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Interrelationship of Heme Oxygenase and the Oxidative Stress in the Cardiac Tissues of Thyroidectomized Rats

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ABSTRACT

Background: Thyroid hormone has profound effects on the cardiovascular system and a regulatory effect on the rate of heme oxidation in the liver. Inducible isoform of heme oxygenase (HO^{-1}) protects the heart and vasculature in pathological conditions. The present study aimed to identify the role of heme oxygenase in cardiac changes in thyroidectomized rats. **Materials and Methods:** Sixty male Wistar rats were equally divided into seven groups; the first and second groups were the control and sham operated groups, respectively while the 3rd and 4th groups were subjected to sham operation then treated with hemin (G_3) and KTZ (G_4). The 5th group (G_5) was thyroidectomized group. The 6th and 7th groups were subjected to thyroidectomy then treated with hemin (G_5) and KTZ (G_6), respectively. **Results:** Hypothyroidism is documented by significant decrease in T_3 accompanied with significant increase in serum TSH levels in thyroidectomized rats. The results obtained revealed that oxidative stress due to hypothyroidism has a detriment effect on cardiac tissue by depending on the result of cardiac Protein Carbonyl Content (PCC) as a marker of tissue damage and a negative significant correlation between cardiac PCC and serum Ferric Reducing Antioxidant Power (FRAP) as marker of Total Antioxidant Capacity (TAC). The Inducible Nitric Oxide Synthase (iNOS) activity in cardiac tissue showed significant decrease in thyroidectomized rats and its value was increased significantly upon treatment with hemin or ketoconazole (KTZ) as compared with sham operated rats. Treatment of thyroidectomized rats with hemin improves the intensity of iNOS immunoreactive cells demonstrating the recovery of some injury. **Conclusion:** There is a positive significant correlation between hepatic HO and iNOS in cardiac tissue. The paradoxical effect of both inducer and inhibitor of HO^{-1} on iNOS needs further studies. Also, to address this inquiry we need further investigation on time and dose-dependent effect of both inducer and inhibitor on cardiac tissue.

Key words: Heme oxygenase, thyroidectomy, cardiac function, hemin, ketoconazole, iNOS

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INTRODUCTION

Thyroid hormones have marked effects on the growth, development and metabolic function of virtually all organs and tissues (Ibrahim *et al*., 2011, 2012; Tousson *et al*., 2012a). A thyroidectomy is an operation that involves the surgical removal of all or part of the thyroid gland. Surgeons often perform a thyroidectomy when a patient has thyroid cancer or some other condition of the thyroid gland (such as hyperthyroidism) or goiter. Hypothyroidism is caused by thyroid hormone deficiency. Thyroid status is also an important determinant of cardiovascular function (Ibrahim *et al*., 2012). Thyroid hormone lowers systemic vascular resistance, increases blood volume and has inotropic and chronotropic effects on cardiac function. Thyroid hormone enhances overall total protein synthesis in the heart; additionally, it regulates the transcription of several specific proteins that are critical for cardiac function such as myosin heavy chain genes (Neves *et al*., 2008).

Triiodothyronine hormone (T_3)-induced changes in cardiac function can result from direct or indirect T_3 effects. Direct effects result from T_3 action in the heart itself and are mediated by nuclear or extranuclear mechanisms. Extranuclear T_3 effects, which occur independent of nuclear T_3 receptor binding and increases protein synthesis, influence primarily the transport of amino acids, sugars and calcium across the cell membrane. Nuclear T_3 effects are mediated by the binding of T_3 to specific nuclear receptor proteins, which results in increased transcription of T_3-responsive cardiac genes (Kahaly and Dillmann, 2005; Otterbein and Choi, 2000).

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Heme oxygenase is a membrane-bound enzyme responsible for catalyzing the first and rate-limiting step in the degradation of heme. Heme oxygenase (HO) catalyzes the oxidative degradation of heme to biliverdin, releasing equimolar amounts of biliverdin-IX, CO and free iron (Otterbein and Choi, 2000). HO^{-1} expression and activity are highly induced by numerous factors such as heavy metals and ultraviolet radiation also by I/R, hypoxia, cytokines, hemin, nitric oxide and angiotensin II (Ishizaka *et al*., 2000). Hemin is a potent inducer of HO^{-1} in the different tissues. Hemin increase cellular heme oxygenase activity in both a time and concentration-dependent manner. Also hemin can induced HO^{-1} and stimulate cGMP production in aortic tissues of hypertensive rats (Ndisang *et al*., 2002).

The tin porphyrins, tin protoporphyrin (SnPP) and several metalloporphyrins (e.g., ZnPPIX and SnPPIX) are potent competitive inhibitors of HO activity (Dover *et al*., 1991). Ketoconazole (KTZ) and other azole antifungal agents are known to have a variety of actions beyond the inhibition of sterol synthesis in fungi. Recently, the antifungal agent KTZ has been reported to have anti-tumor effects in prostate cancer (Wilkinson and Chodak, 2004). The azole antifungal drugs share structural features with a series of novel heme oxygenase (HO) inhibitors. The azole-containing antifungal drugs are potent HO inhibitors (Kinobe *et al*., 2006).

