Biomedical Research 2016; 27 (1): 93-102

ISSN 0970-938X
www.biomedres.info

Thymoquinone protects against hypothyroidism-induced cardiac histopathological changes in rats through a nitric oxide/antioxidant mechanism.

Nasra N Ayuob[a,b*], Nagla A El-Shitany[c,d], Mohammed Nabil Alama[e]

[a]Anatomy Department, Faculty of Medicine, King Abdulaziz University, Saudi Arabia.
[b]Histology and Cytology Department, Mansoura University, Egypt.
[c]Pharmacology and Toxicology Department, Faculty of Pharmacy, King Abdulaziz University, Saudi Arabia.
[d]Pharmacology and Toxicology Department, Faculty of Pharmacy, Tanta University, Tanta, Egypt.
[e]First and former head of cardiology unit, Associate professor of cardiology, Consultant adult interventional cardiologist, King Abdulaziz University Hospital, Jeddah, Saudi Arabia.

Abstract

Background: **The heart is one of the organs which is affected by the thyroid hormones and hence the alterations in the thyroid status influences its function and structure.**

Objectives: **This study aimed to reassess hypothyroidism-induced histopathological cardiac changes and to evaluate the proposed protective role of thymoquinone (TQ) against these changes. In addition, the mechanism of TQ-induced protection is studied regarding the oxidative stress and nitric oxide (NO) pathway.**

Materials and Methods**: A model of propylthiouracil (PTU)-induced hypothyroidism in Wister rats is used in this study. Four groups of rats were used; control, TQ, PTU (hypothyroidism) and PTU+TQ groups. Thyroid hormones, cardiac enzymes, NO and antioxidants were assessed in the blood. Hearts were histopathologically and immunohistochemically examined.**

Results: **A significant increase in plasma cardiac enzymes activity was recorded in hypothyroid rats, which is accompanied by significant histopathological changes in the left ventricle. Treatment of rats with TQ significantly protected the heart muscle against hypothyroidism-induced histopathological and immunohistochemical changes. It also significantly decreased plasma cardiac enzymes activity. TQ caused a reduction in malondialdehyde (MDA) formation, and increased, reduced glutathione (GSH), NO and superoxide dismutase (SOD) production. It also, increased the expression of constitutive nitric oxide synthase (eNOS) activity.**

Conclusion: **hypothyroidism may induce cardiac pathological changes, which is prevented by TQ as it restores thyroid hormones, increases NO formation and eNOS expression, and decreases reactive oxygen species (ROS) production.**

Keywords: Heart-Hypothyroidism, Thymoquinone-Nitric oxide, Reactive oxygen species, Histopathology.

Accepted November 26, 2015

Introduction

Nigella sativa (NS), the promising medicinal plant, commonly known as black seed, was medicinally used for over 2000 years in many middle eastern and eastern Asian countries [1]. NS seeds or its oil was used in the treatment of many diseases, including asthma, inflammations, hypertension and diabetes [2]. Thymoquinone (TQ), the biologically active substance of NS seeds and its oil, was found to possess powerful antioxidant and antiinflammatory activities [3, 4].

Thyroid hormones were considered one of the important modulators of the cardiovascular function. In addition, the heart was the target organ of the thyroid hormones [5]. Hypothyroidism, was a syndrome caused by thyroid hormone deficiency. Recently, Ohga et al. [6], reported left ventricular systolic and diastolic dysfunction in propylthiouracil (PTU)-induced hypothyroidism in rats. Recently, Knapp et al. [7] indicated that hypothyroidism resulted in oxidative stress, and heart diseases. In particular, it was suggested that, hypothyroidism increased the formation of reactive oxygen species (ROS) in the heart and consequently, increased lipid peroxidation [8].

In this study, it is hypothesized that hypothyroidism-induced cardiac pathological changes are caused via oxidative stress mechanism and hence, the use of an antioxidant may protect the heart against these changes. This study aimed to reassess, hypothyroidism-induced cardiac histopathological changes and to evaluate the proposed protective role of thymoquinone (TQ) against these changes. In addition, the mechanism of TQ-induced protection is studied regarding the oxidative stress and nitric oxide (NO) pathway.

Materials and Methods

Chemicals

Thymoquinone from NS (28 mg) was obtained from FRINTON LABORATORIES, INC. It is a yellow powder, which was dissolved in DMSO and diluted to 1:100 in a sterile saline solution (0.9% NaCl) to produce a working stock. PTU was purchased from Sigma Aldrich lnc, USA.

Animals and treatments

This study was approved by the Biomedical Ethics Research Committee at the Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia. Adult male Wister rats (180-200 g) were obtained from the Animal Resources Division of King Fahd Medical Research Center. The rats were housed at 22 ± 3°C and relative humidity of 44%-55% with a 12 h dark/light cycle and were provided with standard laboratory feed and water *ad libitum*. The animals received care, according to institutional guidelines for the care and use of the laboratory animals in King Fahd Medical Research Center. Rats were randomly divided into 4 groups (n=6 each). The control group: rats received normal saline (6 weeks, 2 ml/kg/day, per oral; po); TQ group: rats received TQ (6 weeks, 10 mg/kg/day po) [9]; PTU (hypothyroidism) group: rats received (6 weeks, 6 mg/kg/day, po) as was previously described [10] and PTU+TQ group: received PTU (6 weeks, the same dose, po) and TQ (10 mg/kg/day, po) [11] starting from the 2nd week to 6th week. Body weight of the rats was assessed at the start and at the end of the experiment.

Sample collection

At the end of the 6th week, the rats were decapitated under light ether anesthesia, blood samples were withdrawn by heart puncture. Blood samples were centrifuged and the serum were kept at -80°C until used for the biochemical analysis. Hearts were dissected out, weighted and processed for histopathological examination.

Measurement of serum triiodothyronine (T_3), thyroxine (T_4) and thyroid-stimulating hormone (TSH)

Serum T_3, T_4 and TSH concentrations were measured in El-Safwa Laboratory; Tanta, Egypt, using ADVIA Centaur automated competitive chemiluminescence immunoassay (Bayer HealthCare).

Measurement of plasma lactate dehydrogenase (LDH) and creatine phosphokinase (CPK)

Total plasma LDH and CPK activities were measured using kits of Biodiagnostic, Egypt. LDH activity was measured according to Pesce [12] and the method of Abbot et al. [13] was adopted to determine the CPK enzyme activity.

