Protective Effect of Vitamin E Against Ischemia: Reperfusion Induced Oxidative Stress in Isolated Hearts From Hyperthyroid Rabbits

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Abstract

Background: Hyperthyroidism either clinical or experimentally induced is associated with cardiac problems such as sinus tachycardia and atrial fibrillation in addition to reduction in ventricular contractility which is due to increased free radicals. It is now generally accepted that free radical-lipid peroxidation in biological membranes is associated with variety of important pathological events and aging. Vitamin E is one of the well known antioxidants used in clinical practice.

Aim of the Work: The objective of this work is to study the effect of vitamin E on the functional recovery from in vitro induced ischemia-reperfusion of isolated hearts in hyperthyroid rabbits compared to euthyroid and vitamin E treated hyperthyroid ones.

Material and Methods: In the present study 40 normal male New Zealand rabbits, weighing 2.0-2.25 kg, were used and randomly divided into 4 groups, 10 rabbits each. Group-I (euthyroid “E”): rabbits received intramuscular (i.m.) injection of saline (1ml/kg/day) for 8 days and oral corn oil vehicle (1ml/kg/day) orally for 10 days, Group-II (hyperthyroid “H”): as group-I but rabbits received i.m. injection of l-thyroxin “T4” (200 µg/kg/day) instead of saline. Group-III (vitamin E-treated “VE”): rabbits received i.m. injection of saline (1ml/kg/day) and oral vitamin E (200 µg/kg/day) in corn oil vehicle for 10 days and Group-IV (hyperthyroid vitamin E-treated “HVE”): as group-III but rabbits received i.m. injection of T4 (200 µg/kg/day) instead of saline. At the end of therapy, hearts were isolated, weighed and exposed to 30 min. no flow ischemia followed by 25 min. reperfusion. Pre-ischemic and after 25 min. reperfusion coronary effluent, heart rate (HR) and contractility parameters (amplitude of contractions, LVDP and \( \frac{dP}{dt}_{\text{max}} \)) were assessed then hearts of each group were homogenized to measure the malondialdehyde (MDA) and guanosine 3',5'-cyclic monophosphate (cGMP).

Results: The mean heart weight/BW ratio in (H) and (HVE) groups significantly increased after T4 therapy by +32.4% and +35.1% respectively compared to (E) group. Except for the mean coronary effluent, which showed insignificant changes, all other tested parameters significantly increased in (H) and (HVE) groups compared to corresponding pre-ischemic values of (E) group. All pre-ischemic values of (HVE) group insignificantly changed compared to that of (H) group. The mean coronary effluent significantly increased in (HVE) by +19.4% compared to (H) group after 25 min. reperfusion. However, the mean HR was significantly reduced in (HVE) by -34.7% compared to (H) group after 25 min. reperfusion. The mean contractility parameters “amplitude of contractions, LVDP and \( \frac{dP}{dt}_{\text{max}} \) ” significantly increased in (HVE) by “+41.1%, +106% and +40%” respectively compared to (H) group after 25 min. reperfusion. The mean MDA and cGMP was significantly reduced in (HVE) by -45.7% and -39.5% respectively compared to (H) group.

Conclusion: These results suggested that after exposure of isolated hyperthyroid rabbits’ hearts to ischemia-reperfusion, as a model for oxidative stress, oral vitamin E could improve the contractility parameters, coronary flow as well as tachycardic response to reperfusion in addition to the improvement of the cardiac capability to face oxidative stresses in hyperthyroidism. This might need a further clinical study to prove the role of vitamin E in preventing ischemic cardiac dysfunction in patients with thyrotoxicosis.

Key Words: Vitamin E – Hyperthyroidism – Ischemia - reperfusion – Langendorff heart preparation.

Introduction

THYROID hormone metabolic disarray has been identified as a risk factor for the progression of heart disease and the development of heart failure (HF). Both hyper- and hypothyroidism have been associated with a failing myocardium. Poor cardiac contractility and low cardiac output due to hyperthyroidism is mostly seen in patients with preexisting heart disease. Referred to as a "rate related" phenomenon, hyperthyroid-induced sustained sinus tachycardia or atrial fibrillation may further reduce ventricular contractility [1].