Biliverdin and bilirubin that generated by HO^{-1} protect against oxidative stress. Bilirubin scavenges peroxyl radicals and the antioxidant activity of bilirubin increases in hypoxic conditions. Biliverdin and bilirubin also scavenge other Reactive Oxygen Species (ROS), including superoxide, hydroxides, hypochlorous acid and singlet oxygen. In addition, biliverdin and bilirubin scavenge reactive nitrogen species such as peroxynitrite (Kaur *et al*., 2003).

Nitric Oxide (NO), as a representative endothelium-derived release factor, is deeply involved in the regulation of cardiovascular function and structure. Nitric Oxide Synthases (NOS) catalyze the oxygen- and NADPH-dependent oxidation of L-arginine, leading to production of L-citrulline and nitric oxide (NO). NOS is a family of enzymes with 3 major isoforms: neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS) (Forstermann *et al*., 1994).

Nitric oxide (NO) remains the topic of considerable debate in the pathogenesis of human and experimental heart failure. A functional relation involving the thyroid gland, endothelial cells and NO, which is able to modulate cardiovascular function, has been described (Tousson *et al*., 2012a) and it has been claimed that thyroid hormones are able to regulate heart rate by an NO-mediated mechanism in the absence of autonomic regulation (Fellet *et al*., 2004). Inducible nitric oxide synthase (iNOS or type II) can be expressed by many different cell types, including inflammatory cells, endothelial cells and cardiac myocytes (Balligand and Cannon, 1997).

Carbon monoxide (CO) that generated by the degradation of heme from HO could compete with the NO binding sites on proteins and subsequently increase the free NO. Interactions between NO and CO may illustrate a regulatory network between HO and NOS. HO, may play a role in the prevention of endothelial nitric oxide synthases (eNOS) uncoupling (Dore, 2002). Therefore, the present study aimed at investigating the biochemical role of heme oxygenase in cardiac changes in thyroidectomized rats administered hemin (as HO inducer) or ketoconazole (KTZ) (as HO inhibitor). In addition to, showed the changes in the Inducible Nitric Oxide Synthase (iNOS) immunoreactivity expersion in different groups under study.

MATERIALS AND METHODS

The experiments were performed on 60 male Wistar rats weighing 120±10 g. They were obtained from the farm of Helwan, Egypt. The rats were kept in the laboratory for one week before the experimental study and fed a standardized diet (Barley) *ad libitum*. The temperature in the animal room was maintained at 23±2°C. The laboratory cycle was 12:12 h light-dark cycle. Rats were anesthetized by intraperitoneal injection with thiopental sodium (50 mg kg^{-1}; EIPICO Co., Egypt) for sham operation or thyroidectomy and after surgery, the animals were given Bivatracin (Neomycin, Bacitracin: Topical antibiotic aerosol powder spray; EC4P Co., Egypt) two times/day for 6 days. The experimental protocol was approved by Local Ethics Committee and Animals Research. The rats were randomly and equally divided into the following groups:

Group I: (G_I) Control group; Thirty rats were fed a standardized diet *ad libitum* and equally divided into 3 subgroups as follow:

Group Ia: (G_{Ia}) Sham operated group; rats were subjected to sham operation and did not received any treatment

Group Ib: (G_{Ib}) Sham operated and Hemin-treated group; Rats were subjected to sham operation and after four weeks rats were orally treated with hemin (Ferriprotoporphyrin IX chloride Fe ($C_{34}H_{32}N_4O_4$) Cl, Sigma-Aldrich Co., USA) by a stomach tube (15 mg kg^{-1} day^{-1}) for 4 weeks, hemin was dissolved in 4% alcohol (Ndisang *et al*., 2002)

Group Ic: (G_{Ic}) Sham operated & Ketoconazole (KTZ) treated group; Rats were subjected to sham operation and after four weeks rats were orally treated with KTZ ($C_{26}H_{28}Cl_2N_4O_4$, molecular mass of 531.43 g moL^{-1} from RAMEDA Co., Egypt) by a stomach tube (100 μmol kg^{-1} day^{-1}) for 4 weeks, KTZ was dissolved in distilled water (Kinobe *et al*., 2006)

Group II: (G_{II}) Thyroidectomized group rats were surgically subjected to thyroidectomy (Tousson *et al*., 2012b)

Group III: (G_{III}) Treated thyroidectomized rats with hemin for 4 weeks

Group IV: (G_{IV}) Treated thyroidectomized rats with KTZ for 4 weeks

Briefly, by using a stereomicroscope (Zeiss, Germany) for better observation, the muscle was cut and the trachea was exposed. The parathyroid gland was located, dissected from the thyroid gland and implanted into the surrounding neck muscle. The thyroid gland was carefully dissected out to avoid injury to the laryngeal nerve and was complete excision. The rats were subjected to injection with hemin and KTZ after 4 weeks from thyroidectomy.