Measurement of plasma lipid peroxide (measured as malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO), glutathione peroxidase enzyme activity (GPx), superoxide dismutase enzyme activity (SOD) and catalase enzyme activity (CAT)

Plasma MDA was measured using Biodiagnostic kits, Egypt, according to Uchiyama and Mihara [14]. Plasma GSH was quantified using Biodiagnostic kits, Egypt, according to Ellman [15]. Plasma NO was measured using Biodiagnostic kits, Egypt, according to Tarpey et al. [16]. Plasma GPx activity was measured using Biodiagnostic kits, Egypt, according to Paglia and Valentine [17]. Plasma SOD activity was measured using Biodiagnostic kits, Egypt, according to Nishikimi et al. [18]. Plasma CAT activity was measured using Biodiagnostic kits, Egypt, according to Aebi [19].

Histopathological examination of the heart

After processing the heart for histopathological examination, paraffin blocks were obtained and serially sectioned (3-5 μm), then stained with hematoxylin and eosin (H and E) and Masson's trichrome (MT) stains [20]. The cross sectional area of the cardiomyocytes in the left ventricle was measured in 30 field per animal at magnification 100X objective lens and 10X ocular lens using Pro Plus image analysis software version 6.0. The area percent of the MT-stained collagen fiber (stained blue in color) was assessed using the same software as was described by Afifi and Hanon [21]. The histopathologist who examined the slides was blind to the groups studied.

Immunohistochemical examination of heart

Immunohistochemical staining was done using streptavidine–biotin–peroxidase technique according to Bancroft [20]. Alpha smooth muscle actin (ASMA; Dako Cytomation, Heverlee, Belgium, at a dilution 1/1000), desmin (Dako, Trappes, Frances, at a dilution 1/100) and endothelial nitric oxide synthetase (eNOS; Abcam, Cambridge, MA at a dilution 1/50) antibodies were used in this study. The nuclei were counterstained with hematoxylin. Semiquantitative analysis of the extension of the immunoreactivity was determined by assessing the area percentage using Pro Plus image analysis software version 6.0. ASMA, desmin, and NOS area percent was assessed in 30 field per animal at magnification 40X objective lens and 10X ocular lens as described by Leslie et al. [22]. On assessing the area percent of ASMA or MT-stained collagen fibers, fields with blood vessels were excluded from assessment.

Statistical Analysis

SPSS program (Version 16) was used to statistically analyze all data. The results were expressed as mean ± SDM. Comparisons between different groups were carried out by one-way analysis of variance (ANOVA) followed by Tukey-Kramer test for the parametric data. The morphometric and image analysis data (non-parametric) were analyzed by Kruskal-Wallis ANOVA followed Dunn's test. Person correlation was made between the non-parametric variable. Statistical significance was accepted at $p \leq 0.05$.

Results

The effect of PTU and TQ on body and heart weights, serum total T_3, T_4 and TSH concentrations

Adminstration of PTU significantly reduced (p=0.03) the rats weight gain while it significantly increased (p=0.02) the relative weight of the heart compared to the control. Adminstration of TQ to PTU-treated rats resulted in significant decrease (p=0.04) in the relative weight of the heart compared to the PTU group (Table 1).

Treatments of rats with PTU caused a significant decrease in both serum T_3 and T_4 concentrations (15% and 51%, respectively) compared to the control concentrations (p=0.000 and 0.008, respectively) (Table 1). PTU resulted in a significant increase in serum TSH concentration (~ 6 fold) compared to the control concentration (p=0.001) (Table 1). Adminstration of TQ to PTU-treated rats resulted in a significant increase in both serum T_3 and T_4 concentrations (21% and 82%, respectively) and a significant decrease in serum TSH concentration (87%) compared to PTU injected rats (p=0.01; 0.004 and 0.001 respectively) (Table 1).

Effect of TQ on plasma LDH and CPK activities in PTU-induced hypothyroidism

Treatments of rats with PTU significantly increased both plasma LDH and CPK activities (64% and 73%, respectively) compared to the control activities (p=0.000 and 0.009, respectively) (Table 2). TQ significantly decreased both plasma LDH and CPK activities (42% and 75%, respectively) in PTU=TQ group compared to PTU-treated rats (p=0.045 and 0.008, respectively) (Table 2).

Effect of TQ on plasma MDA, GSH and NO concentrations in PTU-induced hypothyroidism

Treatments of rats with PTU significantly increased both plasma MDA and NO levels (~ 3 and 2 fold, respectively) compared to the control levels (p<0.001 and 0.02, respectively) (Figure 1). On the other hand, treatments of rats with PTU caused a nonsignificant decrease (25%) in plasma GSH concentrations compared to the control rats (p=0.535) (Figure 1). Treatment with TQ after 2 weeks of PTU administration caused a significant decrease (p=0.027) in plasma MDA concentrations (31%) and a significant increase in plasma NO and GSH concentrations (22% and 77%, respectively) compared to PTU treated rats (p=0.016 and 0.035, respectively) (Figure 1).

Effect of TQ on plasma GPx, SOD and CAT activities in PTU-induced hypothyroidism

The results of enzymatic antioxidant analyses were shown in (Table 3). Briefly, the activities of GPX were not changed in all treatment regimens. Treatments of rats

Table 1: *The effect of propylthiouracil (PTU) and thymoquinone (TQ) on the body and heart weights, plasma total T_3, T_4 and TSH concentrations*

Groups	T_3 (ng/ml)	T_4 (ng/ml)	TSH (mIU/L)	Body weight gain (g)*	Relative weight of the heart**
Control	126.6 ± 2.3	3.5 ± 0.9	3.57 ± 1.52	125 ± 11.4	0.31 ± 0.02
TQ	124 ± 2.5	3.7 ± 0.8	3.60 ± 0.79	130 ± 15.2	0.33 ± 0.03
PTU	107.2 ± 0.8 [a]	1.7 ± 0.6[a]	19.60 ± 3.08[a]	106 ± 14.9[a]	0.35 ± 0.03
PTU+TQ	129.6 ± 14.9 [b]	3.1 ± 0.5[b]	2.51 ± 0.54[b]	121 ± 12.3	0.32 ± 0.01[b]

Data are presented as mean ± SDM of six rats.
[a]Significant difference from the control ($P \leq 0.05$).
[b]Significant difference from the PTU (Hypothyroid) ($P \leq 0.05$).
*Weight gain=weight at the end-weight at the start of the experiment
**Weight of the heart/body weight

Table 2: *Effect of propylthiouracil (PTU) and thymoquinone (TQ) on plasma lactic dehydrogenase (LDH), and creatine phosphokinase (CPK) levels*

Groups	LDH (U/L)	CPK (U/L)
Control	1648 ± 518	74 ± 14
TQ	1136 ± 946	62 ± 16
PTU	2696 ± 661[a]	128 ± 32[a]
PTU+TQ	1545 ± 860[b]	32 ± 10[b]

Data are presented as mean ± SDM of six rats.
[a]Significant difference from the control ($P \leq 0.05$).
[b]Significant difference from the PTU (Hypothyroid) ($P \leq 0.05$).

