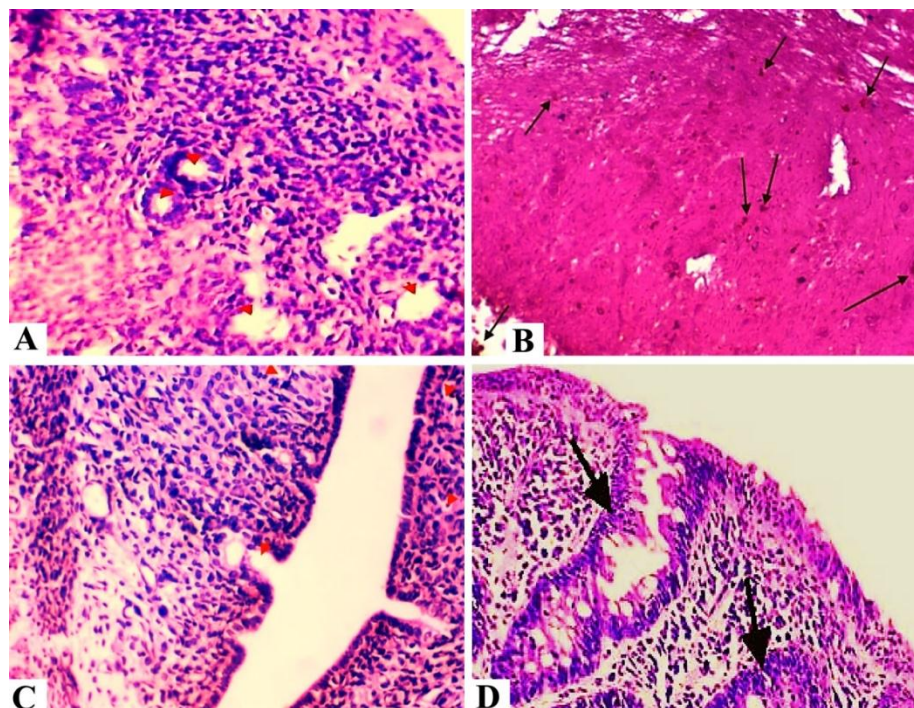
Fig (3): A photomicrograph of a section from uterus of prepubertal female albino rat from (A) negative control, deferoxamine, quercetin and deferoxamine + quercetin treated groups showing; Normal endometrium; round proliferative endometrial glands (arrow head ▼) lined by columnar epithelium and surrounded by sheets of spindle shaped stromal cells. (B) iron treated group showing atrophic endometrial glands and areas of hemorrhage in the myometrium (arrows↓↓↓), (C) iron+ quercetin treated group showing appearance of endometrial glands (arrow head ▼) lined by columnar epithelium and no area of hemorrhage in uterine stroma (D) iron+ deferoxamine+ quercetin group showing highly proliferative glands (arrow↓)((H&E X 400).
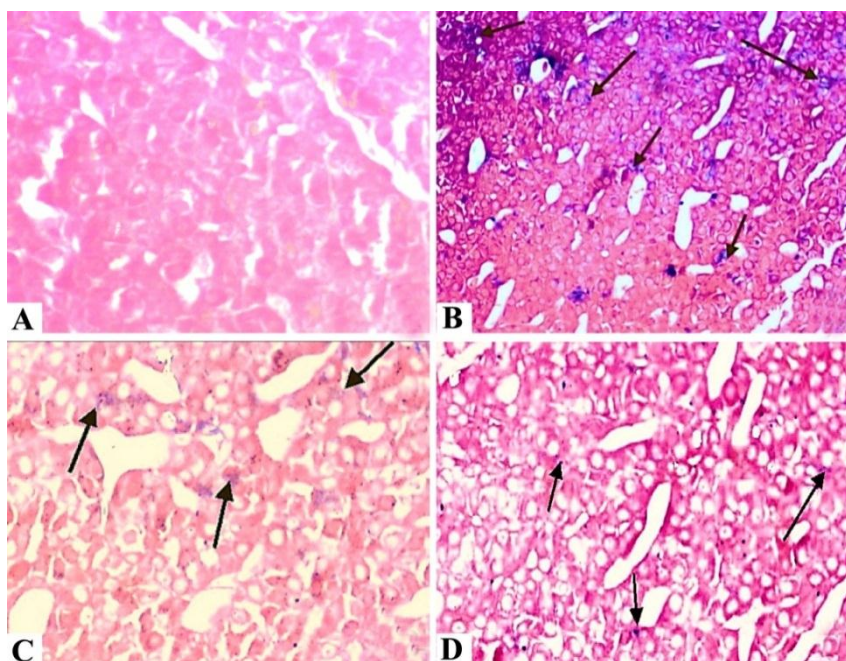