Myocardial ischemia-reperfusion represents a clinically relevant problem associated with thrombolysis, angioplasty and coronary bypass surgery. Injury of myocardium due to ischemia-reperfusion includes cardiac contractile dysfunction, arrhythmias as well as irreversible myocyte damage. These changes are considered to be the consequence of imbalance between the formation of oxidants and the availability of endogenous antioxidants in the heart [2].

It is known from a long time that both clinical [2] and experimental hyperthyroidism [3] alter car-
diac function. Recently, it has been suggested that the hypermetabolic state typical of hyperthyroidism, results in an increased production of reactive oxygen species (ROS) that can lead to oxidative injury of cardiac tissue and progressive organ dysfunction [4]. Actually, hyperthyroidism does not seem to modify H2O2 release by heart mitochondria [8], while it decreases antioxidant capacity of cardiac tissue [6]. This suggests that the increased lipid peroxidation observed in the hyperthyroid heart [6,7] is consequent to its smaller effectiveness in preventing oxidative alterations.

Mitochondria are a possible site of ischemia-reperfusion damage, as the loss of mitochondrial function leads to cell death, whereas an optimal energy metabolism is required to preserve cell viability. Actually, ischemia reduces in vitro mitochondrial respiration [8,9], an effect that is potentiated by reperfusion [9,10]. Furthermore, the reperfusion-induced decline of the mitochondrial function depends on the length of the oxygen deprivation period [9] and the age of the animals [11]. Hyperthyroidism has been associated with a hyperdynamic state of the heart characterized by tachycardia and increased contractile performance [12]. These changes in electrical and mechanical activity of the heart result in an increased total cardiac output and work to match the increased oxygen demand by peripheral tissues owing to the thyroid hormone-induced increase in their metabolism.

Thyroid hormone plays a crucial role in the regulation of mitochondrial oxidative metabolism; the synthesis and degradation of proteins and vitamins; such as vitamin E, vitamin A and β-carotene, the sensitivity of tissues to catecholamines and the regulation of antioxidant enzyme levels [12]. The hypermetabolic state that characterizes hyperthyroidism should accelerate free radical production in the mitochondria and induce changes in the antioxidant defense system [13].

Hyperthyroidism has been implicated as primary cause of cardiomegaly, congestive heart failure and decreased exercise tolerance. Furthermore, it may induce biochemical changes in myocardial tissue that can lead to progressive organ dysfunction. Hyperthyroidism has been reported to decrease heart antioxidant capacity and increase its susceptibility to in vitro oxidative stress [14]. Heart ischemia-reperfusion combined with hyperthyroidism would be one of the conditions that induce an increase in oxidative stress [15].

Vitamin E is the collective name for a set of eight related tocopherols, alpha-tocopherol has been most studied as it has the highest bioavailability, it protects cell membranes against oxidation by reacting with lipid radicals produced in lipid peroxidation chain reaction [16].

Investigations of antioxidant defense system have returned controversial results. Moreover, other thyroid hormone-linked biochemical changes increase tissue susceptibility to oxidative challenge, which exacerbates the injury and dysfunction they suffer under stressful conditions. Mitochondria, as a primary target for oxidative stress, might account for hyperthyroidism linked tissue dysfunction [17].

Studies on free radical production in reperfusion system has suggested that cardiac dysfunction during reperfusion can result from free radical generation and oxidative damage [18]. Nitric oxide (NO), a highly reactive second messenger, plays important role in cardiac muscle physiology [18]. Since thyroid hormone produces biochemical changes that increase tissue susceptibility to oxidative challenge, which exacerbates the tissue injury and dysfunction they suffer under stressful conditions [19]. So, the present study was conducted to investigate the effect of vitamin E as an antioxidant isolated hyperthyroid rabbits’ hearts to face an oxidative stress of ischemia-reperfusion as a model oxidative myocardial injury in comparison to euthyroid and vitamin E treated hyperthyroid rabbits.

Material and Method

Drugs:
1- Vitamin E as D-α tocopheryl acetate soft capsules 400 mg (Pharco Pharmaceutical Company) was dissolved in corn oil (0.25‰).
2- I-thyroxin T4 (Sigma; St. Louis, MO), was dissolved in saline vehicle (0.25‰).
3- Heparin 5000 Ampoule (Nile company).

