EFFECT OF CHRONIC IRON OVERLOADING ON CARDIOVASCULAR SYSTEM IN ADULT MALE ALBINO RATS

By

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ABSTRACT

Background: Iron overload and its accumulation in the heart is a frequently encountered condition, especially in association with certain hematologic conditions such as inherted hemoglobinopathies, i.e thalassemia. This myocardial iron overload may be associated with a decrease in the antioxidant Vit. E and may catalyze oxidant damage to mitochondrial DNA leading to cardiac dysfunction, failure and even death.

Objective: The aim of this study was to determine and explain the possible mechanisms of the effects of chronic iron overloading (CIO) on cardiovascular function in a rat model together with determination of the possibility of using vitamin E supplementation as a potential protective target.

Materials and methods: Thirty healthy adult male albino rats of initial body weight 220-250 gm were included. Rats were randomly and equally divided into 3 groups: Group (1): Vehicle-treated (control) group, group (II): Iron overload group, and group (III): iron overload group treated with vitamin E. Rats were examined for the serum iron, ferritin, lactate dehydrogenase (LDH), creatine phosphokinase (CPK), cardiac troponin I (CTnI), malondialdhyde (MDA), catalase activity (CAT), superoxide dismutase (SOD) activity, tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), and adiponectin (APN) levels. Heart rate, blood pressure, duration of P wave, P-R, R-R and QTc intervals of ECG, and histopathological examination for the heart and aorta were also evaluated.

Results: The present study revealed that chronic iron overloading significantly increased serum LDH, CPK, CTnI, MDA, IL-6 & (TNF-α) levels with a significant positive correlation with serum ferritin level, but significantly decreased serum CAT activity, SOD activity, and APN levels with a significant negative correlation with serum ferritin level and also decreased heart rate and blood pressure together with prolongation of duration of P wave, P-R, R-R & QTc intervals of ECG. Also, iron deposition in the cardiac and aortic tissues with deterioration of their histoarchitecture has been shown. Moreover, it was found that exogenous administration of vitamin E resulted in a significant recovery of all the above-mentioned parameters in the iron overloading group.

Conclusion: The present study demonstated that CIO is associated with deposition of ion in cardiovascular system (CVS) leading to its dysfunction which is termed secondary cardiomyopathy. This dysfunction could be attributed to oxidative stress, inflammation and/or reduced adiponectin levels .Vitamin E as an antioxidant has a protective effect that can strongly ameliorate this dysfunction.

Key words: Iron overload, vitamin E, oxidative stress.

INTRODUCTION

Patients with chronic iron overload such as in thalassemia major suffered from many serious health problems including cardiomyopathy, congestive heart failure and fatal cardiac arrhythmias

which considered the leading causes of death (**Lekawanvijit and Chattipakorn, 2009**).

Iron is a prooxidant and the patho-genesis of harmful reactive oxygen species (ROS) represented an important ultimate factor for heart tissue damage (**Nikolaus and Peter, 2015**). Excess ROS produced by high intracellular iron produces lipid peroxidation, protein fragmentation as a result of amino acids oxidation, and DNA damage (**Bartfay et al., 1999**). However, the associated mechanism seen in iron-overload heart is still unclear (**Lin et al., 2013**).

Other studies further demonstrated that iron overload could enhance arachidonic acid release and eicosanoids production in cultured cardiomyocytes, and suggested a causal connection between these signals and electromechanical abnormalities in iron-overload cardiomyopathy (**Mattera et al., 2001**). It is possible that iron may also stimulate macrophage infiltration and activate tissue inflammation (**Harada et al., 2005**).

Adiponectin (APN) is an antioxidant and anti-inflammatory adipocytokine. Although it has been shown that APN protects the heart in acute cardiac diseases, its effects in iron overload cardiomyopathy (IOC) are unknown (**Lin et al., 2013**). Determining the pathogenic mechanisms of IOC leads us to valuable recognition of new therapeutic strategies (**Nikolaus and Peter, 2015**).

Antioxidants may represent a potential therapy to cardiac iron overload induced oxidative injury. It has been found that antioxidants supplementations such as taurine decrease the ROS induced cardiovascular dysfunction and enhance

the heart endurance in iron overloaded mice (**Oudit et al., 2004**).

Vitamin E exerts strong anti-oxidative and anti-inflammatory effects and has been shown to be decreased in patients with hereditary hemochromatosis and in experimental iron overload (**Brown et al., 1997**).

Vitamin E plays an important role to preserve cells against oxidative stress. It diminishes lipid peroxide intermediates by giving hydrogen, and this prevents hydrogen removal from polyunsaturated fatty acids (PUFAs). This contributes to stoppage of perpetual lipid peroxidation chain reaction (**Das et al., 2004**).

The present study was designed to determine the possible mechanisms of the effects of chronic iron overloading on cardiovascular function in a rat model, together with determination of the possibility of using vitamin E supplementation as a potential therapeutic target. The level of biomarkers of cardiac injury, certain cytokines, antioxidant enzyme activities, as well as cardiac and aortic histoarchitecture were studied in a trial to elucidate possible elaborated mechanisms.

MATERIAL AND METHODS

30 healthy adult male albino rats of local strain weighing 170 -190 g, were obtained from the animal house of Faculty of Veterinary Medicine- Zagazig University. Rats were kept in steel wire cages (40 x 30 x 18 Cm- 4/cage) under hygienic conditions. They were fed the commercial rodent chow with free access to water, kept at room temperature and were maintained on a 12 h light/dark cycle. Rats were adapted to the new

environment for one week before the experiment going on. The animal experiments were approved by the Institutional Research Board.