Fig (4): A photomicrograph of a section from the pituitary gland of prepubertal female albino rat from (A) negative control group, showing absence of iron pigments in the pituitary cells and stroma, (B) iron treated group showing marked irregular distributed iron pigments in the pituitary cells and stroma (C) iron + quercetin treated group showing moderate scattered iron pigments in the pituicytes & stroma and (D) iron + deferoxamine + quercetin group showing faint scattered iron pigments in the pituitary cells & stroma (Prussian blue X 400).
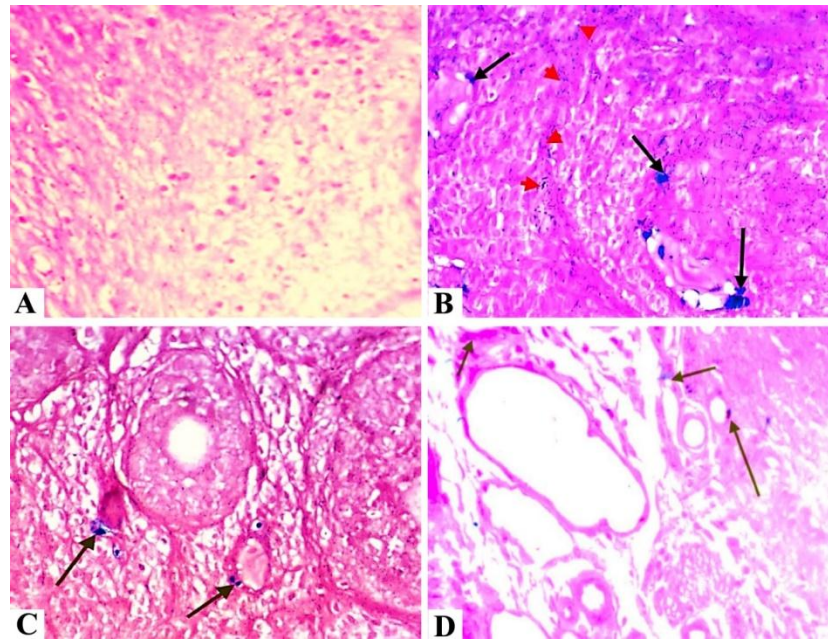
Fig (5): A photomicrograph of a section from ovary of prepubertal female albino rat from (A) negative control group showing absence of iron pigments in the follicles and ovarian stroma, iron treated group (B) showing marked widely distributed iron deposits in the ovarian tissue (C) iron + quercetin treated group showing moderate iron deposits in the graafian follicles and in the ovarian stroma and (D) iron + deferoxamine + quercetin treated group showing scanty iron deposits in the graafian follicles and in the ovarian stroma (Prussian blue X 400).
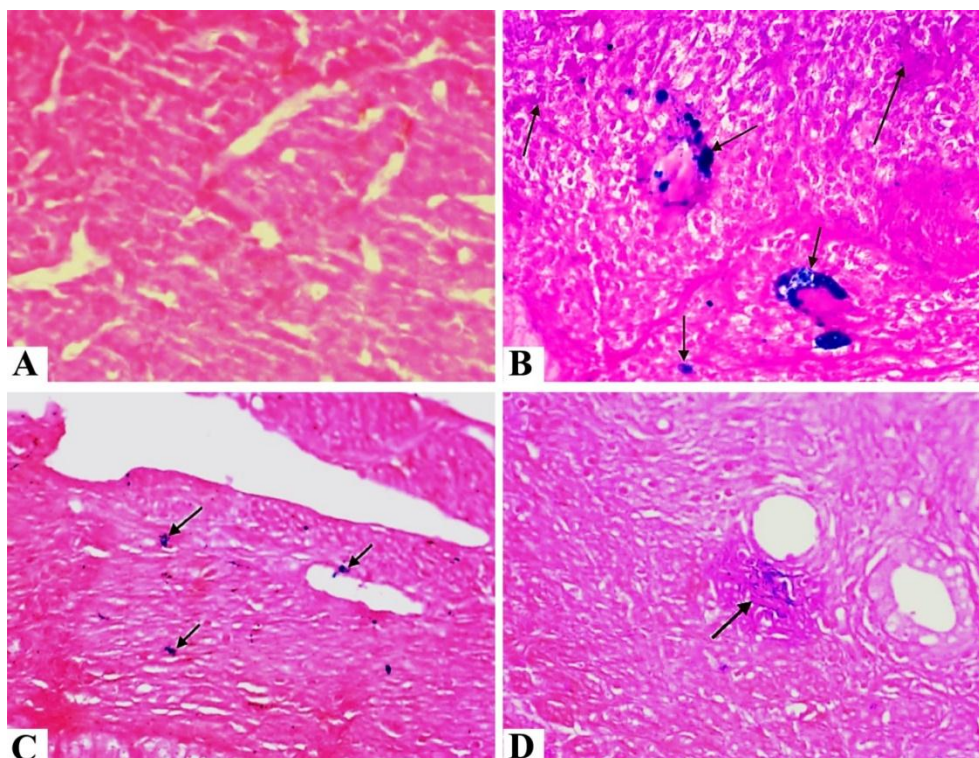