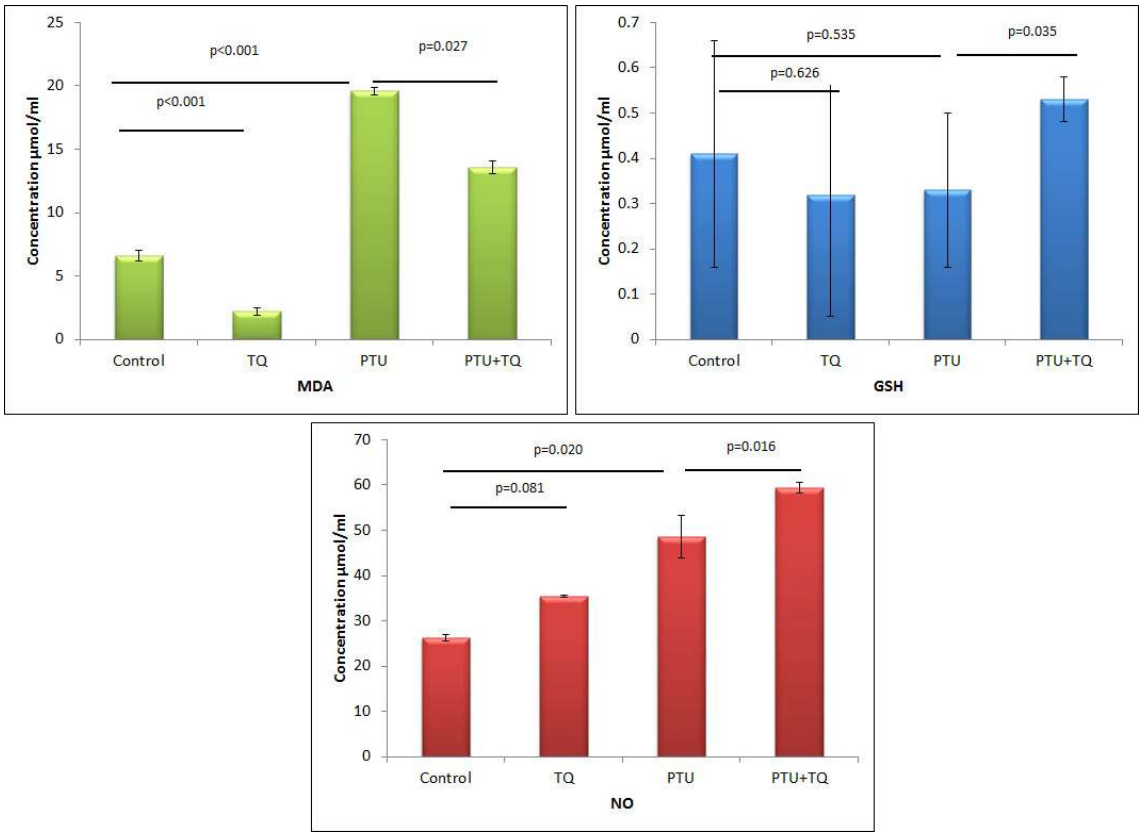

Figure 1. *Effect of propylthiouracil (PTU) and thymoquinone (TQ) on plasma lipid peroxides (MDA), reduced glutathione (GSH) and nitric oxide (NO) concentrations. Each point represents the mean ± SD. Significance is considered at p≤0.05*

Table 3: *Effect of propylthiouracil (PTU) and thymoquinone (TQ) on plasma glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activities*

Groups	GPX (mU/ml)	SOD (U/ml)	CAT (U/L)
Control	156.4 ± 24.7	285.7 ± 27.3	294.5 ± 84
TQ	171.2 ± 32	304.9 ± 75.9	239.5 ± 45.5
PTU	182.9 ± 26.1	104.5 ± 48.2[a]	541.7 ± 99.4[a]
PTU+TQ	159.5 ± 48.4	228.6 ± 98.3[b]	511 ± 76.1

Data are presented as mean ± SDM of six rats.
[a]Significant difference from the control (P ≤ 0.05).
[b]Significant difference from the PTU (Hypothyroid) (P ≤ 0.05).

with PTU significantly decreased plasma SOD activity (63%) compared to the control rats (p=0.000). Treatment with TQ after 2 weeks of PTU adminstration significantly increased plasma SOD activity (~ 2 fold) compared to PTU injected rats (p=0.035). In addition, treatments of rats with PTU caused significantly increased plasma CAT activity (84%) compared to the control rats (p=0.000). Treatment with TQ after 2 weeks of PTU administration caused a nonsignificant decrease in plasma CAT activity (6%) compared to PTU injected rats (p=0.599).

Effect of TQ on histopathological changes of hearts in PTU-induced hypothyroidism

Left ventricle of the control rats showed intact branching and anastomosing cardiac muscle fibers (CMFs) which possess acidophilic sarcoplasm with regular striations and oval central nuclei. Left ventricular CMFs of TQ group showed no histological changes compared with that of the control group Figure (2A, B). The left ventricle of PTU group showed some myocytes with hydropic changes in the form of lost striations and pyknotic nuclei. Continuity of the adjacent myocytesas well as lateral alignment of the myofibrils was lost in some areas together with nuclear displacement. Cellular infiltration and hemorrhage were also observed. The cross sectional area of the CMFs of this group was significantly decreased compared with that of the control rats (Figure 2C-E, G). The PTU+TQ group showed intact myocytes in almost all left ventricle with fewer cellular infiltrate and hemorrhages compared to the PTU group and the cross sectional area of the CMFs was insignificantly increased compared with that of the PTU group (Figure 2F). A significant increase in area percent of collagen fibers was observed in the left ventricle of PTU group compared with that of the control group while the PTU+TQ group showed a significant decrease compared with that of PTU group (Figure 3). In addition, significant