At the end of the experimental period, rats were euthanized with intraperitoneal injection with sodium pentobarbital (50 mg kg^{-1}). Blood samples were individually collected from the inferior vena cava of each rat and serum was separated. Hearts of rats were removed and divided into parts for biochemical and immunohistochemical investigations. Also, livers of rats were removed and washed by saline.

Determination of serum triiodothyronine hormone (T_3) concentration: Determination of serum T_3 was carried out according to Chopra *et al*. (1971).

Determination of serum thyroid stimulating hormone (TSH) concentration: Determination of serum TSH was carried out according to Engall (1980).

Determination of serum total bilirubin concentration: Serum total bilirubin concentration was determined by Biomerieux kit, France according to Jendrassik and Graf (1938).

Determination of serum and heart thiobarbituric acid reactive substances (TBARS) Level: Lipid peroxidation in serum and heart tissue was estimated colorimetrically by measuring TBARS by method of Esterbauer and Cheeseman (1990).

Determination of serum and heart protein carbonyl content (PCC): The protein carbonyl content was estimated according to Fagan *et al*. (1999).

Estimation of serum and heart total antioxidant capacity (TAC) by ferric reducing antioxidant power (FRAP): The ferric reducing antioxidant power was estimated by method of Benzie and Strain (1996).

Isolation of liver microsome: Isolation of hepatic microsome was carried out according to method of (Schenkman and Cinti, 1978).

Estimation of liver microsomal total protein content: The protein content was determined according to the folin-Lowry method (Lowry *et al*., 1951).

Estimation of liver microsomal heme oxygenase activity (LHO): Activity of hepatic microsomal heme oxygenase is carried out by modification of Tenhunen *et al*. (1969).

After decapitation animals were dissected, heart (left ventricle) were removed from different groups and fixed in 10% neutral buffered formalin, dehydrated, cleared and embedded in paraffin. Sections of 5 microns thickness were cut and mounted on clean positive slides to identify the distribution of iNOS in left ventricle sections from different groups under study. Sections were deparaffinized, rehydrated, washed in Phosphate Buffered Saline (PBS) (3×5 min) and peroxidase activity was quenched using 0.3% H_2O_2 in methanol for 30 min. Subsequently, washed in PBS and incubated with blocking solution at room temperature for 10 min, incubated with biotinylated mouse anti-iNOS primary antibody in moist chamber for 30-60 min and then rinsed with PBS. Samples were incubated with streptavidin peroxidase at room temperature for 10 min and washed with PBS. The antibody-peroxidase complex was developed using DAB chromogen at 18-24°C for 2-5 min. Finally, the sections were washed with PBS, counterstained with methyl blue for 1 min, washed with tap water, dehydrated, cleared and cover-slipped with Mount-Quick (Daido Sangyo, Tokyo). All stained slides were viewed by using Olympus microscope and images were captured by a digital camera (Cannon 620).

Statistical analysis: Data were expressed as mean values±SE and statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) tests to assess significant differences among treatment groups. The criterion for statistical significance was set at $p<0.05$. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS® Inc., USA).

RESULTS

Table 1 Showed that the serum T_3 levels in thyroidectomized rats were significantly decreased as compared with the sham groups. The serum TSH levels were significantly increased in thyroidectomized rats when compared with the sham groups. Also, there was significant increase in serum Thiobarbituric Acid Reactive Substances (TBARS) level in thyroidectomized group when compared with the sham group. Table 1 also showed the changes in serum Protein Carbonyl Content (PCC) in the different groups and changes in serum Total Antioxidant Capacity (TAC) that was estimated by measuring Ferric Reducing Antioxidant Power (FRAP) in the different groups. There were significant increase in serum FRAP in thyroidectomized that treated with hemin when compared thyroidectomized group and significant decrease in serum FRAP in thyroidectomized that treated with KTZ when compared thyroidectomized group. There was in significant changes in serum total bilirubin (TB) concentrations in all groups under study (Table 1).

Table 2 showed the significant decrease in heart TBARS level in thyroidectomized that treated with hemin when compared thyroidectomized group. Table 2 showed also the changes in heart Protein Carbonyl Content (PCC) in the different groups. There were significant increase in heart FRAP in thyroidectomized and hemin group when compared thyroidectomized group and significant decrease in heart FRAP in thyroidectomized that treated with KTZ when compared thyroidectomized group.

Table 3 showed that, a significant decrease in liver microsomal Heme Oxygenase activity (HO) in thyroidectomized group when compared with the sham group, significant increase in HO activity in thyroidectomized that treated with hemin when compared thyroidectomized group and significant decrease in thyroidectomized that treated with KTZ when compared thyroidectomized group.