Fig (6): A photomicrograph of a section from uterus of prepubertal female albino rat from (A) negative control group, showing absence of iron pigments in the endometrial glands and uterine stroma, (B) iron treated group showing marked iron pigments deposition in the stroma tissue and in the smooth muscle fibers, (C) iron+ quercetin treated group showing mild deposits of iron pigments scattered around endometrial glands and myometrium (arrow ↑) and (D) iron+ deferoxamine + quercetin treated group showing localized deposits of iron pigments around well-formed proliferated endometrial glands (arrow↑) (Prussian blue X400).
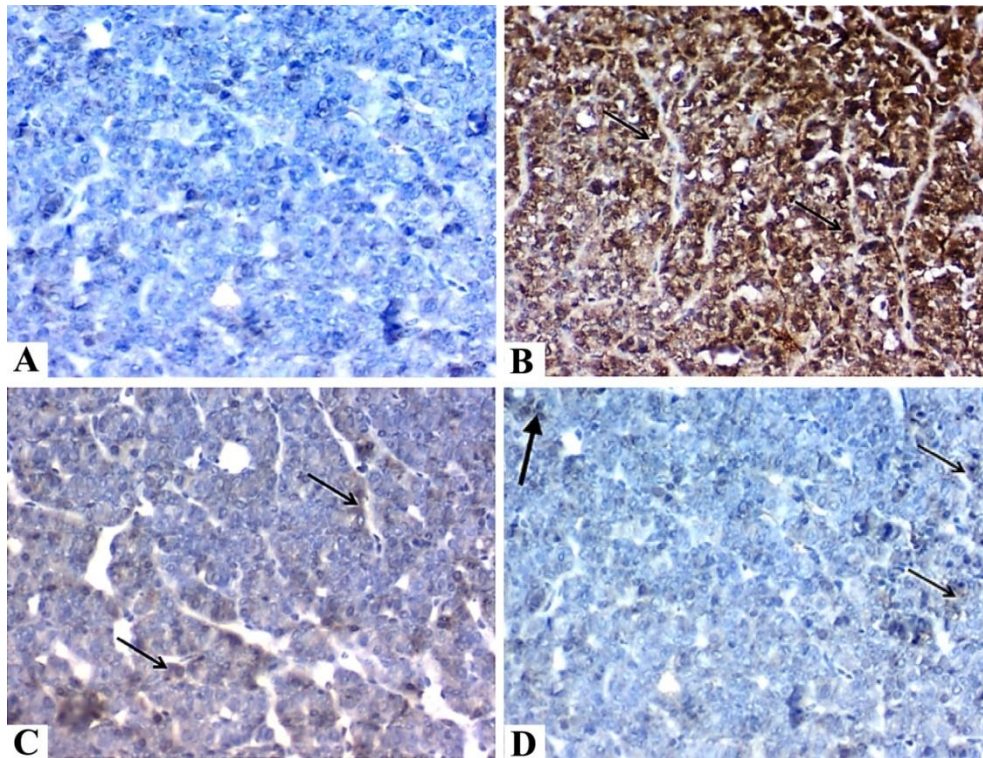
Fig (7): A photomicrograph of a section from the pituitary gland of prepubertal female albino rat from (A) negative control group, showing negative immunostaining for caspase 3, (B) iron treated group showing marked immunostaining for caspase 3 (arrow ↑), (C) Iron + quercetin group showing moderate immunostaining for caspase 3, (D) Iron + deferoxamine + quercetin group showing scanty immunostaining for caspase 3 (immune stain caspase 3 X 400).
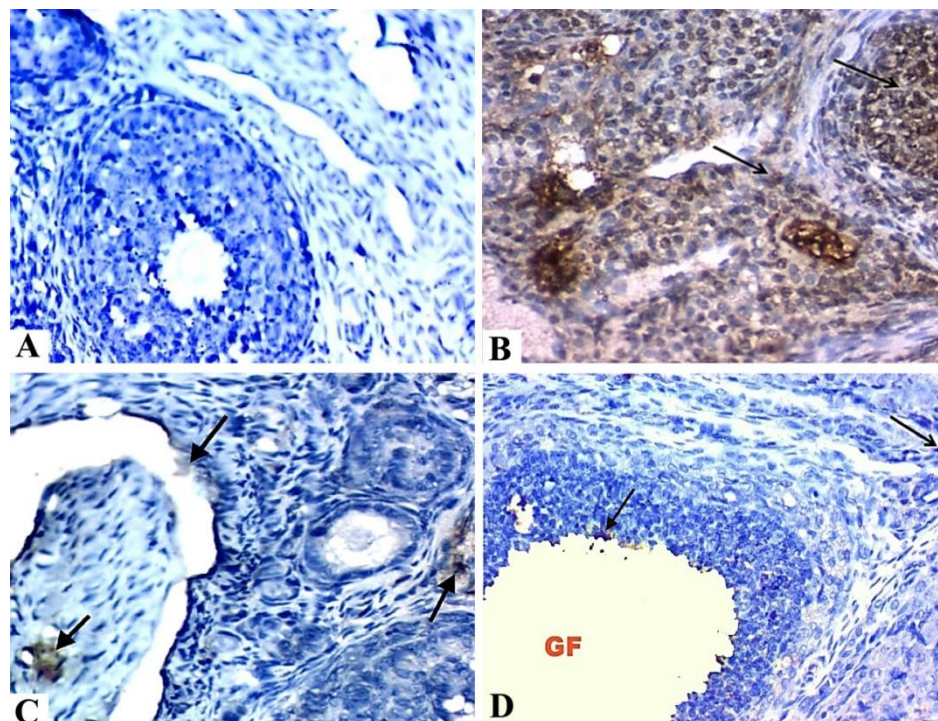