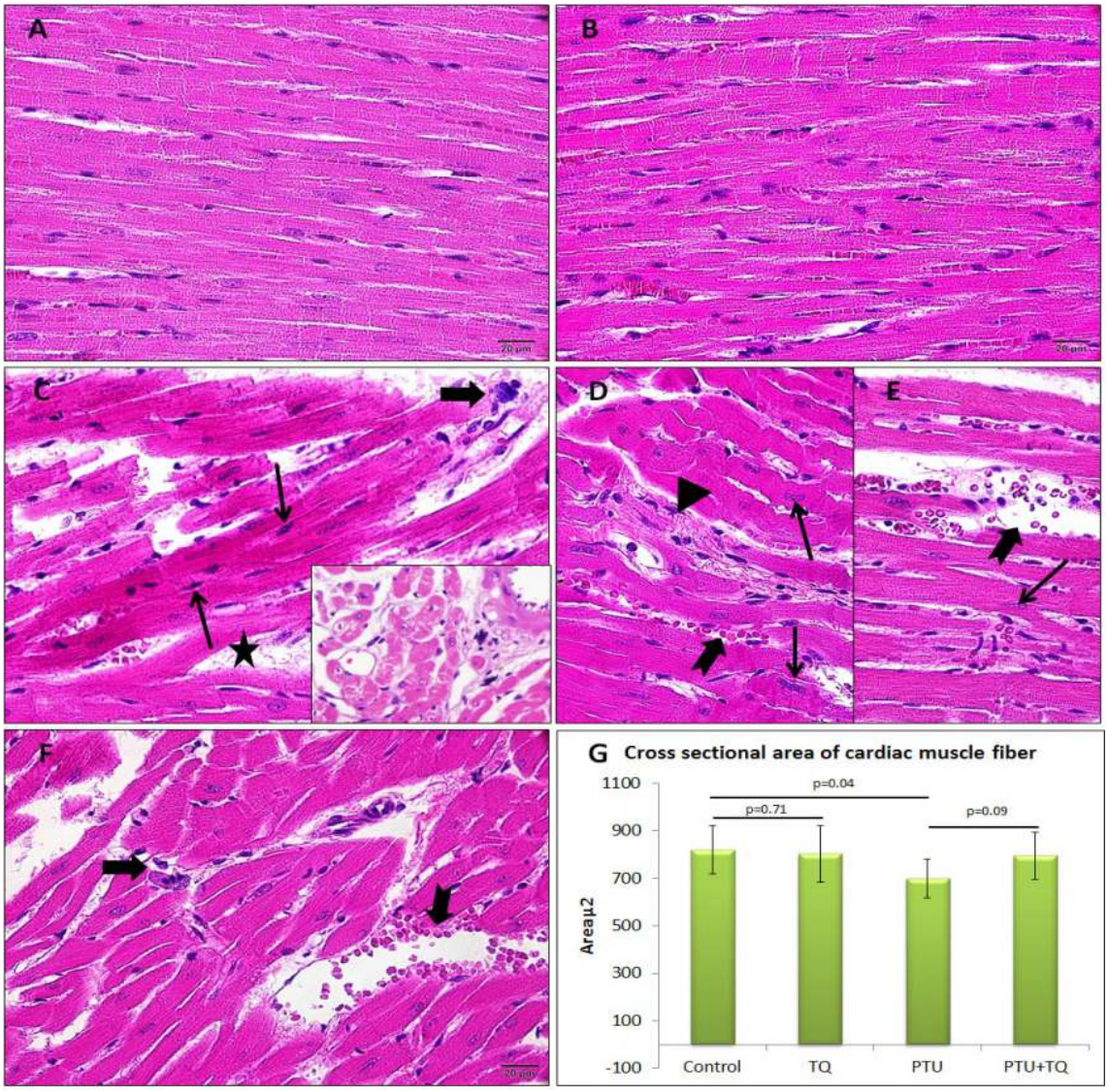

Figure 2. *Sections in rat left ventricle of the control (A) and the TQ group (B) showing branching and anastomosing cardiac muscle fibers (CMFs) with normal myofibrillar structure, regular striations and continuity with adjacent myofibrils. Sections of PTU (hypothyroid) rats (C, D, E) show some myocytes with hydropic changes (seen in the insert), pyknotic nuclei (arrow), lost striations and loss of continuity with adjacent myocytes. Some cellular leukocyte infiltration (thick arrow), edema (star) and hemorrhage (bifid arrow) are observed. Fibrous tissue (arrow head) is observed between myocytes. Irregular arrangement of muscle ibers and nuclear displacement (thin arrow) are observed in some areas. The section from TQ+PTU rats (F) show that most myocytes appear intact with few leucocytes and hemorrhage in between (H&E X600, insertX1000). Histogram (G) shows a significant decrease in diameter of the CMFs of the PTU rats compared to the control.*

positive correlations existed between area percent of collagen fibers and blood levels of LDH (r=0.532, p=0.01) and CPK (r=0.496, p=0.01).

Effect of TQ on PTU-induced immunohistochemical changes in ASMA, desmin and eNOS expression

Blood vessel wall showed strong ASMA expression in the left ventricle of both the control and TQ groups, whereas the CMFs showed negative expression. Some CMFs of PTU group showed moderate ASMA expression while fewer CMFs showed weak expression in the PTU+TQ group. Both groups showed many branched myofibroblasts between the CMFs with a strong ASMA expression. A significant increase ($p<0.001$) was observed in ASMA expression in PTU group compared with the control group and TQ administration significantly reduced it ($p<0.001$) (Figure 4). A significant positive correlation ($r=0.916$, $p<0.001$) was recorded between area percent of ASMA expression and that of collagen fibers in the left ventricle (Figure 3F). In addition, significant positive correlations existed between area percent of ASMA expression and blood levels of LDH ($r=0.580$, $p<0.003$) and CPK ($r=0.678$, $p<0.001$).

A strong desmin expression was observed in CMFs of both control and TQ groups. Most of CMFs of PTU and PTU+TQ groups showed a strong desmin expression while few of them showed weak or no expression. A significant decrease ($p<0.001$) in desmin expression was observed in