Animals:
Forty normal male New Zealand rabbits weighing 2.0-2.25 kg were kept in individual cages on a 12:12 light dark cycle in a room temperature at 26°C±1°C and were given free access to water and food in the animal house of Kasr Al-Aini Faculty of Medicine, Cairo University. Rabbits were randomly assigned to one of 4 groups, each group comprised of 10 rabbits, as follows:
• Group-I (control group “Euthyroid”): rabbits received intramuscular (i.m.) injection of saline (1ml/kg/day) for 8 days and oral corn oil vehicle (1ml/kg/day) for 10 days.
• Group-II (Hyperthyroid): rabbits received i.m. injection of T4 (200 µg/kg/day) [20] for 8 days [20] and oral corn oil vehicle (1ml/kg/day) for 10 days.
• **Group-III** (Euthyroid + vitamin E): rabbits received i.m. injection of saline (1ml/kg/day) for 8 days and oral vitamin E (200 γ/kg/day) [21] in corn oil vehicle for 10 days [21].

• **Group-IV** (Hyperthyroidism + vitamin E): rabbits received i.m. injection of T4 4 (200 γ/kg/day) [20] for 8 days [20] and oral vitamin E (200 γ/kg/ day) [21] in corn oil vehicle for 10 days [21].

Induction of hyperthyroidism in group II and IV was confirmed by measurement of thyroid hormone level in blood samples obtained from ear veins at 8th day of the study by using serum T4 ELISA kit according to the manufacturer’s instructions (MONOBIND Co., USA). Rabbits with serum T4 > 24 γ/dl were considered as hyperthyroid one [22]. All rabbits were weighed after therapy once more.

Experimental protocol:

1. **Heart preparation:** At the end of therapy all rabbits were anesthetized by urethane. (1.5 g/kg, i.p) [23]. heparinized (100 IU/100 g body weight i.p.) and a left thoracotomy was performed to expose the heart. Each heart was rapidly excised, flushed to get rid of blood for 1 min with Krebs-Henseleit (KH) solution containing in mmol/l: NaCl 118, NaHCO3 24, KCl 4.7, MgSO4 1.2, NaH2PO4 1.2, CaCl2 2.5, EDTA 0.5 and glucose 11, pH 7.4. Then hearts were weighed, cannulated and perfused retrogradely with (KH) solution through the aortic root under a pressure of 70 mmHg according to Langendorff as previously described [24] at 37 ºC and gassed with 5% CO2 in O2. The hearts were also maintained at 37°C using a water reservoir surrounding the hearts in which the open end was covered to maintain temperature and humidity [8]. To record intraventricular pressures a saline filled latex balloon (size No. 4, Hugo Sachs Elektronik, Germany), connected to a catheter which was inserted into the left ventricle via an incision in the left atrial appendage. The catheter was then connected to a pressure transducer (Gold statum). Pressure changes were analyzed and displayed on an electronic polygraph (NEC-Sanei instruments Ltd, 2238). The preparation was equilibrated for 30 minutes. Then the hearts were subjected to 30 minutes of no-flow global ischemia by clamping the perfusion line-during this period the temperature was kept constant at 37°C as mentioned above, followed by 25 minutes [15] of reperfusion. The investigation conforms to the Guidelines for Care and Use of Laboratory Animals of the Kasr Al- Aini Faculty of Medicine, Cairo University.

2. **Evaluation of cardiac function and post-ischemic recovery:** The following parameters were measured; the amplitude of contraction the heart rate (HR), left ventricular developed pressure (LVDP) designated as the difference between systolic and diastolic left ventricular pressures. The peak rate of maximum left ventricular pressure rise dP/dtmax was also measured from digital signals [28], it is considered a good index of contractility.

The cardiac function parameters were recorded two times one at the end of the equilibration and the other at the end of reperfusion (25 th minute). Coronary flow was measured by timed collection of the coronary effluent in a sealed graduated cylinder during each recording of the cardiac function parameters. At the end of the perfusion protocol heart great vessels, valves and atria of each group were trimmed away and the ventricles were cut open, rinsed free of liquid and kept frozen at -40°C. Frozen myocardial tissue samples of each group were ground to a fine powder in a stainless steel mortar and divided into two equal weights for estimation of the mean MDA and cGMP.