Table 4 showed the correlations between different parameters in all groups under study. There were positive correlations between the following:

Table 1: Changes in serum triiodothyronine (T_3), thyroid stimulating hormone (TSH), thiobarbituric acid reactive substances (TBARS), protein carbonyl content (PCC), ferric reducing antioxidant power (FRAP) and total bilirubin (TB) levels in the different groups under study

Parameters/groups	T_3 (ng dL^{-1})	TSH (μIU mL^{-1})	Serum TBARS (nmol mL^{-1})	Serum PCC (μmol protein carbonyl mg^{-1} protein)	Serum FRAP (μmol Fe^{+2} L^{-1})	Serum TB (mg dL^{-1})
Group Ia: Sham operated	133.40±3.66	0.62±0.05	1.13±0.22	0.66±0.10	0.28±0.05	0.16±0.06
Group Ib: Sham operated and Hemin	142±1.03	0.45±0.03	2.01±0.13	0.76±0.02	0.24±0.02	0.13±0.02
P[1]	<0.021	<0.002	NS	NS	NS	NS
Group Ic: Sham operated and ketoconazole (KTZ)	136±1.52	0.59±0.02	3.02±0.58	0.87±0.06	0.24±0.03	0.25±0.03
P[1]	NS	NS	< 0.002	NS	NS	NS
Group II: Thyroidectomized	62.80±3.94	1.30±0.04	4.43±0.18	0.86±0.16	0.33±0.04	0.18±0.04
P[1]	<0.001	<0.001	<0.001	NS	NS	NS
Change (%)	-52.92	109.68	292.04	30.30	17.86	12.5
Group III: Thyroidectomized and hemin	49.80±1.77	1.56±0.03	1.85±0.31	0.87±0.12	0.45±0.05	0.21±0.04
P[1]	<0.001	<0.001	NS	NS	<0.003	NS
P[2]	<0.002	<0.001	<0.001	NS	<0.033	NS
Change (%)	-62.66	151.61	63.72	31.82	60.71	31.25
Group IV: Thyroidectomized and ketoconazole (KTZ)	43.20±2.73	1.61±0.03	2.94±0.63	1.02±0.10	0.33±0.04	0.17±0.05
P[1]	<0.001	<0.001	<0.003	NS	NS	NS
P[2]	<0.001	<0.001	<0.013	NS	NS	NS
P[3]	NS	NS	NS	NS	<0.031	NS
Change (%)	-67.62	159.68	160.18	54.55	17.86	6.25

P[1]: Sham operated (Group Ia), P[2]: Thyroidectomized (Group II), P[3]: Thyroidectomized and Hemin (Group III). Change (%): Compare with Sham operated (Group Ia)

Table 2: Changes in heart thiobarbituric acid reactive substances (TBARS), protein carbonyl content (PCC) and ferric reducing antioxidant power (FRAP) levels in the different groups under study

Parameters/groups	Heart TBARS (nmol g^{-1} tissue)	Heart PCC (μmol protein carbonyl mg^{-1} protein)	Heart FRAP (μmol Fe^{+2} g^{-1} tissue)
Group Ia: Sham operated	40.60±6.73	3.32±0.29	1.04±0.12
Group Ib: Sham operated and Hemin	32.22±1.27	3.57±0.21	1.53±0.29
P[1]	NS	NS	NS
Group Ic: Sham operated and ketoconazole (KTZ)	44.36±4.25	7.43±0.43	1.28±0.04
P[1]	NS	<0.001	NS
Group II: Thyroidectomized	31.62±3.92	1.75±0.11	1.41±0.36
P[1]	NS	<0.001	NS

Table 2: Continue

Parameters/groups	Heart TBARS (nmol g^{-1} tissue)	Heart PCC (μmol protein carbonyl mg^{-1} protein)	Heart FRAP (μmol Fe^{+2} g^{-1} tissue)
Change (%)	-22.12	-47.29	35.58
Group III: Thyroidectomized and hemin	19.87±1.95	1.87±0.25	1.63±0.16
P^1	<0.001	<0.001	NS
P^2	<0.050	NS	NS
Change (%)	-51.06	-43.67	56.73
Group IV: Thyroidectomized and ketoconazole (KTZ)	37.95±4.01	2.87±0.23	1.32±0.35
P^1	NS	NS	NS
P^2	NS	<0.006	NS
P^3	<0.004	<0.014	NS
Change (%)	-6.53	-13.55	26.92

P^1: Sham operated (Group Ia), P^2: Thyroidectomized (Group II), P^3: Thyroidectomized and Hemin (Group III), Change (%): Compare with Sham operated (Group Ia)

Table 3: Changes in liver microsomal Total Protein (TP) content and liver microsomal heme oxygenase activity (HO) levels in the different groups under study