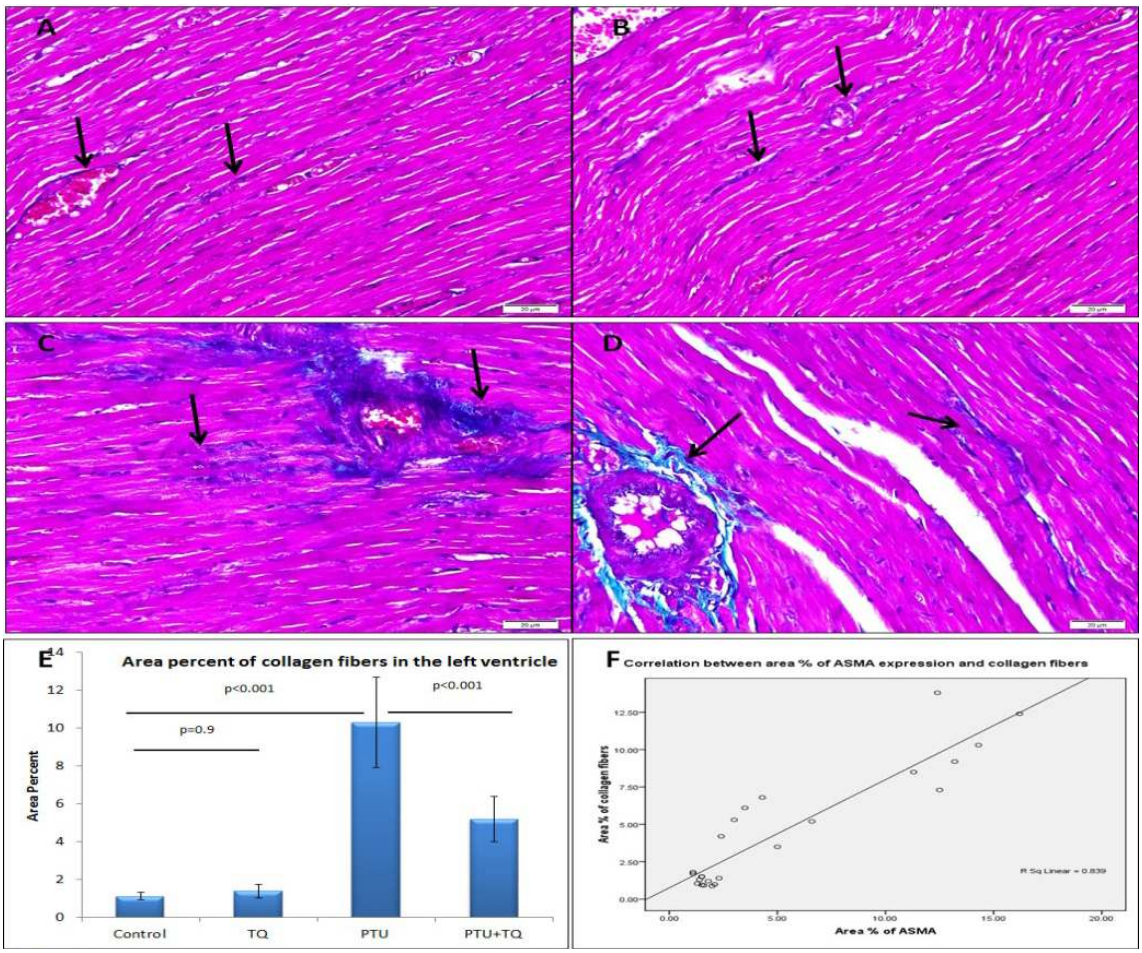

Figure 3. *Sections in the left ventricle of control (A) and TQ group (B) rats showing a minimal amount of collagen fibers (thin arrow) around the blood vessels and between the cardiac muscle fibers while those of PTU (hypothyroid) rats (C) or PTU+TQ rats (D) showing increased amount of collagen fibers in both areas (MT stain X 400). Histogram (E) shows significant increase in area percent of collagen fibers in the left ventricle of PTU rats compared with that of the control as well as a significant decrease in TQ+PTU rats compared with PTU rats. Histogram (F) shows significant positive correlation (r=0.916, p<0.001) between the area percent of collagen fibers and the area percent of ASMA expression in the left ventricle.*

the left ventricular CMFs of PTU group compared with the control, whereas those in the PTU+TQ group showed a significant increase ($p<0.05$) compared with the PTU group (Figure 5).

Concerning eNOS, a moderate expression was observed in the CMFs and the endothelial lining of the blood vessels of the control group. PTU group showed an increased expression that was found to be statistically significant ($p=0.04$) when compared to the control group. The left ventricle of the PTU+TQ group showed strong eNOS expression that was significantly higher ($p=0.01$) when compared to those of the PTU group (Figure 5).

Discussion

This study was conducted first to reassess the impact of experimentally-induced hypothyroidism on the adult rat heart. An experimental model of thyroid hormone deficiency was made by the reversible goitrogen PTU which decreased the conversion of T_4 to T_3. The hypothyroid state was ensured as the serum T_3 and T_4 levels were significantly lower, whereas the serum TSH level was significantly higher compared to control rats. A significant increase in plasma LDH and CPK enzymes activity was recorded in hypothyroid rats compared to control rats which indicate cardiac muscle affection. This was found to be accompanied by significant histopathological changes in left ventricle included hydropic changes in CMFs, cellular infiltration and multiple hemorrhages. These findings were in line with those of Massoud et al. [23]. In this study the hypothyroid rats weight gain was significantly reduced and this was in line with the previous studies [24]. On the other hand, the relative weight of the heart was significantly increased and this could be attributed to the infiltration of the inflammatory cell observed on the microscopic examination.

In the present study, a significant decrease in the CMFs cross sectional area was observed. In previous studies myocardial atrophy in hypothyroidism and marked reduction in muscle fiber mass in the PTU (hypothyroid) rats myocardium was reported [22]. This myocardial atrophy could be explained in the light of Klein and Danzi findings [25]. They reported that thyroid hormone increased total protein synthesis in the cardiac muscle and controls the transcription myosin heavy chain genes that are important for heart function.