3. **Measurement of Malondialdehyde (MDA):** It is one of end products formed via the decomposition of lipid peroxidation. MDA was measured in cardiac tissue homogenate after precipitation of protein by addition of trichloracetic acid then thiobarbituric acid (TBA). TBA reacted with MDA to form thiobarbituric acid product which was measured at 532 nm according to Draper and Hadley [26]. The level of peroxidation was expressed as the amount of MDA in nmol/g wet mass.

4. **Guanosine 3',5'-cyclic monophosphate (cGMP) assay:** It proportionally reflects the degree of NO production in heart preparations. The weighed frozen ventricular powder was homogenized in 10 volumes of 0.1 M HCl to stop the action of phosphodiesterases. Centrifugation was done at 30,000 r.p.m. at room temperature and the supernatant was then collected for quantitative immunoassay of cGMP level according to general principle of ELISA technique [27] and the manufacturer's instructions (CG-200, Sigma-Aldrich, Inc. USA). The assay used a polyclonal antibody to cGMP to bind, in a competitive manner, cGMP in samples and standards covalently attached to alkaline phosphatase. After incubation with the p-nitrophenyl phosphate substrate a yellow colour yielded; its intensity was measured at 405 nm. The amount of cGMP was expressed as pmol/g wet mass.

**Statistical analysis:**

All data are shown as mean ± SD. Statistical analysis of the results is performed by using student t-test for comparison of two groups. Comparison among more than two groups is carried out using
one way ANOVA, p<0.05 is considered statistically significant [28].

Results

Effect of hyperthyroidism on the mean body weight (BW) and heart weight (HW)/body weight ratio, compared to different studied groups:

The mean BW (kg), before any therapy, of all groups showed insignificant differences compared to the corresponding value of control group-I. Administration of either saline alone or with vitamin E in groups I or III resulted in insignificant increase the mean BW by 4.2% and 4.3% respectively compared to the corresponding value before therapy within the same group. There was insignificant change in the mean BW after therapy in group-III compared to group-I. However, T4 induced hyperthyroidism either alone or with vitamin E in groups II or IV significantly reduced the mean BW by (-13.4% & -12.9%) and (-13.1% & -14.7%) compared to (the corresponding value before induction of hyperthyroidism within the same group and to the corresponding value in control group-I) respectively. There was insignificant reduction in the mean BW after induction of hyperthyroidism by -2.0% in group-IV compared to group-II. Vitamin E treated group-III showed significant increase in the mean BW by 12.8% compared to that of hyperthyroid group-IV [Table (1) & Fig. (1)].

The mean HW/BW ratio (g/kg) was insignificantly increased by +6.7% after vitamin E treatment in group-III compared to control group-I. T4 induced hyperthyroidism either alone or with vitamin E in groups II or IV significantly increased the mean HW/BW ratio by 32.4% and 35.1% respectively compared to control group-I. There was significant reduction (-19.4%) in the mean HW/BW ratio in group-III compared to group-II. However, group-IV showed insignificant increase (2.04%) in the mean HW/BW ratio compared to group-II [Table (1) & Fig. (2)].

Effect of vitamin E on the mean amplitude of contractions, coronary flow, heart rate (HR), Left ventricular developed pressure (LVDP) and the peak rate of maximum left ventricular pressures rise dP/dtmax at pre-ischemic state of isolated rabbits’ hearts and after 30 minutes of no-flow global ischemia that was followed by 25 minutes reperfusion, of different studied groups:

Except for the HR in hyperthyroid group-II, the mean value of each tested parameter of isolated hearts in all groups after 25 min. reperfusion showed a significant reduction compared to the corresponding pre-ischemic value within the same group [Table (2) & Figs. (4-8)].

T4 induced hyperthyroidism either alone in group-II or with vitamin E in group-IV showed a significant increase in the mean amplitude of contractions (cm) of isolated rabbits’ hearts at pre-ischemic state by 33.7% and 29.5% respectively compared to the corresponding value of control group-I. However, after 25 min. reperfusion, the mean amplitude of contractions significantly reduced by -33.3% in group-II but insignificantly reduced by -5.9% in group-IV compared to the corresponding value of control group-I. Administration of vitamin E alone in group-III produced insignificant increase in the mean amplitude of contractions at pre-ischemic state and after 25 min. reperfusion by +7.3% and +3.9% respectively compared to the corresponding value in control group-I. Despite of the reduction in the mean amplitude of contractions at pre-ischemic state either significant in group-III (-19.7%) or insignificant in group-IV (-3.1 %) compared to the corresponding value in hyperthyroid group-II, the mean amplitude of contractions after 25 min. reperfusion significantly increased by +55.9% and +41.1% in vitamin E treated groups III and IV respectively compared to the corresponding value in hyperthyroid group-II [Table (2) & Figs. (3-4)].