Rats were randomly assigned to three equal groups: **Group (I)**: Vehicle-treated (control) group, each rat received a single interaperitoneal injection of 0.1 ml/100g body weight saline three times per week for six weeks, **group (II)**: Iron overloading group, IP injected with ferric hydroxide polymaltose (Haemojet, Amriya Pharma. Ind. for European Egyptian Pharma. Ind., Alexandria, Egypt.), as one dose (100mg /kg body weight) every other day (three times per week) for six weeks (**Zhao et al., 2005**), and **group (III):** Iron overloading group treated with vitamin E, in which chronic iron overload was persuaded as in the second group, but with coadministration of IP injected vitamin E (α – tocopherol (Sigma Aldrich Co.- USA) at a dose of 10mg/100gm body weight (**Mustacich et al., 2006**).

Measurement of Blood Pressure and Electrocardiography: Rats were anesthetized by intraperitoneal (IP) injection of urethane (0.13g /100g body weight) and the blood pressure was estimated by using pressure catheter coupled to a PowerLab 4/20 (data acquisition system) with *MLT844* physiological pressure transducer (AD Instruments Pty Ltd, Australia). ECG was done by using cutaneous needle electrodes coupled also to the PowerLab data acquisition system.

Sample collection:

Retro-orbital venous plexus blood samples were obtained then serum was separated by allowing the blood samples to clot then centrifuged at 3000 rpm for 20 minutes, kept at (-20° c) and used to measure the serum levels of iron, ferritin, lactate dehydrogenase (LDH), creatine phosphokinase (CPK), cardiac troponin I, malondialdhyde (MDA), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), catalase, superoxide dismutase (SOD) and adiponectin. After collecting blood samples, the animals were sacrificed by cervical dislocation under mild ether anesthesia. Heart and aorta were excised and processed for histopathological studies. **Biochemical Analysis**

1. **Serum iron level** was measured as reported by **Burits and Ashwood (1999),** using commercial kit provided by Spinreact, co., Spain.

2. **Serum ferritin level** was measured as reported by **Linpisarn et al. (1981),** using commercial FE ELISA (Enzyme Amplified Sensitivity Immunoassay) Kits (Catalog Number: MBS722921, BioSource Europe S.A., Belgium),

3. **Serum LDH level** was measured as reported by **Kachmar and Moss (1976),** using spectrophotometer (spectronic 3000 Array, Germany) at 340 nm**,** and using commercial kit (Catalog Number 279 001, provided by Egyptian Company for Biotechnology), measured

4. **Serum CPK level** was measured as reported by **Gerhardt and Waldenstr?m (1979),** using commercial kit**.** (Catalog Number 1001050 provided by spinreact, co., spain),

5. **Serum Cardic Troponine-I level** was measured as reported by **Etievent et al. (1995),** using commercial kit. (Catalog Number SE120134 provided by Sigma-Aldrich Co.).

6. **Serum MDA level** was measured as reported by **Satoh (1987**), using Biodiagnostic kit (Biodiagnostic company, Dokki, Giza, Egypt)

7. **Serum SOD activity** was measured as reported by **Nishikimi et al. (1972**), using kit provided by (Biodiagnostic company, Dokki, Giza, Egypt)**.**

8. **Serum catalase activity** was measured as reported by **Aebi (1984)**, using kit provided by (Biodiagnostic company, Dokki, Giza, Egypt)

9. **Serum TNF-α level** was measured as reported by **Engvall and Perlmann (1971),** using commercial ELISA kit, (Catalog Number RAB0480, provided by Sigma-Aldrich Co).

10. **Serum IL-6 level:** was measured according to the method of **Engvall and Perlmann (1971), using** commercial ELISA kit, (Catalog Number RAB0306, provided by Sigma-Aldrich Co).

11. **Serum adiponectin level** as reported by **Engvall and Perlmann (1971),** using commercial ELISA kit, (Catalog Number RAB0005, provided by Sigma-Aldrich Co).

Histopathological examination:

Heart and aorta were immediately excised, rinsed with ice-cold normal saline (4 ℃) to exclude the blood cells, blotted and dried with filter paper then kept in 10% buffered formalin- saline at 4℃ for at least one week (1ry fixation), then the specimens were dehydrated with a series of ascending grade ethanol from 75 to 100%. Tissues were placed, there-after, in xylol and embedded in paraffin wax.

Longitudinal sections of about 1-2 µm thickness of heart and cross sections of about 1-2 µm thickness of aorta were processed on slides and stained with the following techniques for routine microscopic examination

1. Hematoxylin and eosin (H & E) stain to examine the general microscopic features of the heart and aorta (**Drury and Wallington, 1980).**

2. Perl's Prussian blue stain to study the iron deposition in heart and aorta (**Perls, 1867**).

The stained slides were examined under light microscope by a blinded pathologist.

Statistical Analysis:

The data Results were presented as mean ± SD for and analyzed using version 18 SPSS program (SPSS Inc. Chicago, IL, USA). One way Analysis of variance (ANOVA) was used followed by student-least significant differences (LSD) test to compare statistical differences between groups. P value less than 0.05 was considered to be significant. Pearson's test was done to detect correlations between parameters.