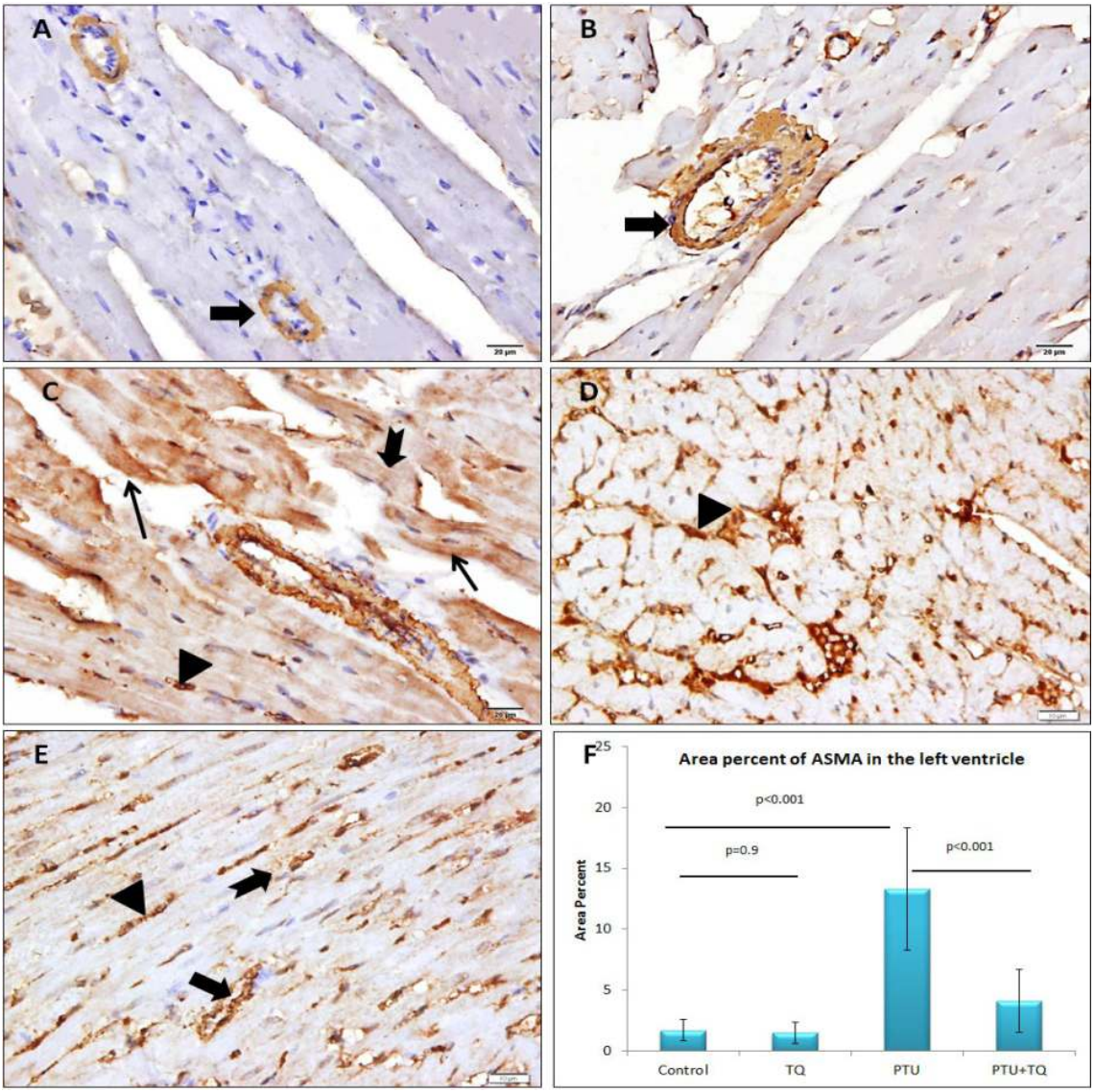

Figure 4. *Sections in the left ventricle of control (A) and TQ group (B) showing strong alpha smooth muscle actin (ASMA) expression in the wall of the blood vessels (thick arrow), while some areas of the left ventricle of PTU (hypothyroid) rats (C) show moderate (thin arrow) and weak (bifid arrow) expression in some CMFs and strong expression in the myofibrolasts (arrow head) with their branches anastomosing as seen in the cross section (D). Left ventricle of TQ+PTU rats (E) showing weak (bifid arrow) expression in the cardiac muscle fibers (CMFs) and strong expression in the myofibrolasts (arrow head) (ASMA immunohistochemistry X 600). Histogram (F) shows significant increase in ASMA area percent in the left ventricle of PTU rats compared with that of the control.*

A significant increase in ASMA expression was observed in the left ventricle of hypothyroid rats in this study indicating further fibrosis. The area percent of collagen fibers was positively correlated with ASMA expression in all the studied groups, suggesting that the collagen fibers were derived from cells expressing ASMA. This was in line with a recent study which reported that in myocardial stress, and heart diseases, cardiomyocytes are lost due to the myofibroblasts initiated reparative fibrosis with subsequent increase in ASMA-positive cells [26]. It was described that ASMA, a protein that is present in embryonal/fetal heart muscle cells, but absent in adult cardiomyocytes, is reexpressed in cardiomyocytes, undergo dedifferentiation, during heart hypertrophy induced by cardiac overload [27] and this could explain for ASMA expression observed in some left ventricular myocytes in this study.

The significant decrease in desmin expression was observed in the left ventricle CMFs of hypothyroid rats, in this study, indicating affection of the structural integrity of these fibers as was reported by Paulin et al. [28]. In addition, Capetanaki et al. [29] reported that mice lacking desmin develop numerous muscle architectural and ultrastructural defects, especially in extensively used muscles such as the heart and among the structural abnormalities observed were loss of lateral alignment of myofibrils and loss of nuclear shape and positioning that was observed in the present study.

The second aim of the present work was to assess the suggested cardioprotective action of TQ against hypothyroidism-induced cardiac affection. In this study, treatment of rats with TQ significantly protects the heart