Fig (8): A photomicrograph of a section from ovary of prepubertal female albino rat from (A) negative control group showing negative immune stain for caspase 3, (B) iron treated group showing marked immune stain for caspase 3, (C) iron + deferoxamine showing mild immune stain for caspase 3, while (D) iron + deferoxamine + quercetin treated group showing faint positive immune stain for caspase 3 (Immune stain caspase 3 X 400)
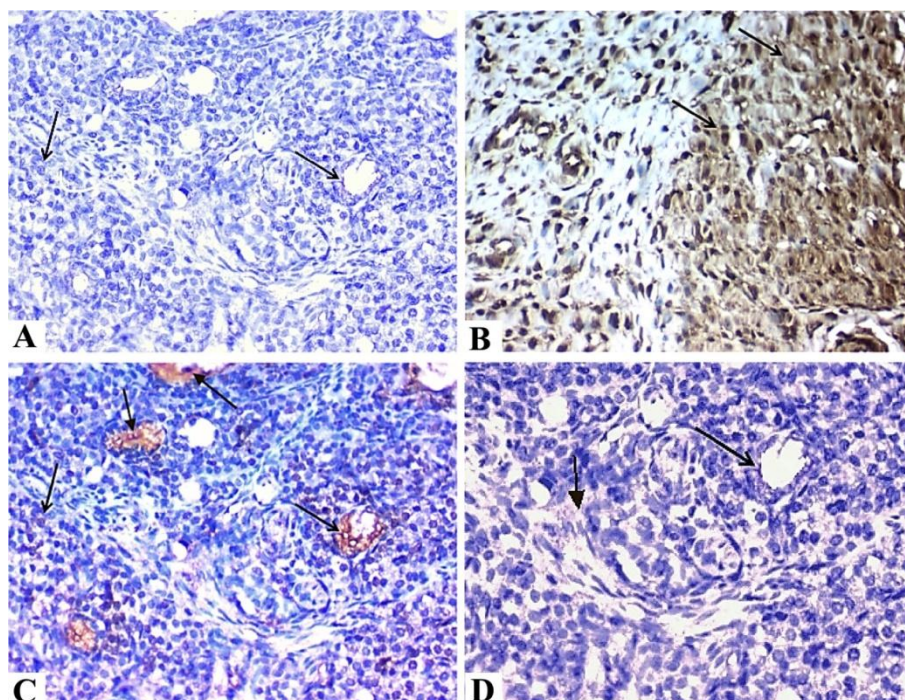
Fig (9): A photomicrograph of a section from uterus of prepubertal female albino rat from (A) negative control group showing negative immune stain for caspase 3,(B) iron treated group showing marked immunostaining of the stromal cells for caspase 3, (C) iron+ quercetin treated group showing mild immunostaining of the endometrial glands and surrounding stromal cells, (D) iron + deferoxamine + quercetin treated group showing faint immunostaining of the endometrial glands and surrounding stromal cells [immune stain caspase 3 X 400].