Parameters/groups	Liver TP (mg g^{-1})	Liver HO (nmol $min^{-1}mg^{-1}$)
Group Ia: Sham operated	58.94±3.43	6.26±0.29
Group Ib: Sham operated and hemin	47.38±4.49	9.07±0.35
P^1	NS	<0.001
Group Ic: Sham operated and ketoconazole (KTZ)	51.06±5.04	3.24±0.29
P^1	NS	<0.001
Group II: Thyroidectomized	27.24±2.75	3.95±0.32
P^1	<0.001	<0.001
Change (%)	-53.78	-36.90
Group III: Thyroidectomized and hemin	58.08±2.99	5.75±0.40
P^1	NS	NS
P^2	<0.001	<0.001
Change (%)	-1.46	-8.15
Group IV: Thyroidectomized and ketoconazole (KTZ)	58.34±6.43	1.93±0.17
P^1	NS	<0.001
P^2	<0.001	<0.001
P^3	NS	<0.001
Change (%)	-1.02	-69.17

P^1: Sham operated (Group Ia), P^2: Thyroidectomized (Group II), P^3: Thyroidectomized and hemin (Group III), Change (%): Compare with Sham operated (Group Ia)

- T_3 and H.TBARS; H.PCC; H.FRAP; L.HO; H.iNOS
- TSH and S.TBARS; S.PCC; S.FRAP; S.TB
- S.TBARS and H.TBARS; S.PCC; S.TB
- H.TBARS and S.PCC; H.PCC; H.iNOS
- S.PCC and S.FRAP; H.FRAP; S.TB
- H.PCC and S.TB; H.iNOS
- S.FRAP and H.FRAP; S.TB
- H.FRAP and L.HO; H.iNOS
- L.HO and H.iNOS

On the other hand, there were negative correlations between the following:

- T_3 and TSH; S.TBARS; S.PCC; S.FRAP; S.TB
- TSH and H.TBARS; H.PCC; H.FRAP; L.HO; H.iNOS
- S.TBARS and H.PCC; S.FRAP; H.FRAP; L.HO; H.iNOS
- H.TBARS and S.FRAP; H.FRAP; S.TB; L.HO
- S.PCC and H.PCC; L.HO; H.iNOS
- H.PCC and S.FRAP; H.FRAP; L.HO
- S.FRAP and L.HO; H.iNOS
- H.FRAP and S.TB
- S.TB and L.HO; H.iNOS

The detection and distribution of iNOS immunoreactivity (iNOS-ir) in the left ventricular in the different groups under study were revealed in Fig. 1(a-f). Myocardium in sham, sham treated with hemin and sham treated with KTZ groups showed strong positive reaction for iNOS-ir (grade 4) in sarcoplasm (Fig. 1(a-c). Finite negative reactions for iNOS-ir (grade 1) were detected in the sarcoplasm of the ventricular sections in the thyroidectomized rats group (Fig. 1d). On the other hand, treatment of thyroidectomized rats with hemin showed mild positive reaction against iNOS-ir (grade 3) while the intensity of iNOS-ir were decreased (grade 2) in the cardiac myocytes of thyroidectomized rats that treated with KTZ for four weeks (Fig. 1e and f, respectively).

Table 4: Correlations between the different studied parameters

	T_3	TSH	S.TBARS	H.TBARS	S.PCC	H.PCC	S.FRAP	H.FRAP	S.TB	L.HO	iNOS
T_3											
r											
p											
TSH											
r	-0.995**										
p	0.001										
S.TBARS											
r	-0.322	0.285									
p	0.073	0.127									
H.TBARS											
r	0.334	-0.310	0.041								
p	0.072	0.095	0.831								
S.PCC											
r	-0.294	0.285	0.034	0.032							
p	0.115	0.127	0.858	0.866							
H.PCC											
r	0.0624**	-0.599**	-0.010	0.364*	-0.010						
p	0.001	0.001	0.958	0.048	0.957						
S.FRAP											
r	-0.544**	0.571**	-0.029	-0.118	0.296	-0.494**					
p	0.002	0.001	0.878	0.534	0.112	0.006					
H.FRAP											
r	0.014	-0.010	-0.367	-0.117	0.147	-0.058	0.212				
p	0.940	0.958	0.046	0.539	0.439	0.763	0.260				
S.TB											
r	-0.043	0.056	0.236	-0.187	0.126	0.237	0.157	-0.200			
p	0.822	0.770	0.210	0.321	0.507	0.207	0.408	0.288			
L.HO											
r	0.523**	-0.539**	-0.403*	-0.136	-0.294	-0.139	-0.125	0.198	-0.138		
p	0.003	0.002	0.027	0.473	0.114	0.464	0.510	0.295	0.467		
iNOS											
r	0.667**	-0.615**	-0.667**	0.168	-0.087	0.519**	-0.218	0.209	-0.008	0.438*	
p	0.001	0.001	0.001	0.375	0.649	0.003	0.247	0.267	0.966	0.015	