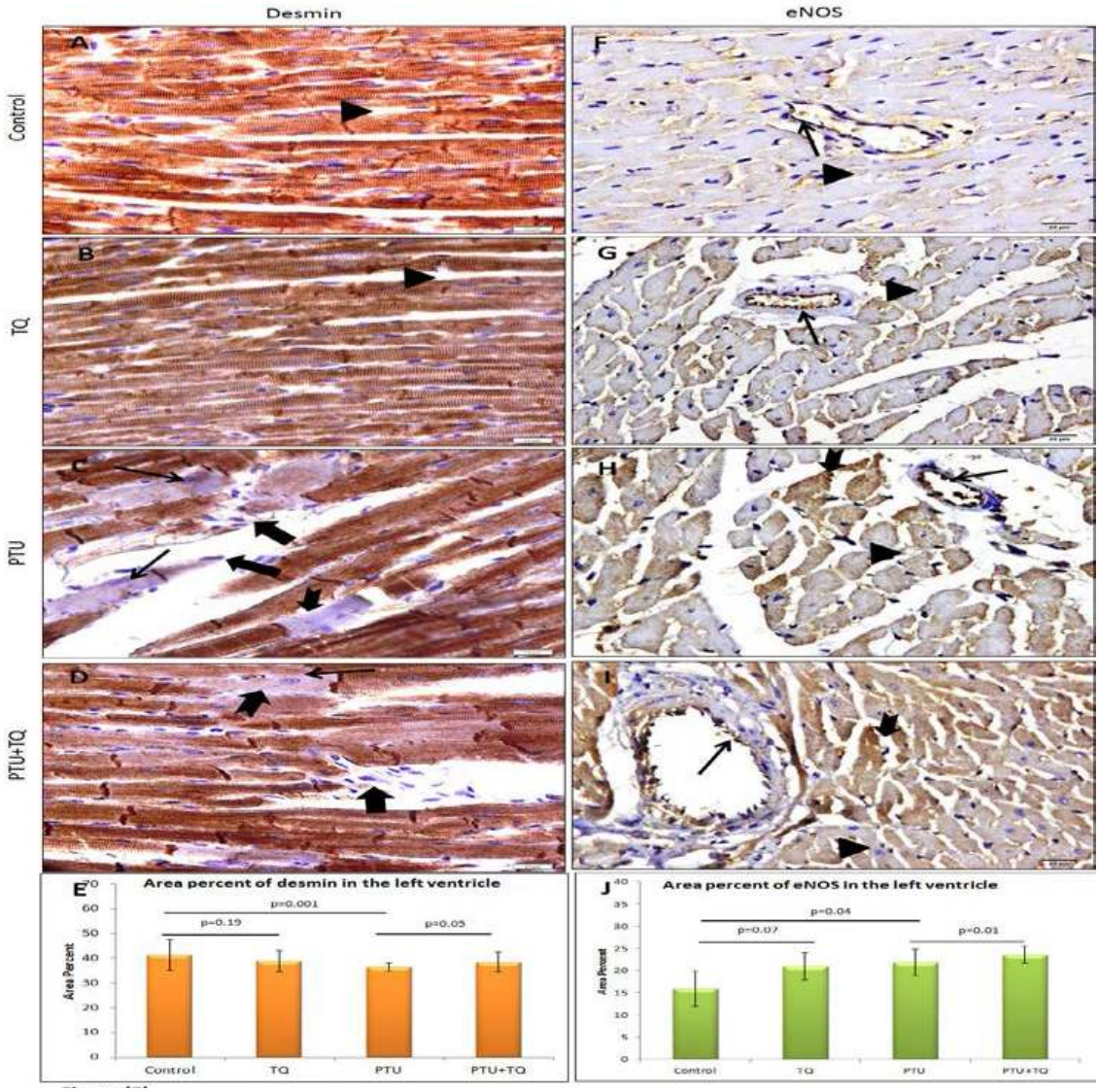

Figure 5. *Sections in left ventricle of control (A) and TQ (B) rats showing strong desmin expression (arrow head) in CMFs while that of PTU (C) and that of PTU+TQ rat (D) show weak or no expression (bifid arrow) in some CMFs. Note the loss of the lateral alignment of the myofibrils and myocytes (thick arrow) and nuclear displacement (thick arrow) in the desmin-negative CMFs (desmin immunohistochemistry X 600). Histogram (E) shows significant decrease area percent (AP) of desmin in the left ventricle of PTU group compared with that of the control while that of PTU+TQ rats shows significant increase compared with that of PTU group. Left ventricle of control (A) and TQ (B) rats show moderate eNOS expression in CMFs (arrow head) and endothelial lining (thin arrow) of the blood vessels while that of PTU rat (C) show moderate expression (arrow head) in the majority of CMFs and strong expression (bifid arrow) in few CMF as well as the endothelial lining of the blood vessels (thin arrow). Left ventricle of PTU+TQ rat (D) shows strong expression in majority of CMFs (bifid arrow) (eNOS immunohistochemistry X 600). Histogram (E) shows significant increase in AP of eNOS in the left ventricle of PTU group compared to that of the control rats and in that of PTU+TQ rats compared to those of PTU group.*

muscle against hypothyroidism-induced histopathological and immunohistochemical changes. It also significantly decreased plasma LDH and CPK enzymes activity compared to PTU group. This study reported a protective effect of TQ against PTU-induced hypothyroidism and the associated cardiac damage and this is supported by Nagi and Mansour [30] who reported a protective role of thymoqinone against adriamycin-induced cardiotoxicity.

Finally, the mechanism of TQ -induced protection is studied regarding the ROS and NO formation. In the present study, the effect of PTU on T_3, T_4 and TSH seems to be reversed in rats treated with TQ as the levels of the T_3, T_4 and TSH tends to be near the normal levels. This finding is in line with that of Shariatifar et al. [31] who reported that NS significantly increases the levels of T_3 and T_4 and decreases the TSH in mice. The role of thyroid hormones in metabolic pathways and antioxidant enzyme activities was well known in many species [32]. In the present study PTU-induced hypothyroidism caused an increase in the plasma MDA formation (a product of lipid peroxidation) and CAT activity and a decrease in plasma SOD activity while both GSH content and GPX activity were not altered. Increased MDA formation suggested enhancement of oxidative stress in hypothyroidism. This is in line with

the results of Yilmaz et al. [8], who reported increased plasma, liver, heart and muscle MDA level in hypothyroid rats. In addition, Chattopadhyay et al. [33] reported increased CAT activity in hypothyroidism, heart, which is suggested to be a reflex mechanism against increased oxidative stress-induced by hypothyroidism. Choudhury et al. [34] reported a fall in SOD and CAT activity in hypothyroid rats, but in addition of T_3, only the catalase activity was restored. Chattopadhyay et al. [33] described that, a decreased thyroid function may disturb SOD/CAT ratio and hence imbalance the oxidant/antioxidant state resulting in generation of oxidative stress. Petrulea et al. [35] also reported that GSH didn't differ significantly in serum and different tissues of hypothyroid rats and this was observed also in this study. Thymoquinone effect seems to be mediated through a reduction in MDA formation, and increased GSH and SOD production, hence it restores the oxidant/antioxidant balance. The mechanism underlying TQ protective effect in this study could be attributed to the normalization of T_3, T_4 and TSH. Thymoquinone acts as a potent free radical scavenger against superoxide, hydroxyl and singlet oxygen radical [36].

NO is an important vasodilator produced by coronary endothelial cells, which exerts a cardioprotective effect. It serves as an oxygen free radical scavenger, hence minimizing the deleterious effects of the oxidative stress [37]. In addition, it inhibits contractile tone and the proliferation of underlying vascular smooth muscle cells and promotes diastolic relaxation [38]. This study hypothesized a role of NO in TQ induced protection against hypothyroidism induced pathological cardiac changes. The present results showed that PTU-induced hypothyroidism increased NO formation, which seems to be a protective mechanism, and TQ treatment resulted in a further increase in NO formation. The immunohistochemical results were in line with this as the eNOS expression was increased in the left ventricle of rats received PTU and further increased in TQ rats. This finding is in concordance with some previous studies which reported an increase in NOS activity in both ventricles of the chronic hypothyroidism models and in the atria, endothelial and smooth muscle of the aorta of the acute model of thyroidectomy [39]. Increased NOS activity was described to be a compensatory mechanism stimulated by decreased plasma T_3 levels which plays a protective role in maintaining blood flow and reduces cardiac after load that resulted in an increase in the cardiac output and attenuation of pulmonary edema, thus markedly improving survival [39].