RESULTS

The present study showed that chronic iron overload significantly increased serum LDH, CPK, cardiac troponin I, MDA, IL-6 and TNF-α levels (p<0.001) with a significant positive correlation with serum ferritin level (r=0.866, r=0.844, r=0.832, r=0.768, r=0.910, and r=0.910 respectively, p < 0.01) but significantly decreased serum catalase, SOD, and adiponectin levels (p < 0.001) with a significant negative correlation with

serum ferritin level(r=-0.861, r=-0.796 and r=-0.785 respectively, p<0.01). Heart rate and both systolic and diastolic blood pressures also significantly decreased together with prolongation of duration of P wave, P-R, R-R & QTc intervals of ECG (p<0.001) in the iron overloaded group in comparison to the normal control group (Tables 1, 2 & 3, and figures 1,2,3)

Moreover, it was found that vitamin E administration resulted in a significant decrease in serum LDH, CPK, cardiac troponin I, MDA, (p<0.01) , interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), (p<0.001) levels with a significant positive correlation of LDH, CPK, MDA, interleukin-6 (IL-6) (r=0.739, r=0.661, r=0.642 and r=0.748, respectively., p<0.05) and cardiac troponin I r=0.947& TNF-α,r= r=0.871, (p<0.01)

with serum ferritin level but significantly increased serum catalase activity, SOD activity (p<0.01) and adiponectin levels (p<0.001) with a significant negative correlation with serum ferritin level (r=0.871, r=-0.648 and r=-0.634 respectively, (p<0.05) in the treated group in comparison to iron overloaded untreated group. Vitamin E also significantly increased systolic blood pressure (p<0.01), heart rate and diastolic blood pressure (p<0.001) together with decreases in duration of P wave (p<0.001), P-R(p<0.05), R-R (p<0.01) and QTc intervals(p<0.001) of ECG but no significant differences in serum iron or ferritin levels(p>0.05) in the treated group in comparison to iron overloaded untreated group (Tables 1, 2 & 3).

Table (1): Serum iron, ferritin, LDH, CPK and cTnI in all studied groups.

Parameters	Groups	Group I	Group II	Group III
Serum iron (μg/dL)	$\overline{X} \pm SD$	185.20±13.983	2040.20±153.363	1815.20±280.094
	P value of LSD		P < 0.001[a]	P < 0.001[a] NS[b]
Serum ferritin(ng/ml)	$\overline{X} \pm SD$	49.70±10.382	180.80±48.808	161.0±40.158
	P value of LSD		P < 0.001[a]	P < 0.001[a] NS[b]
Serum LDH (U/L)	$\overline{X} \pm SD$	202.2 ±42.2	423.3 ±39.5	329 ± 54.7
	P value of LSD		P < 0. 001[a]	P < 0.01[a,b]
	r	r=0.570, P>0.05	r=0.866[**], P<0.01	r=0.739[*], P<0.05
Serum CPK (U/L)	$\overline{X} \pm SD$	151.5 ± 18.17	330.1±79.05	234.5 ± 53.31
	P value of LSD		P < 0. 001[a]	P < 0.01[a,b]
	r	r=0.046, p> 0.05	r=0.844[**], p< 0.01	r=0.661[*], p< 0.05
Serum cTnI(pg/ml)	$\overline{X} \pm SD$	2.58 ± 0.72	12.75±2.89	5.68 ±1.64
	P value of LSD		P < 0. 001[a]	P < 0.01[a,b]
	r	r=0.152, P > 0.0	r=0.832[**], P < 0.01	r=0.947[**], P < 0.01

a = versus group I   = versus group II   **NS = non-significant (P > 0.05)**
*= p<0.05   ****= p< 0.01**   *****= p<0.001**   r = correlation with serum ferritin

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Table (2): Serum TNFα, IL.6, APN, MDA, SOD and CAT in all studied groups.

Parameters / Groups		Group I	Group II	Group III
Serum MDA (nmol/ml)	$\overline{X} \pm SD$	43.18 ±12.36	72.15± 13.84	55.25 ± 11.94
	P value of LSD		$P < 0.001^a$	$P < 0.05^a P < 0.01^b$
	r	r=0.552, P > 0.05	r=0.768[**], p<0.01	r=0.642[*], P < 0.05
Serum SOD (U/L)	$\overline{X} \pm SD$	44.14 ±9.28	24.65± 5.048	33.60± 4.35
	P value of LSD		$P < 0.001^a$	$P < 0.01^{a\,b}$
	r	r= -0.571,P >0.05	r=-0.796[**],p<0.01	r=-0.648[*], p < 0.05
Serum CAT (mmol/l)	$\overline{X} \pm SD$	59.17 ± 13.12	36.00 ± 9.80	48.67 ± 8.20
	P value of LSD		$P < 0.001^a$	$P < 0.01^{a,b}$
	r	r=0-.696[*],P < 0.05	r=-0.861[**]·P< 0.01	r=-0.739[*]·P< 0.05
Serum TNFα (pg/ml)	$\overline{X} \pm SD$	44.9 ±5.42	58.6 ±5.35	51.60 ±5.038
	P value of LSD		$p < 0.001^a$	$p < 0.001^{a,b}$
	r	r=0.605, P > 0.05	r=0.910[**], P <0.01	r=0.871[**], P < 0.01
Serum IL.6 (pg/ml)	$\overline{X} \pm SD$	8.49 ±1.21	24.97±2.92	18.52 ± 3.218
	P value of LSD		$P < 0.001^a$	$P < 0.001^{a,b}$
	r	r=0.605, P > 0.05	r=0.910[**], P <0.01	r=0.748[*], P < 0.05
Serum APN (μg/ dL)	$\overline{X} \pm SD$	6.88±0.77	3.46±0.51	4.68±0.69
	P value of LSD		$P < 0.001^a$	$P < 0.001^{a,b}$
	r	r=-0.712[*], P<0.05	r=-0.785[**],P< 0.01	r=-0.634[*],P < 0.05

Table (3): Heart rate, systolic blood pressure ,diastolic blood pressure P wave duration and P-R, R-R and QT_C intervals of all studied groups.