*,**Correlation is significant at the 0.05 and 0.01 level, respectivly (2-tailed)

DISCUSSION

Hypothyroidism is caused by deficient thyroid hormone secretion. Heme oxygenase (HO) is a heme-catabolizing enzyme. HO^{-1} and its reaction products protect the heart and vasculature in pathological conditions. The present study represented a contribution to declare the effect of low thyroid hormone status on hepatic HO level, oxidative stress parameters and total antioxidant parameters and the impact of these biomarkers on the heart of hypothyroid rats. Also, the present study was extended to elucidate the role of hemin and ketoconazole (KTZ) supplementation as HO inducer and inhibitor respectively to indicate the role of HO. To achieve this target we made a deficient state of thyroid hormones by thyroidectomy and to ensure the hypothyroid state, we regularly determined the serum triiodothyronine hormone (T_3) and thyroid stimulating hormone (TSH) after thyroidectomy where serum T_3 concentration is depressed and serum TSH concentration is significantly elevated in rats. This finding is compatible with Tousson *et al.* (2012b) that used thyroidectomy to achieve hypothyroid state. The increase in TSH level can be explained by decreased production of T_3 from the thyroid gland that minimizes TSH feedback inhibition resulting in an increase in its secretion by the anterior pituitary gland (Choksi *et al.*, 2003).

The current study showed a significant decrease in the level of hepatic microsomal heme oxygenase activity in thyroidectomized group as compared to sham operated group. Also, the activity was increased in the thyroidectomized group treated with hemin and decreased in the thyroidectomized group treated with KTZ when compared to thyroidectomized group.

Seelig *et al.* (1981) reported that thyroid hormone attenuates certain specific messenger RNA sequences while enhancing others in rat liver. This finding indicates that thyroid hormone can regulate heme oxygenase activity. Also, the hormone can act in a synergistic fashion to enhance the response of hepatic heme oxygenase to a chemical inducer of the enzyme (Smith *et al.*, 1982). The present study showed a positive significant correlation between T_3 and HO activity and documents the impact of