Table (7):- Statistical comparison of the mean values of caspase3 optical density in the pituitary gland, ovary and uterus among different studied groups using ANOVA test:-

	ve control		+ ve control		Q	DFO	Q+DFO	F	p. value
	(4weeks)	(8weeks)	(4weeks)	(8weeks)					
	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD		
Pituitary	0.69±0.2	0.68±0.5	0.67±0.2	0.66±0.9	0.66±0.2	0.65±0.3	0.64±0.2	0.065	0.978
Ovary	0.67±0.2	0.66±0.7	0.65±0.2	0.66±0.01	0.66±0.1	0.64±0.2	0.61±0.2	0.147	0.931
Uterus	0.23±0.07	0.24±0.01	0.23±0.07	0.22±0.4	0.19±0.09	0.18±0.08	0.21±0.06	0.5	0.686

values are expressed as Mean± SD. **SD:** Standard deviation $P > 0.05$ non-significant.
$P < 0.05$ significant * $P < 0.001$ highly significant   Q: quercetin
 DFO: deferoxamine   Q + DFO: quercetin + deferoxamine.

Table (8):- Statistical comparison of the mean values of caspase 3 optical density in the pituitary gland, ovary and uterus among different studied groups:

Studied groups	Pituitary Mean ±SD	Ovary Mean ±SD	Uterus Mean ±SD
-ve control	0.69±0.2	0.67±0.2	0.23±0.07
Fe	16.7±2.5	16.2±2.3	15.67±4.2
Fe + DFO	6.8±0.9	7.03±0.9	6.94± 1.06
Fe + Q	6.3±1.4	6.9±4.3	6.56± 1.5
Fe + DFO +Q	0.9±0.8	0.89± 1.1	0.61± 0.5
F	136.024	46.892	55.123
P. value	< 0.001**	< 0.001**	< 0.001**
LSD	Control versus Fe (p≤ 0.001**) Control versus Fe + DFO (p≤ 0.001**) Control versus Fe + Q (p≤ 0.001**) Control versus Fe + DFO + Q (p= 0.11) Fe versus Fe + DFO (p= 0.001**) Fe versus Fe + Q (p≤ 0.001**) Fe versus Fe + DFO + Q (p≤ 0.001**) Fe + DFO versus Fe + Q (p= 0.576) Fe+ DFO versus Fe +DFO+Q (p≤ 0.001**) Fe + Q versus Fe + DFO+ Q (p≤0.001**)	Control versus Fe (p≤ 0.001**) Control versus Fe + DFO (p≤ 0.001**) Control versus Fe + Q (p≤ 0.001**) Control versus Fe + DFO + Q (p=0.78) Fe versus Fe + DFO (p≤ 0.001**) Fe versus Fe + Q (p≤ 0.001**) Fe versus Fe + DFO + Q (p≤ 0.001**) Fe + DFO versus Fe + Q (p= 0.891) Fe + DFO versus Fe+ DFO+ Q (p= 0.002*) Fe+ Q versus Fe + DFO + Q (p=0.003*)	Control versus Fe (p≤ 0.001**) Control versus Fe + DFO (p≤ 0.001**) Control versus Fe + Q (p≤ 0.001**) Control versus Fe + DFO + Q (p=0.81) Fe versus Fe+ DFO (p≤ 0.001**) Fe versus Fe+ Q (p≤ 0.001**) Fe versus Fe+ DFO+ Q (p≤ 0.001**) Fe + DFO versus Fe + Q (p= 0.732) Fe + DFO versus Fe+ DFO + Q (p= 0.002*) Fe+ Q versus Fe + DFO + Q (p=0.004*)

values are expressed as Mean± SD. **SD:** Standard deviation   P > 0.05 non-significant.
P < 0.05 significant *   P < 0.001 highly-significant.   Fe: iron   Fe+ Q: iron+ quercetin
Fe+ DFO: iron+ deferoxamine   Fe+ DFO+ Q: iron+ deferoxamine + quercetin.

IV. DISCUSSION

The present study had been conducted to evaluate the role of with deferoxamine (DFO) and quercetin (Q) in the treatment of toxicity and oxidative stress of the pituitary, ovaries and uterus induced by short term chronic exposure to iron.