| Parameters / Groups		Group I	Group II	Group III
Heart rate (beat/min)	$\overline{X} \pm SD$	391.83± 18.804	260.86±33.306	306.860±13.341
	P value		$P < 0.001^a$	$P < 0.001^{a,b}$
Systolic blood pressure(mmHg)	$\overline{X} \pm SD$	120.6 ± 4.90	74±11.643	104±8.014
	P value		$P < 0.001^a$	$P < 0.01^{a,b}$
Diastolic blood pressure(mmHg)	$\overline{X} \pm SD$	76± 6.62	51.70 ± 9.27	67.50 ± 4.95
	P value		$P < 0.001^a$	$p < 0.05^a P < 0.001^b$
P wave duration (ms)	$\overline{X} \pm SD$	16.30 ±2.526	26.44±1.917	19.69 ± 2.759
	P value		$p < 0.001^a$	$p < 0.001^{a,b}$
P-R interval (ms)	$\overline{X} \pm SD$	27.6 ± 6.60	39.1 ± 5.17	33.40 ± 5.34
	P value		$P < 0.001^a$	$P < 0.05^{a,b}$
R-R interval (ms)	$\overline{X} \pm SD$	155.60± 12.25	240.2 ± 41.6	205.3 ±14.34
	P value		$p < 0.001^a$	$p < 0.001^a \, p< 0.01^b$
QT_C (ms)	$\overline{X} \pm SD$	73.10±7.880	230± 12.275	155.30± 6.977
	P value		$p < 0.001^a$	$p < 0.001^{a,b}$
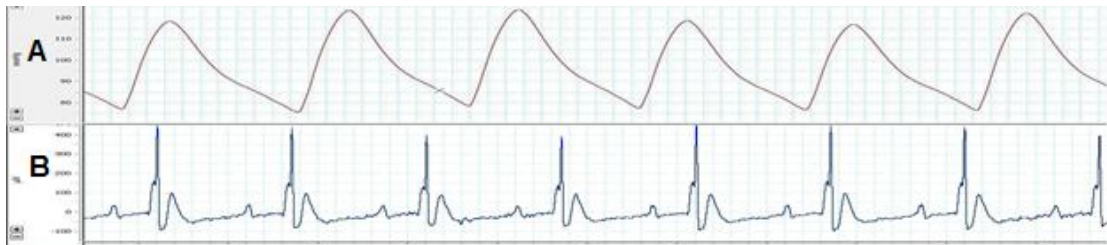
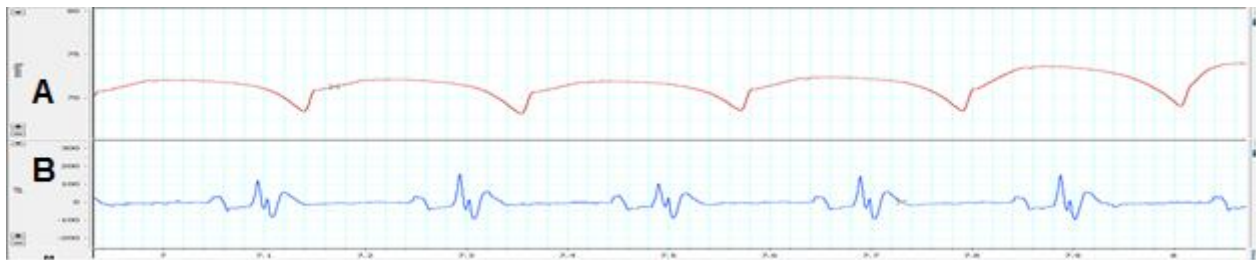**Figure (1):** A record of blood pressure (A) and ECG (B) for group1 (control group).

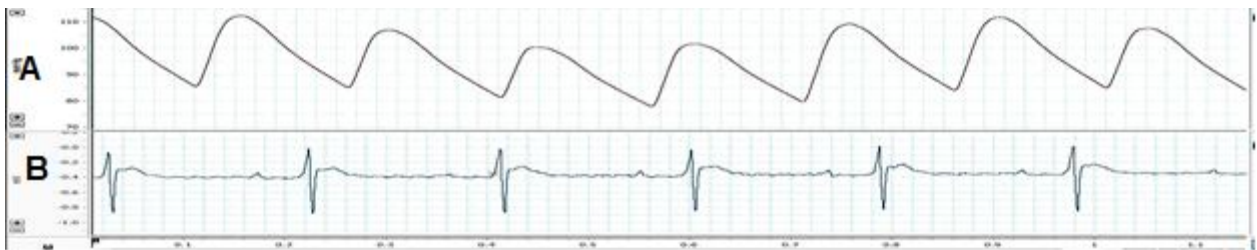**Figure (2):** A record of blood pressure (A) and ECG (B) for group 2 (iron overload group).

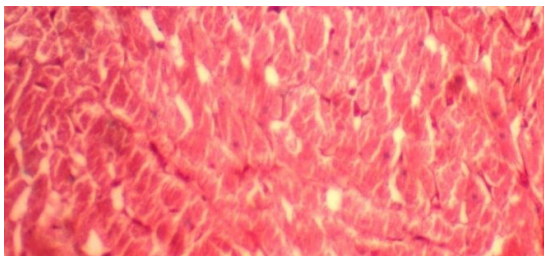**Figiure (3):** A record of blood pressure (A) and ECG (B) for group 3 (iron overload + vit E group).