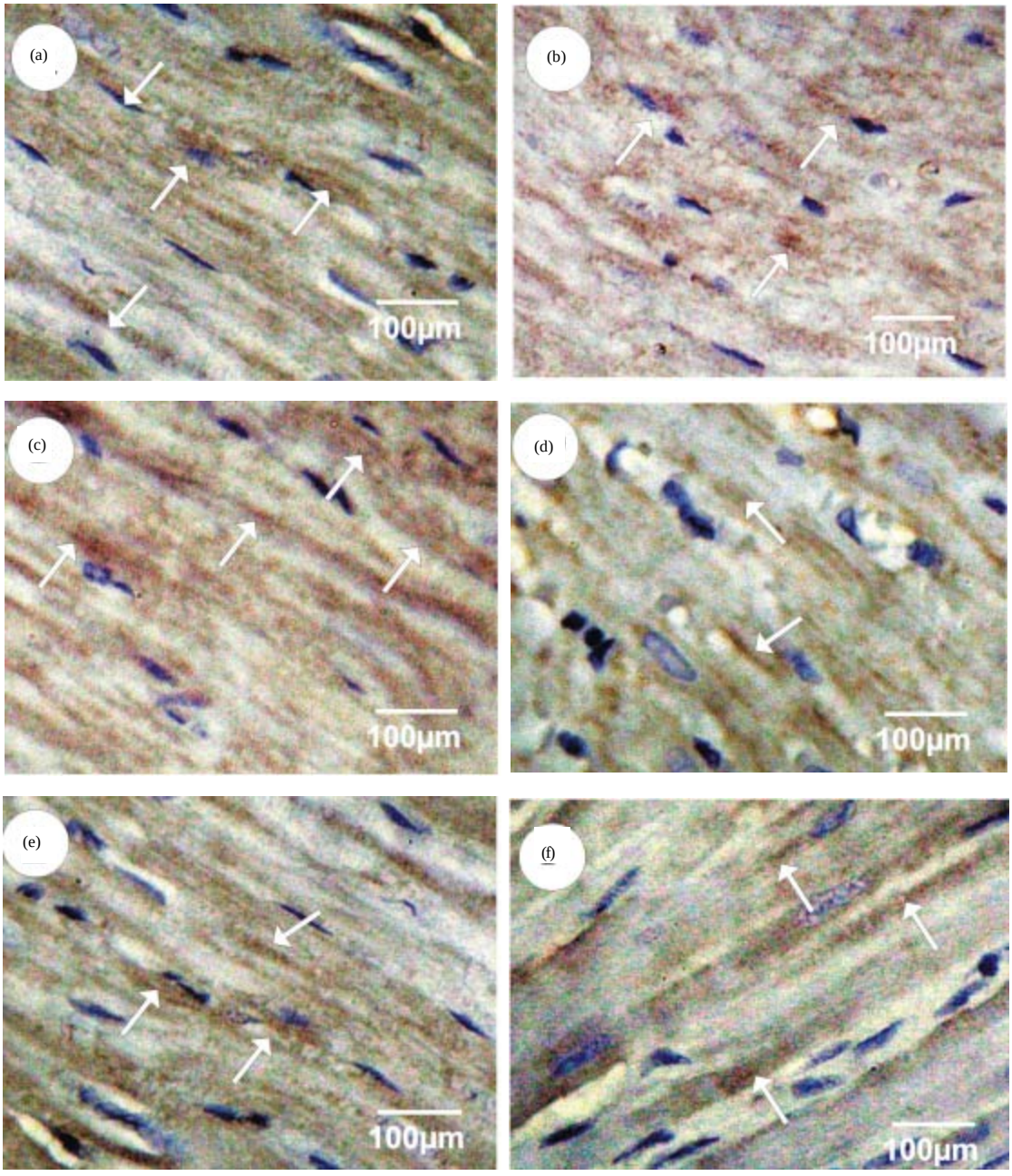

Fig. 1(a-f): Photomicrographs of the cardiac myocytes of rat left ventricle showed the detection and distribution of iNOS immunoreactivity (iNOS-ir) in the different groups under study. (a-c): Positive reaction for iNOS-ir (grade 4) in the cardiac myocytes of rat left ventricle in sham, sham treated with hemin and sham treated with KTZ groups respectively. (d): Finite negative reactions for iNOS-ir (grade 1) in the cardiac myocytes in the thyroidectomized rats. (e): Moderate positive reaction for iNOS-ir (grade 3) in the cardiac myocytes in thyroidectomized rats treated with hemin. (f): Mild positive reaction for iNOS-ir (grade 2) in the cardiac myocytes in thyroidectomized rats treated with KTZ

T_3 on HO^{-1}. Consistently, Yang *et al.* (2008) reported that hemin induces heme oxygenase-1 over expression in rodent testes after torsion-detorsion injury involves nuclear factor-E2 related factor-2 (Nrf2), nuclear factor-B and extracellular regulated kinase.

The decrease in HO activity in the thyroidectomized group treated with KTZ is compatible with Kinobe *et al.* (2006) who reported that; KTZ and other azole antifungal agents are known to have a variety of actions beyond the inhibition of sterol synthesis in fungi. These drugs share structural features with a series of HO inhibitors. Therapeutically used azole-based antifungal drugs are effective HO inhibitors. The azole-containing antifungal drugs are potent HO inhibitors. Because these drugs were effective within the concentration range observed in humans, it is possible that inhibition of HO may play a role in some of the pharmacological actions of these antimycotic drugs.

Kinobe *et al.* (2006) explored the possibility that KTZ-induced inhibition of HO activity might be due to a direct interaction between the KTZ imidazole moiety and heme iron resulting in a complex that is not accessible to the HO catalytic site. Another possibility was that inhibition of HO activity by KTZ was mediated through inhibition of cytochrome P450 NADPH reductase (NADPH CPR), which serves as an accessory enzyme during the oxidative breakdown of heme and the conversion of NADPH to $NADP^+$ (Yoshida *et al*., 1980). The result of serum TBARS reflect an enhanced oxidative stress in hypothyroidism and is in agreement with the result of Sarandol *et al.* (2005) who reported increased plasma, liver and muscle MDA levels in adult hypothyroid rats but, the result of heart TBARS is in a disagreement with these studies. Nevertheless, data on the oxidant status of hypothyroidism are limited and controversial (Gredilla *et al*., 2001). This is because of a decrease in free radical production in hypothyroidism is expected because of the metabolic suppression brought about by the decrement in thyroid hormone levels (Messarah *et al*., 2007).

The present study demonstrated that the HO has a protective effect due to a negative significant correlation between HO and serum TBARS. This finding is compatible with Moustafa *et al.* (2009) who studied the oxidative stress and thyroid hormones in patients with liver diseases and reported that oxidative stress could play a role in the pathogenesis and progression of liver diseases. Moustafa *et al*. (2009) demonstrated that there is a negative correlation between T_3 and MDA. The present study demonstrated the role of hemin in decrease the oxidative stress and that finding is in agreement with study of An *et al*. (2011) that reported that HO^{-1} up-regulation by hemin plays a protective role in ventilator-induced lung injury by suppression of inflammatory process and oxidative stress.

The damage to cellular proteins by oxygen free radicals play an important role in cell aging and death and underlie pathogenesis of many diseases. The present study demonstrated a negative significant correlation between heart PCC and serum ferric reducing antioxidant power (FRAP as marker for total antioxidant capacity). The glycoxidation and lipoxidation reactions might contribute to the formation of protein carbonyls (Miyata *et al*., 1998). The present results showed that assessment of heart PCC is valid as marker of tissue damage but not serum PCC due to in significant results of serum PCC.