Conclusion

This study concluded that hypothyroidism may induce pathological cardiac changes, which were prevented by TQ via increased thyroid hormones and NO, and decreased ROS production.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Haq A, Abdullatif M, Lobo PI, Khabar KS, Sheth KV, al-Sedairy ST. *Nigella sativa*: effect on human lymphocytes and polymorphonuclear leukocyte phagocytic activity. Immunopharmacolog 1995; 30: 147-155.
2. Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. Phytother Res 2003; 17: 299-305.
3. Houghton P, Zarka R, Las Heras B, Hoult J. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes membrane lipid peroxidation. Plant Med 1995; 61:33-36.
4. Leong XF, Rais Mustafa M, Jaarin K. *Nigella sativa* and Its Protective Role in Oxidative Stress and Hypertension. Evid Based Complement Alternat Med 2013.
5. Dillmann WH. Cellular action of thyroid hormone on the heart. Thyroid 2002; 12: 447-452.
6. Ohga Y, Sakata S, Takenaka C, Abe T, Tsuji T, Taniguchi S, Takaki M. Cardiac dysfunction in terms of left ventricular mechanical work and energetics in hypothyroid rats. Am J Physiol Heart Circ Physiol 2002; 283: H631-641.
7. Knapp M, Lisowska A, Sobkowicz B, Tycińska A, Sawicki R, Musiał WJ. Myocardial perfusion and intima-media thickness in patients with subclinical hypothyroidism. Adv Med Sci 2013; 58: 44-49.
8. Yilmaz S, Ozan S, Benzer F, Canatan H. Oxidative damage and antioxidant enzyme activities in experimental hypothyroidism. Cell Biochem Funct 2003; 21: 325-330.
9. Badary OA, Abdel-Naim AB, Abdel-Wahab MH, Hamada FM. The influence of thymoquinone on doxorubicin-induced hyperlipidemic nephropathy in rats, Toxicol 2000; 143: 219-226.
10. Villar D, Rhind SM, Dicks P, McMillen SR, Nicol F, Arthur JR. Effect of propylthiouracil-induced hypothyroidism on thyroid hormone profiles and tissue deiodinase activity in cashmere goats. Small Ruminant Research 1998; 29: 317-324.
11. Ebru U, Burak U, Yusuf S, Reyhan B, Arif K, Faruk TH, Emin M, Aydin K, Atilla II, Semsettin S, Kemal E. Cardioprotective effects of *Nigella sativa* oil on cyclosporine A-induced cardiotoxicity in rats. Basic Clin Pharmacol Toxicol 2008; 103: 574-580.
12. Pesce AJ. Lactate dehydrogenase: In: Methods in Clinical Chemistry. The C. V. Mosby Comany, St. Louis., 1987; pp.903-906.
13. Abbot B. Creatinine kinase: In: Clinical chemistry. St Louis, Toronto, Princeton: Mosby 1984; pp. 1112e6.
14. Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Analytical Biochemistry 1978; 86: 271-278.
15. Ellman GL. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics 1959; 82: 70-77.
16. Tarpey M, Wink DA, Grisham MB. Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in

vivo considerations. The American Journal of Physiology-Regulatory Integrative and Comparative Physiology 2004; 286: R431-R444.

17. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70: 158-169.
18. Nishikimi M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun 1972; 46: 849-854.
19. Aebi H. Catalase in vitro. Methods Enzymol 1984; 105: 121-126.
20. Bancroft J, Gamble. Theory and Practice of Histology Techniques. 6th Ed Churchill Livingstone Elsevier 2008.
21. Afifi NM, Hanon AF. Histological and immunohistochemical study on the possible cardioprotective role of acetylcysteine in oral formalin myocardial toxicity in adult albino rats. The Egyptian Journal of Histology 2011; 34: 859-869.
22. Leslie K, Taatjes DJ, Schwarz J, vonTurkovich M, Lowt RB. Cardiac Myofibroblasts Express Alpha Smooth Muscle Actin during Right Ventricular Pressure Overload in the Rabbit. Am J Pathol 1991; 139: 207-216.
23. Massoud AA, El-Atrash A, Tousson E, Ibrahim W, Abou-Harga H. Light and ultrastructural study in the propylthiouracil-induced hypothyroid rat heart ventricles and the ameliorating role of folic acid. Toxicology and Industrial Health 2012; 28: 262-270.
24. Cakc-Milosevic M, Korac A, Davidovi V. Methimazole-Induced Hypothyroidism In Rats: Effects On Body Weight And Histological Characteristics Of Thyroid Gland. Jugoslov Med Biohem 2004; 23: 143-147.
25. Klein I, Danzi S. Thyroid disease and the heart. Circulation 2007; 116: 1725-1735.
26. Weber KT, Sun Y, Bhattacharya SK, Ahokas RA, Gerling IC. Myofibroblast mediated mechanisms of pathological remodelling of the heart. Nat Rev Cardiol 2012; 10: 15-26.
27. Gosteli-Peter MA, Harder BA, Eppenberger HM, Zap fJ, Schaub MC. Triiodothyronine induces over-expression of alpha-smooth muscle actin, restricts myofibrillar expansion and is permissive for the action of basic fibroblast growth factor and insulin-like growth factor I in adult rat cardiomyocytes. J Clin Invest 1996; 98: 1737-1744.
28. Paulin D, Huet A, Khanamyrian L. Desminopathies in muscle disease. J Pathol 2004; 204: 418-427.
29. Capetanaki Y, Milner DJ, Weitzer G. Desmin in muscle formation and maintenance: Knockouts and consequences. Cell Struct Funct 1997; 22: 103-116.
30. Nagi MN, Mansour MA. Protective effect of thymoquinone against doxorubicin-induced cardiotoxicity in rats: a possible mechanism of protection. Pharmacol Res 2000; 41: 283-289.
31. Shariatifar A, Riazi M, Ebnolelm M, Jahromy MH. Effects of *Nigella sativa* L. Seed Extract on Fatigue, Blood Biochemical Parameters and Thyroid Function in Male Mice. Chinese Medicine 2014; 5: 16-21.
32. Nazifi S, Mansourian M, Nikahva lB, Razavi SM. The relationship between serum level of thyroid hormones, trace elements and antioxidant enzymes in dromedary camel (Camelusdromedarius). Trop Anim Health Prod 2009; 41: 129-134.
33. Chattopadhyay S, Zaidi G, Das K, Chainy GBN. Effects of hypothyroidism induced by 6-n-propylthiouracil and its reversal by T_3 on rat heart superoxide dismutase, catalase and lipid peroxidation. Indian J Exp Biol 2003; 41: 846-849.
34. Choudhury S, Chainy GB, Mishro MM. Experimentally induced hypo- and hyper-thyroidism influence on the antioxidant defence system in adult rat testis. Andrologia 2003; 35: 131-140.
35. Petrulea MS, Duncea IH, Georgeta D, Gheorghe D, Nicoleta MA. Oxidative stress in experimental hypothyroidism: effect of vitamin e supplementation. Clujul Medical 2010; 83: 245.
36. Badary OA, Taha RA, Gamal el-din AM, Abdel-Wahab MH. Thymoquinone is a potent superoxide anion scavenger. Drug Chem Toxicol 2003; 26: 87-98.
37. Rassaf T, Poll LW, Brouzos P, Lauer T, Totzeck M, Kleinbongard P. Positive effects of nitric oxide on left ventricular function in humans. Eur Heart J 2006; 27: 1699-1705.
38. Balligand JL, Cannon PJ. Nitric oxide synthases and cardiac muscle. Autocrine and paracrine influences. Arterioscler. Thromb Vasc Biol 1997; 17: 1846-1858.
39. Fellet AL, Arza P, Arreche N, Arranz C, Balaszczuk AM. Nitric oxide and thyroid gland: modulation of cardiovascular function in autonomic-blocked anaesthetized rats. Exp Physiol 2004; 89: 303-312.

***Correspondence to:**

Nasra Ayoub

Department of Anatomy

Faculty of Medicine

King Abdulaziz University

Saudi Arabia.