Photo (1): Photomicrograph of a normal cardiac tissue showing intercommunicating cardiac muscles formed of central nuclei,and abundant eosinophilic cytoplasm (H&E- X 400).
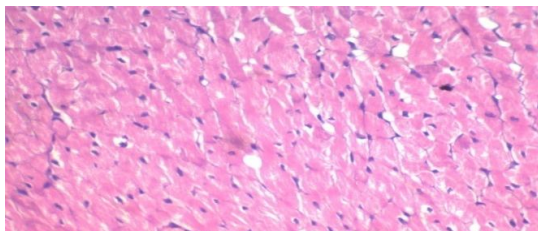

Photo (2): Photomicrograph of normal cardiac tissue showing absence of iron deposit in the normal cardiac muscles (Prussian blue- X 400).
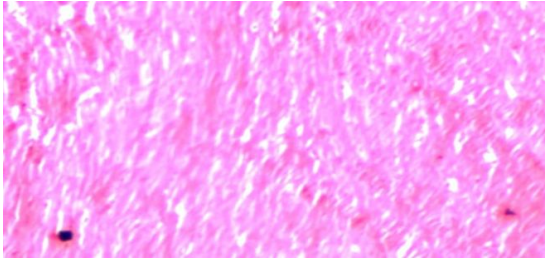
Photo (3): Photomicrograph of cardiac tissue of iron injected rat showing degenerated cardiac muscles with pyknotic nuclei (H&E- X 400).
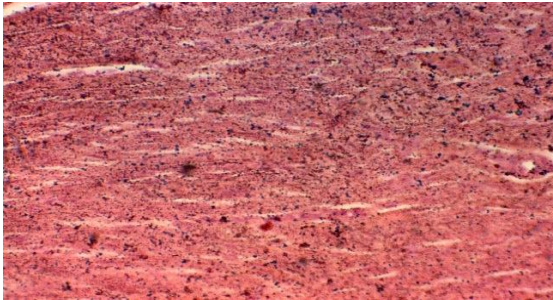

Photo (4): Photomicrograph of cardiac tissue of iron injected rat stained showing heavy iron deposition in the degenerated cardiac muscles with pyknotic nuclei. (Prussian blue- X 400)
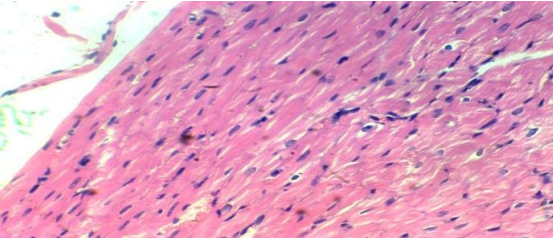

Photo (5): Photomicrograph of cardiac tissue of iron overload group treated with vit E showing return of the cardiac muscle to its normal state (H&E- X 400).
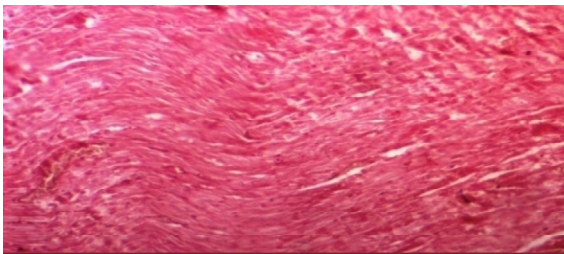

Photo (6): Photomicrograph of cardiac tissue of iron overload group treated with vit E showing return of the cardiac muscle to its normal state with trace dots of iron (prussian blue- X 400)
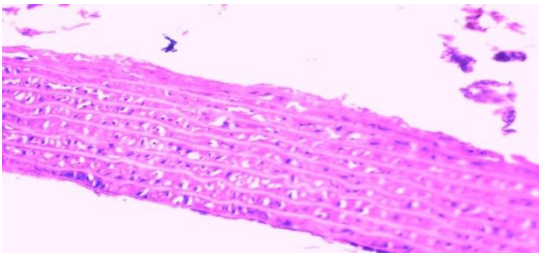

Photo (7): Photomicrograph of a segment of normal aorta formed of intima (flattened endothelial cells), media (longitudinal bundles of smooth muscle fibers) and outer adventitia (connective tissue) (H&E- X 400).
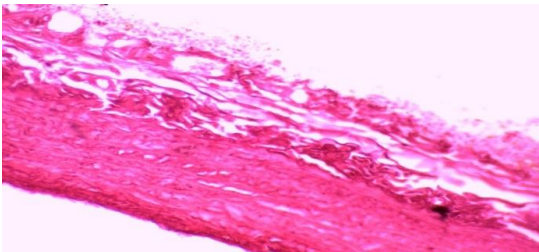

Photo (8): Photomicrograph of a segment of normal aorta stained with Prussian blue showing absence of iron from all layers of the aorta (Prussian blue- X 400).
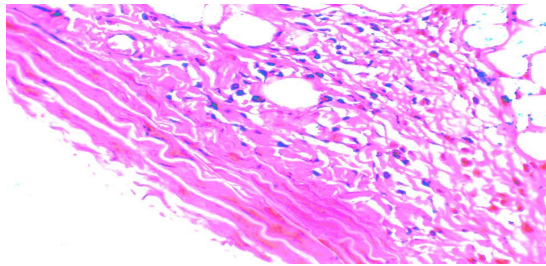
Photo (9): Photomicrograph of a segment of aorta of iron overload group showing atrophy of smooth muscles fibers with pyknotic nuclei (H&E- X 400).
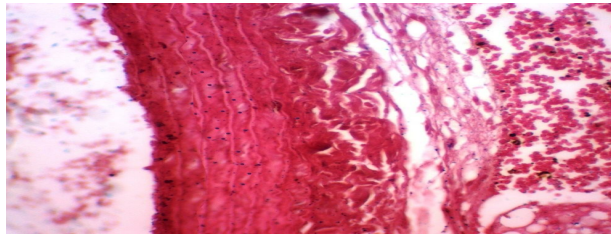

Photo (10): Photomicrograph of a segment of aorta of iron overload group showing excess iron deposition in the aortic layers (Prussian blue- X 400).
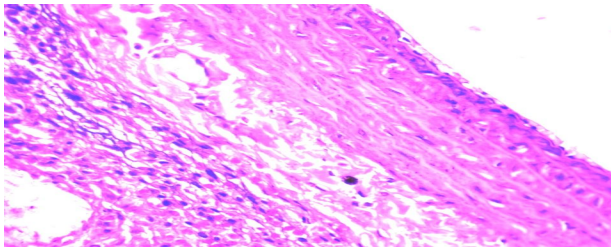

Photo (11): Photomicrograph of a segment of aorta of iron overload group treated with vit E showing aorta returned to normal state with its three layers (H&E- X 400).
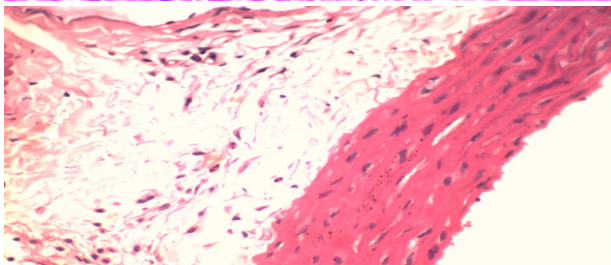

Photo (12): Photomicrograph of a segment of aorta of iron overload group treated with vit E showing normal aorta with its three layers (Prussian blue- X 400).