The results showed that exposure to iron (300 mg/kg) daily for 4 weeks caused significant increase in some iron parameters compared to control group as serum iron concentration, ferritin level and non-transferrin bound iron (NTBI) which constitutes a labile form of iron and indicator of the biologic damage associated with iron overload. These results were in agreement with that of El-Maraghy et al. (2009) who recorded that administration of ferric nitrilotriacetate induced a significant deposition of iron in rat liver associated with increase serum iron concentration and serum ferritin levels. Furthermore, Wassem et al. (2011) reported that, the serum ferritin and non-transferrin bound iron (NTBI) were significantly increased in iron overloaded patients, as compared with the control group. In the present study, treatment with DFO or Q induced a significant decrease in serum iron, ferritin and NTBI   levels when compared with iron treated group. There was a non-significant difference between (Fe + DFO) group and (Fe + Q) which indicate either treatment is nearly equally effective in decreasing high iron parameters. These results of the present study were supported by Evans et al. (2010) who reported that, DFO is effective at lowering serum ferritin levels and hepatic iron concentration. Also Kalpravidh et al. (2010) reported that, Q reduced ferritin protein in cultured liver cells and serum iron concentration, suggesting that iron chelation may be an additional mode of action of flavonoid. The results of this study showed that combined treatment of iron with DFO and Q showed a highly significant decrease in serum iron, ferritin and NTBI levels when compared with (Fe + DFO) and (Fe + Q) groups indicating more effectiveness than single treatment. These results were in agreement with Ho et al. (2011) who found that, combination of flavonoid and DFO showed a greater efficacy in promoting iron excretion than usage of chelator alone, as well as rapid reduction of iron overload in the heart and liver and reversal of cardiac dysfunction in iron overloaded patients.

In the present study, serum estradiol, FSH and LH levels showed a highly significant decrease in iron treated group when compared with other groups. These results indicated an impaired endocrinal functions of iron overloaded rats that is in agreement with Sylvia, (2017) who found that, hormonal imbalance in individuals with primary or secondary iron overload presented with insufficient secretion of gonadotropins luteinizing hormone (LH), follicle stimulating hormone (FSH) is mainly due to the selective accumulation of iron in gonadotropic cells of the ant pituitary gland.

In this study, treatment of iron intoxicated groups with DFO or Q resulted in a highly significant increase in serum LH, FSH and estradiol levels in these groups when compared with (Fe) group. These results agreed with Sohn et al. (2008) who found that, usage of deferoxamine in iron overloaded patients caused improvement in pituitary gland dysfunction and an increase in LH and estrogen hormone as it chelates excess iron, reduces its toxic potential and improves mitochondrial function in the endocrine glands. As regard Q Farhomand and Sadeghi (2015) found that, Q has an improver effect on plasma gonadotropin concentration and increase (Testosterone, LH, FSH) hormones in rats. Also the result of the present work demonstrated that combined treatment with DFO and Q in iron treated group (Fe + DFO + Q) showed a highly significant increase in serum LH, FSH and estradiol levels when compared with (Fe + DFO), (Fe + Q) groups indicating more efficiency than single treatment. These results were in harmony with Waseem et al. (2011) who documented that, treatment with combination of antioxidants like quercetin and iron chelators could neutralize the deleterious effects of reactive oxygen species (ROS) and probably reverse endocrine complications, improving reproductive ability and fertility potential.

The results of present study showed a highly significant increase in the mean values of serum thiobarbituric acid reactive substance (TBARS) and a highly significant decrease in the mean values of serum total antioxidant capacity (TAC) in iron treated group when compared with control group. These results indicated an impaired function of antioxidant defense system; iron generates reactive oxygen species (ROS) via the Fenton and Haber– Weiss reactions. ROS react directly with proteins, lipids and nucleic acids and induce oxidative stress by depleting cellular stores of antioxidants. The results of the present study were in line with Furze and Rankin, (2008) who reported that, iron toxicity resulted in a significant increase in the cytotoxic and reactive aldehydic by-products of LPO as MDA, thiobarbituric reactive substances (TBARS) which impair cellular function and protein synthesis. In this study, treatment of iron group with DFO or Q induced a highly significant decrease in serum thiobarbituric acid reactive substances levels (TBARS) and high significant increase in total antioxidant capacity (TAC) in these groups when compared with (Fe) group. There was non-significant difference between (Fe + DFO) and (Fe + Q) groups indicating that both are equally effective in improving oxidative stress. The combined treatment with DFO and Q in (Fe + DFO + Q) group showed a highly significant decrease in (TBARS) and a high significant increase in (TAC) when compared with (Fe + DFO) and (Fe + Q) groups indicating more improvement compared with single treatment. These results were in agreement with Budzyn et al. (2011) who reported that, lipid peroxidation is dependent on the autoxidation of Fe2+ to Fe3+, and the binding of iron by DFO can inhibit the formation of the hydroxyl radical and reduce oxidative stress. Kuppusamy and Tan (2011) anticipated a protective effect of deferoxamine against iron mediated oxidative stress along with iron chelator property.