Despite Reactive Oxygen Species (ROS) deleterious effects are kept under check by a delicate balance between the rate of their production and the rate of their elimination by antioxidant defense systems, the excessive addition of these substances to the tissue defense system resulting in tissues oxidative damage. This imbalance can be an effect of depletion of endogenous antioxidants and/or increased formation of free radicals and other reactive species (Shrinivas *et al*., 2000).

Ferric Reducing Antioxidant Power (FRAP) represents a single assay to evaluate Total Antioxidant Capacity (TAC). In the present study, there was a significant increase in serum FRAP in thyroidectomized group treated with hemin as compared to thyroidectomized one and in significant change in serum FRAP in thyroidectomized group treated with KTZ as compared to thyroidectomized group. This reflects the role of hemin in decreasing the oxidative stress and that finding is in agreement with the study of An *et al*. (2011). This reflects oxidative stress associated with hypothyroidism as indicated by the negative correlation between serum and heart MDA (marker of oxidative stress) and serum FRAP. Also, there was a negative significant correlation between heart PCC and serum FRAP. The data of the present work reveal a negative significant correlation between serum FRAP and T_3 level and this finding may be explained by the role of hemin in restoring FRAP without restoration of euthyroid state as presented in the present study.

The significant increase in FRAP of heart homogenate in thyroidectomized group is in contrast to Venditti *et al.* (1997) who reported that, the whole antioxidant capacity of tissues decreased, but significantly only in liver and heart. Continuing of the same theme a positive correlation between T_3 and heart FRAP has been reported. This finding confirms what previously mentioned that, thyroid hormones regulate protein and antioxidant enzymes synthesis and degradation (Varghese *et al*., 2001).

There were in significant changes in total bilirubin concentration neither in thyroidectomized group nor treated thyroidectomized groups when compared with sham operated group and thyroidectomized group,

respectively. These results may be explained by Nag *et al.* (2009) who showed that when Reactive Oxygen Species (ROS) generated at mild elevated level, it possibly lowers the biliverdin reductase (BVR) activity and lowers bilirubin production. The biliverdin-bilirubin interconversion is interrupted and the bilirubin present may not be sufficient to counteract the total ROS generated at that level and gets transformed to biliverdin, resulting in mild elevation of bilirubin level. However, this finding is in contrast to Nag *et al.* (2009) who reported from a part of study that bilirubin can act as antioxidant.

The current results showed that iNOS immunoreactivity was significantly decreased in left ventricular after thyroidectomy in response to the decrease in thyroid hormone. A reciprocal relationship between HO^{-1} and iNOS expression has been suggested Rat astrocyte-conditioned media increases HO^{-1} expression in rat microglia, while at the same time there is a reduction in the levels of iNOS protein and nitrite production (Min *et al*., 2006). In the work of Min *et al.* (2006) CO, a hemeoxygenase-1 product, does not affect iNOS protein expression, but CO inhibits nitrite levels in the primary rat microglia.

In lipopolysaccharide (LPS) stimulated macrophages, there is an induction of HO^{-1} mRNA and protein expression, while there is an inhibition of iNOS mRNA and protein expression (Srisook *et al*., 2006). Therefore it seems possible that HO^{-1} protein is involved in the inhibition of iNOS expression. Also, Weiss *et al.* (1994) reported that increased levels of intracellular iron decrease iNOS expression and NO generation in these cells while its deprivation results in enhanced iNOS expression and NO formation. Parhizgar (2007) demonstrated that iron inhibits NOS_2 mRNA transcription and iNOS protein expression. All these reports may explain the significant increase of iNOS immunoreactivity upon treatment of thyroidectomized rat with KTZ.

Unexpectedly, treatment of hypothyroid rats with hemin also increased the iNOS immunoreactivity in thyroidectomized rat. This finding can be explained by the stimulatory effect of hemin on inflammatory gene expression and subsequently increased iNOS via an NF-κB-dependent mechanism as occur in cultured astrocytes (Tousson *et al*., 2012b; Melissa *et al*., 2008). This paradoxic effect of HO^{-1} on iNOS immunoreactivity presented here in need of further investigation.

CONCLUSION

Hypothyroidism is associated with oxidative stress that has a detriment effect on cardiac tissue documented by changes in histopathological, TBARS, PCC, FRAP results. The current work declare the impact of HO^{-1} activity on the serum oxidative stress parameter with no effect on cardiac one however, there is a positive significant correlation between liver HO and iNOS in cardiac tissue that has been suggested to ameliorate the histopathological changes that have been detected in the hearts of thyroidectomized rats. The paradoxical effect of both inducer and inhibitor of HO^{-1} and iNOS needs further studies. Also, to address this inquiry we need further investigation on time and dose-dependent effect of both inducer and inhibitor on cardiac tissue.

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