DISCUSSION

The present study revealed that chronic iron overload significantly increased serum iron, ferritin, lactate dehydrogenase (LDH), creatine phosphokinase (CPK), cardiac troponin I, malondialdhyde (MDA), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) levels but significantly decreased serum catalase, Serum superoxide dismutase (SOD), adiponectin levels, heart rate and blood pressure together with prolongation of duration of P wave, P-R, R-R and QTc intervals of ECG. Also, iron deposition in the cardiac and aortic tissues with deterioration of their histoarchitecture has been shown. Furthermore, it was found that exogenous administration of vitamin E produced a significant improvement of all the aforementioned parameters which were encountered in the iron overload group except serum iron and ferritin levels.

The significant increases in serum LDH, serum CPK and serum cardiac troponin I levels in iron overload group of the present study are in agree with the findings of **Sakha et al. (2008), Zamily et al. (2010) and Shahramian et al. (2013)** who reported their increase in cases of

iron overload such as in thalassemic patients compared to the healthy controls.

Increased cardiac troponin I is one of the best diagnostic indices in myocardial necrosis even without elevation of ST segment in ECG (**Bertrand et al., 2000**). Moreover, leakage of intracellular CPK and/or LDH is indicative of local tissue damage induced by free radicals, oxygen lack or trauma (**Zamily et al., 2010**).

The first possible mechanism that explains the deterioration of cardio-vascular function observed in the present study may be chronic iron overload induced oxidative stress. This possibility was proved in our study by the presence of a significant decrease in serum CAT and SOD levels together with a significant increase in MDA serum level. Similar results were also reported by **Hussein et al. (2013)** who revealed that iron overload resulted in significant decreases in liver glutathione peroxidase, CAT and SOD activities. Iron overload leads to a severe deficiency of total antioxidant capacity. This phenomenon can be noticed in most iron overload animal models (**Dabbagh et al., 1994**) and in patients with hereditary haemochromatosis (**Young et al., 1994**) and thalassemia (**Livrea et al., 1996**).

In chronic iron overload, iron toxicity is dose-dependent (**Bartfay et al., 1999**). Non transferrin-bound iron (NTBI) enters the cardiomyocytes through the voltage gated long lasting Ca^{2+} channels (L-type Ca^{2+} channels) and divalent metal transporter leads to IOC (**Oudit et al., 2003**) . Free (redox active) iron enters fenton-type reactions that lead to the transformation of Fe^{2+} into Fe^{3+} and generate free radicals including the highly reactive hydroxyl radicals (**Papanikolaou**

and Pantopoulos, 2005). That produces widespread damage to cellular lipids (lipid peroxidation, or lipoperoxidation), proteins, sugar and DNA (**Emerit et al., 2001**).

Moreover, the elevated aldehyde products from cellular lipid peroxidation such as elevated MDA make the low-density lipoprotein cholesterol (LDL-C) more susceptible to form peroxides leading to enhancement of cardiovascular disease (CVD) and organ damage even in patients with lower total cholesterol (TC) and LDL-C concentrations (**Sengsuk et al., 2014**).

The second possible mechanism that may also explain the deterioration of cardiovascular function observed in the present study, in iron overload, is the aggravation of the inflammatory infiltration that was also indicated by the presence of a significant increase in serum IL-6 and TNF-α level, showed a significant positive correlation with serum ferritin levels.

Iron loading can induce thromboxane A2 (TXA2) that act as a paracrine facilitator of tumor necrosis factor alpha (TNF-α) expression through the thromboxane receptor (TP) calcium/ calcineurin signaling pathway (**Katagiri et el., 2008**). TNF-α then activates calcineurin-nuclear factor of activated T cell (NFAT) signaling cascades in the heart with recruitment of macrophages and aggravation of the inflammatory infiltration, which further increased the ROS and gear up the inflammation-fibrosis circuit that results in aggravation of IOC and cardiac fibrosis (Harada et al., 2005). Furthermore TNF-α can enhance the activation of NFκB and caspases

which help in the development of apoptosis. NFκB stimulates the expression of various cytokines and inducible enzymes e.g. Cyclo oxygenase -2 and inducible nitric oxide synthase (**Srinivasan and Ramarao, 2007**).

ROS also increased expression of adhesion molecules and impair nitric oxide (NO) bioavailability. This provoked hypercoagulability and decreased NO-dependent, flow-mediated dilatation (**Aggeli et al., 2005**).

Our study had also demonstrated a significant negative correlation between serum ferritin and Adiponectin (APN) levels. This result was supported by the findings of **Gabrielsen et al. (2012)** which suggests that fat cell iron negatively regulates APN transcription via Forkhead box protein O1(FOXO-1) mediated repression. APN is an antidiabetic, antiatherogenic, antioxidative, and anti-inflammatory protein. It also ameliorates iron deposition in the heart through a peroxisome proliferator activated recep-tor-alpha peroxisome-gamma coactivator-1alpha (PPARα-PGC-1)-dependent mechanism and exerts beneficial effects to IOC (**Kahn et al., 2010 and Lin et al., 2013**). Thus, it is possible that diminished APN levels may be the third possible mechanism that explains the deterioration of cardiovascular function demonstrated in this study.