Additionally Niu et al. (2016) reported that, Q prevents iron- induced oxidative damage in human hepatic cells and endocrinal gland by decreasing iron content and increasing antioxidants, including GPx, SOD, and total antioxidant capacity. David et al. (2008) provided evidence that, combined treatment with DFO and Q is capable of decreasing oxidative load on neuronal cell in the striatum as evidenced by an increase in the total GSH and SOD levels.

The histopathological changes observed in pituitary in iron treated rats were in the form of atrophy of the cellular components surrounded by wide area of connective tissue stroma, accompanied with marked iron deposition in pituitary tissues detected by special stain for iron (Prussian blue) with marked immunostaining for caspase 3 (marker of apoptosis). These changes were improved with treatment with either DFO or Q in the form of regenerated pituitary cells and narrowing of connective tissues spaces between cells, mild to moderate deposition of iron in pituitary tissue with mild caspase 3 marker

expression. Combined treatment of iron treated rats with DFO and Q showed more improvement than single one. These results were in line with Modell and Kief (2017) who showed the pathological changes of the pituitary gland due to iron overload in the form of destruction of gonadotrophs and subsequent reduction of their hormone content and secretory granules which were evident by both light and electron microscopy with absence of any significant fibrosis or alteration in the volume of the gland. In Prussian blue stained sections, the iron was most often present in gonadotrophic cells. Also Delvecchio and Cavallo (2010) demonstrated, preferential localization of iron deposits in gonadotrophs compared with other cell types at the ultrastructural level and positive immune stain caspase 3 marker displayed selective severe cellular injury which explain the cause of hypogonadism frequently associated with hemochromatosis was probably pituitary insufficiency in the form of decrease in gonadotrophins (LH, FSH) release.

As showed in this study treatment of iron treated rats with DFO, improved histopathological changes of pituitary induced by iron. These results were supported by Farmaki et al. (2011) observed that, chelation therapy with DFO improved endocrine tissue structure including the pituitary gland supported by histologic findings of a selective decrease in iron deposition in pituitary gonadotrophs and by the reversibility of hypogonadism in secondary hemochromatosis. Farhomand and Sadeghi (2015) reported the protective potentials of Q in improving the testicular and pituitary functions and maintaining the cellular components of DNA, RNA, nucleic acids and lipid in the membranes through the reduction of lipid peroxidation and increased antioxidants in the tissues.

In this study, the histopathological changes observed in ovarian follicles and premature ovarian failure. The stroma was infiltrated by extensive areas of hemorrhage, diffuse marked iron deposition in ovarian tissue and marked immunostaining for caspase 3.

These findings agreed with Podratz et al. (2015) & Zheng et al. (2016) showed that, female rats exposed to iron displayed impaired ovarian follicular development and hormonal signature associated with increased oxidative stress and apoptosis in ovarian follicles with positive immunostaining. These ovarian histopathological changes could be attributed to Fe2+ released from the iron depot that could directly arrest the cell cycle and inhibit the granulosa cell proliferation through releasing the reactive oxygen species (Yang et al., 2017).

The current study showed that, iron induced ovarian pathological changes were partially improved with treatment with either DFO or Q. Combined treatment with DFO and Q showed more improvement in these histopathological changes.

These results were in harmony with Mora-Esteves and Shin (2013) who reported that, DFO is effective in oxidative stress-induced female-factor infertility, with respect to improving ovarian and uterine pathology induced by iron overload and subsequent increase pregnancy rates. Naseer et al. (2017) found that, Q supplementation significantly improves the follicular development and minimize granulosa cells apoptosis.

The results of the present study showed that iron produced histopathological changes in the uterus in the form of atrophy of endometrial gland and stroma infiltrated by extensive areas of hemorrhage, diffuse marked iron deposition in uterine stroma with marked immunostaining for caspase 3. These changes were improved by using DFO or Q. The improvement was more evident when DFO and Q were used in combination.