Moreover, our investigation results revealed a significant decrease in heart rate, both systolic and diastolic blood pressures together with a significant increase in duration of P wave and P -R, R-R & QTc intervals of ECG, with iron overload. These findings were consistent with those of some investigators (**Sellan et al., 2009 and Rose et al., 2011**), but at variance with those of others (**Cardoso et al., 2005 and Turbino-Ribeiro et al., 2013**).

The inconsistency between our findings and those of others may be due to differences in the chosen dose, route and/or duration of administration.

Bradycardia in patients with iron overload could originate from extrinsic or intrinsic factors to the heart. Intrinsic electrical activity of the specialized pacemaker myocytes located in the sinoatrial node (SAN) could be disrupted by iron as a result of modulation of a number of membrane currents or Ca^{2+} handling (**Mangoni and Nargeot, 2008**). Alternatively, elevated iron could affect autonomic nervous system activity, possibly by impairing neuronal function (**Madsen and Gitlin, 2007**) or interfering with other elements of the baroreceptor reflex pathways external to the heart (**Cardoso et al., 2005**).

Rose et al. (2011) revealed that the reductions in heart rate as well as the conduction deficits seen in CIO result from selective reductions in $Ca_V1.3$-mediated L-type Ca^{2+} current ($I_{Ca,L}$) with no changes in pacemaker current (I_f current).

The action potential duration also shortened due to late fast sodium current diminution that may give an explanation to the delayed impulse conduction and produce widening of the QRS complex and other arrhythmias (**Laurita et al., 2003 and Cardoso et al., 2005**). These electrophysiological incongruities with the patchy iron deposition in the heart may produce re-entry and develop fatal arrhythmias ((**Laurita et al., 2003**).

Furthermore, impaired Na^+-K^+ ATPase activity increases the intracellular Na that increase the Na^+ outflux via Na^+-Ca^{2+} exchange, resulting in Ca^{2+} influx that may be maintained due to impaired Ca^{2+} extrusion produced by iron-inhibited sarcolemmal Ca^{2+} pump activity, and may lead to cardiac arrhythmias (**Kaneko et al., 1989**).

Moreover, iron competes with Ca^{2+} for ryanodine-sensitive Ca^{2+} channels leading to inhibition of Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum (SR) and weakness of the heart contraction (**Kim et al., 1995**). On the other hand, oxygen free radicals also diminish cardiac SR Ca^{2+} ATPase activity which may produce diastolic dysfunction (**Zeitz et al., 2002**).

Bradycardia could also contribute to the impaired cardiac contractility seen with CIO (independent of the effects of iron on myocardial contractility and cardiac fibrosis) as a consequence of the force-frequency relationship (**Matthes et al., 2004**).

Neurohormonal abnormalities have also been suggested to contribute to cardiac disease in thalassemic patients. **Veglio et al. (1998) and Franzoni et al. (2004)** found diminished sympathetic activity and decreased plasma norepinephrine. An increased atrial natriuretic peptide level with left ventricular diastolic dysfunction has also been proposed (**Derchi et al., 1992**). These neurohormonal dysfunctions together with various channel affections provide a fourth mechanism for the cardiovascular dysfunction noticed in the present study.

The histopathological examination performed in the present study also showed increased iron deposition in the cytoplasm of cardiac muscle cells with pyknosis of their nuclei, and increased interstitial tissue between the cardiac muscle cells with excess iron deposition in all aortic layers especially in the media.

Iron usually deposits in a patchy manner primarily in the subepicardial layer then to the subendocardial layer in the ventricles before the atria (**Bartfay et al., 1999 and Wood, 2008**). Iron deposits in the conduction system have also been demonstrated causing bradyarrhythmias and requiring pacemaker placement (**Schwartz et al., 2002**).

Iron is probably a proarrhythmic by itself (**Wood et al., 2005**), and this effect together with the patchy deposition of iron in the tissues lead to variation in conduction velocity or repolarization that may be responsible for atrial and ventricular tachyarrhythmias that have been frequently demonstrated in patients with iron overload (**Wu et al., 2004 and Hahalis et al., 2005**). Left ventricular dilation with systolic dysfunction can also exacerbate the ventricular arrhythmias (**Horwitz and Rosenthal, 1999**).

In addition, similar to the cardiac iron deposition, there is an evidence of iron deposition in pancreatic beta cells as well as in the anterior pituitary (**Argyropoulou et al., 2001**). Patients with excessive iron burdens also suffer from many endocrino-pathies including insulin-dependent diabetes mellitus (**Mula-Abed et al., 2008**), Hypo-parathyroidism, (**Chang et al., 2001**) and hypothyroidism (**Shupnik et al., 1996**).

On the other hand, iron overload increased oxidative stress appeared to be a detrimental factor leading to insulin resistance and eventually type 2 diabetes

mellitus (T$_2$D) in thalassemic patients **(Tangvarasittichai et al., 2012)**. In this regard we have performed another study in which we proved that CIO was associated with type 2 Diabetes mellitus, insulin resistance, hypothyroidism and hypogonadism **(Asala et al., 2016).** All previous endocrinopathies may further aggravate the direct iron overload induced cardiomyopathy and provide another explanation for the observed deterioration in CV function in the present study.

Iron chelators are the most important line of treatment in chronic iron overload. However, another potential therapy is represented by antioxidants after demonstrating the role of oxidative stress in cardiac iron overload. It has been found that antioxidant supplementation reduced oxidative injury in the heart of iron overloaded mice **(Oudit et al., 2004)**.

Our results also demonstrated that vitamin E co-administration during induction of chronic iron overload in rats produced a significant improvement of all the parameters measured in chronic iron overload group (Vit E untreated group). **Hadi et al. (2012)** also reported the safeguarding effect of Vitamin E against the oxidative stress induced cardiac injury by doxorubicin (anticancer drug) in rat.

Vitamin E has potent anti-oxidative and anti-inflammatory effects. It decreases lipid peroxidation and superoxide (O^{2-}) production by impairing the assembly of nicotinamide adenine dinucleotide phosphate oxidase (reduced form; NADPH) as well as by decreasing the expression of scavenger receptors that help in the genesis of foam cells **(Teupser et al., 1996)**.

It has been also shown that vitamin E decreases the release of proinflammatory cytokines as IL-8, plasminogen activator inhibitor-1 and C-reactive protein levels and could have valuable role on cardiovascular diseases in a high-risk population **(Singh et al., 2005).**

Taking the present findings together, it can be concluded that chronic iron overload can deteriorate CV function as indicated by a significant decrease in heart rate, both systolic and diastolic blood pressure together with ECG abnormalities (prolonged p wave, and duration of P-R, R-R & QTc intervals). The probable mechanisms include 1). Increased lipid peroxidation by its pro-oxidant effect with degradation of all cellular constituents leading to systolic dysfunction, 2). The aggravation of the inflammatory infiltration, 3). Decreased APN level that promotes cardiac inflammation and endothelial dysfunction, 4). Impairment of sympathovagal balance together with various channel affections and 5). Iron overload induced many endocrinopathies including diabetes mellitus, hypoparathyroidism and hypothyroidism .

Further investigations on alterations of ion channels are required to explain the association of molecular and electro-physiological changes at the cellular level in iron overload. Further studies are also required to test the use of vitamin E and APN as potential protective targets.

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الحمل الزائد المزمن للحديد على جهاز القلب والأوعية الدموية فى الجرذان البالغة

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خلفية البحث: زيادة الحديد المزمنة وتراكمه في القلب كثير الحدوث وخاصة مع بعض الأمراض الدموية مثل الثلاسيميا. ويرافق هذا التزايد فى عضلة القلب إنخفاض في فيتامين هـ المضاد للأكسدة، ويؤدى ذلك الى أكسدة الحامض النووي مما يؤدي إلى ضعف القلب وفشله حتى الموت.

الهدف من البحث : تحديد وشرح الآليات الممكنة لآثار الحمل الزائد المزمن للحديد على وظيفة القلب والأوعية الدموية في نموذج الجرذان مع تحديد إمكانية إستخدام فيتامين (هـ) كمضاد للأكسدة كهدف وقائي محتمل.

مواد وطرق البحث: تم إجراء هذه الدراسة على عدد ثلاثين من ذكور الجرذان البيضاء البالغة التي يبلغ وزنها ١٧٠-١٩٠ جم وقد تم تقسيمها بشكل عشوائي وعلى قدم المساواة الى المجموعة الأولى التى إستخدمت كمجموعة ضابطة، و المجموعة الثانية هى مجموعة الحديد الزائد، والمجموعة الثالثة هى مجموعة الحديد الزائد مع فيتامين (ه) .

في نهاية فترة التجربة تم تسجيل رسم كهربى للقلب، و تسجيل ضغط الدم الشريانى وفصل مصل الدم لتعيين بعض القياسات البيوكيميائية: مستوى الحديد والفيريتين وبعض إنزيمات القلب مثل لاكتات دي هيدروجيناز وكيرياتين فسفوكيناز وبروتين القلب تروبونين اى وبعض دلائل الإجهاد التأكسدى مثل المالون داى الدهيد وسوبر اوكسيد ديسميوتيز والكاتاليز وبعض السيتوكينات مثل إنترلوكين٦ وعامل نخر الورم ألفا والديبونكتين، كما تم عزل القلب والأورطى وتحضير أنسجتها للدراسة الميكروسكوبية للكشف عن ترسيب الحديد.

النتائج: أدى الحديد الزائد المزمن إلى إرتفاع في مستوي إنزيمات القلب (لاكتات دي هيدروجيناز وكيرياتين فسفوكيناز) وبروتين القلب (تروبونين اى) ومؤشرات الإلتهاب، والإجهاد التأكسدى (المالون داى الدهيد)، وبعض السيتوكينات مثل إنترلوكين ٦، وعامل نخر الورم ألفا مع وجود إرتباط إيجابي كبير مع مستوى الفيريتين في المصل، ولكن إنخفض بشكل ملحوظ نشاط سوبر أوكسيد ديسميوتيز والكاتاليز ومستويات أديبونكتين مع وجود إرتباط سلبي كبير مع مستوى الفيريتين في الدم،

وإنخفض أيضـا معدل ضـربات القلب وضـغط الـدم مـع إطالـة مدة فترات موجـة P، RR وQTc في تخطيط القلب. وقد تبين أيضا ترسب الحديد في أنسجة القلب والشريان الأورطى. وعلاوة على ذلك، فقد وجد أن إستخدام فيتـامين (هـ) أدى إلـى إنتعـاش كبيـر لجميـع النتـائج المذكورة أعلاه فـي مجموعـة الحديد الزائد.

الاستنتاج: زيادة الحديد المزمنة ترتبط مع ترسب أيون الحديد في القلب والأوعية الدمويـة ممـا يؤدي إلـى خلل وظيفي والـذى يسمى إعتلال عضلة القلب الثانوي. ويمكن أن يعزى هذا العجز إلـى زيـادة إجهاد الأكسدة و الإلتهاب و / أو إنخفاض مستويات أدييونيكتين، كمـا أن إستخدام فيتـامين هـ كمضـاد للتأكسد لديه تأثير وقائي ويمكن أن يحسن بشدة هذا الخلل.