The observations of the present study were in agreement with Liu et al. (2015) who reported that, iron overload caused abnormal uterine development which had been associated with an increase in oxidative stress and lower enzymatic antioxidant defense mechanisms. As regard effect of Q the current results were in parallel with Shahzad et al. (2015) & Yiğitaslan et al . (2016) who provided evidence of possible clinical benefits of Q in individuals with estrogen deficiency as in cases of iron overload., Q, is a phytoestrogen, had also been found to stimulate both ERα and ERβ, with a higher capacity for stimulating ERβ causing hypertrophic cells in the lamina propria of the endometrium which were spindle-shaped and had a dark nucleus, and there were numerous mitotic cells confirmed by histological analysis of uterus of female rats.

V. CONCLUSION

DFO and Q have a role in the treatment of toxicity and oxidative stress of the pituitary, ovaries and uterus induced by iron. Combined treatment of iron toxicity with DFO and Q are better than single treatment.

VI. RECOMMENDATIONS

It is recommended to use Q concurrently with DFO in treatment of iron toxicity to improve results and decrease dose and side effects of DFO periodic check of patients who use iron for long times or need repeated blood transfusion. More studies are needed to investigate the protective role of Q against the different toxic effects of iron. Prepare medicinal preparations in Egypt that contain pure Q to be used in different toxicities and cancer treatment.

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دور الديفيروكزامين والكيرستين على التسمم المزمن قصير المدى للحديد فى الغدة النخامية والمبايض والرحم فى الجرذان البيضاء الغير بالغة

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الملخص العربى

يعتبر الحديد من المعادن المهمة للغدة النخامية والأعضاء التناسلية . الديفيروكزامين يعمل مضادا للأكسدة بالإضافه إلى الإستخلاب. الكيرسيتين يعمل مضادا للأكسدة. الهدف من الدراسة هو تقييم الدور العلاجى للديفيروكزامين والكيرستين ضد التأثيرات السامه والأكسدة الخلوية فى الغدة النخامية والمبايض والرحم نتيجة التعرض المزمن القصير المدى للحديد فى إناث الجرذان البيضاء الغير بالغة. تم إجراء البحث على ١١٠جرذ غير بالغ من الإناث مقسمة إلى خمس مجموعات المجموعة الاولى وتتكون من٤٠ جرذ (٢٠ فى كل مجموعة) إلى (أ)←(مجموعة ضابطة سالبة) (ب)←(مجموعة ضابطة موجية). المجموعة الثانية (مجموعة الديفيروكزامين)← وتتكون من ١٠ جرذ (١٢٥مجم/كجم/يوميا) عن طريق الحقن البروتونى لمدة ٤ أسابيع.المجموعة الثالثة (مجموعه الكيرستين)← وتتكون من ١٠ جرذ (٢جم /كجم /يوميا) عن طريق الفم لمدة ٤ أسابيع. المجموعة الرابعة (الديفيروكزامين والكيرستين)← وتتكون من ١٠ جرذ تم إعطاء الجرذان الديفيروكزامين و الكيرستين لمدة ٤ أسابيع. المجموعة الخامسة:← وتتكون من ٤٠ جرذ مقسمة إلى ٤ مجموعات كالأتى (مجموعه معالجه بالحديد) تم إعطاء الجرذان حديد عن طريق الحقن البريتونى بجرعة (٣٠٠مجم/كجم/يوميا) لمدة ٤ اسابيع ثم ذبحت وأخذ منها عينات دم وأنسجة. (مجموعة الحديد والديفيروكزامين) الحديد عن طريق الحقن البريتونى لمدة ٤ أسابيع ثم أعطت الديفيروكزامين عن طريق الحقن البريتونى لمدة ٤ أسابيع أخرى. ج) (مجموعه الحديد و الكيرستين):- تم إعطاء الجرذان الحديد عن طريق الحقن البريتونى لمدة ٤ أسابيع ثم أعطت الكيرستين عن طريق الفم لمدة ٤ أسابيع أخرى. (مجموعة الحديد والديفيروكزامين والكيرستين) تم إعطاء الجرذان الحديد عن طريق الحقن البريتونى لمدة ٤ أسابيع ثم أعطت الديفيروكزامين يوميا عن طريق الحقن البريتونى مع الكيرستين عن طريق الفم لمدة ٤ اسابيع اخرى. أظهرت النتائج فى مجموعة الحديد إرتفاعا فى دلائل الحديد ومواد الأكسدة وقلة فى هرمونات الخصوبة وتغيرات هستوباثولوجية فى أنسجة الغدة النخامية والمبايض والرحم وزيادة تركيز الحديد والكاسبيز ٣ فى الأنسجة. العلاج بمادة الديفيروكزامين أو الكيرسيتين كل على حدة أظهر تحسن فى التغيرات البيوكيميائية والهستوباثولوجية وأن الإستخدام المزدوج للمادتين معا أظهر تحسن أكثر من استعمال كل مادة على حده.

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