The mean coronary flow (ml/min) of isolated rabbits’ hearts of vitamin E treated group-III, at pre-ischemic state and after 25 min. reperfusion, was significantly increased by 75% and 30.8% respectively compared to the corresponding value of control group-I. However, T4 induced hyperthyroidism either alone in groups II or IV produced insignificant changes in the mean coronary flow at pre-ischemic state by 3.7% and -2.5% respectively compared to the corresponding value of control group-I. Moreover, group-II or IV showed significant reduction in the mean coronary flow after 25 min. reperfusion by -30.7% and -17.3% respectively compared to the corresponding value of control group-I. On the other hand groups II or IV showed significant changes in the mean coronary flow at pre-ischemic state by 3.7% and -2.5% respectively compared to the corresponding value of control group-I. However, after 25 min. reperfusion by (-6.0% & +19.4%) respectively compared to the corresponding value in hyperthyroid group-II [Table (2) & Fig. (5)].

Hyperthyroidism in group-II produced significant increase in the mean HR at pre-ischemic state and after 25 min. reperfusion by 22.8% and 80.5% respectively compared to the corresponding value of control group-I. However, vitamin E therapy in group-III produced insignificant increase in the mean
HR by 2.6% and 6.1% at pre-ischemic state and after 25 min. reperfusion, respectively and compared to the corresponding value of control group-I. Vitamin E treated hyperthyroid group-IV showed significant increase in the mean HR at pre-ischemic state by 20.9% and insignificant increase after 25 min. reperfusion by 1.3% compared to the corresponding value of control group-I. There was significant decrease in the mean HR in group-III at pre-ischemic state and after 25 min. reperfusion by -16.4% and -48% respectively compared to the corresponding value in hyperthyroid group-II. Vitamin E in hyperthyroid group-IV resulted in an insignificant and a significant reduction at pre-ischemic state and after 25 min. reperfusion respectively by -1.5% and -34.7% compared to the corresponding value in hyperthyroid group-II [Table (2) & Fig. (6)].

The mean (LVDP and \(dP/dt_{\text{max}}\)) at pre-ischemic state, significantly increased in hyperthyroid group-II by (+42.6% and +39%) and in hyperthyroid group-IV with vitamin E by (+40.9% and +35.7%) respectively compared to the corresponding value of control group-I. However, after 25 min. reperfusion the mean (LVDP and \(dP/dt_{\text{max}}\)) significantly reduced by (-54.5% and -36%) in group-II but insignificantly reduced by (-6.4% and -10.5%) in group-IV respectively compared to the corresponding value of control group-I. Administration of vitamin E in group-III produced insignificant increase in the mean (LVDP and \(dP/dt_{\text{max}}\)) by (+4.7% and +3%), at pre-ischemic state and (+6.3% and +2.7%) after 25 min. reperfusion, respectively compared to the corresponding value of control group-I. Compared to hyperthyroid group-II, the mean (LVDP and \(dP/dt_{\text{max}}\)) at pre-ischemic state significantly decreased by (-26.6% and -26%) in group-III and insignificantly decreased by (-1.2% and -2.3%) in group-IV respectively. However, after 25 min. reperfusion, vitamin E treated groups III and IV showed significant increase in the mean (LVDP and \(dP/dt_{\text{max}}\)) by (+134% and +60.7%) in group-III and (+106% and +40%) in group-IV respectively compared to the corresponding value in hyperthyroid group-II [Table (2) & Figs. (7-8)].

The mean MDA was increased by 98% in T4 treated group-II compared to the corresponding value of control group-I. However, treatment with vitamin E alone in group-III or in hyperthyroid group-IV produced insignificant changes in the mean MDA by -7.2% and +7.3% respectively and compared to the corresponding value of control group-I. Vitamin E in group-III and group-IV produced a significant reduction in the mean MDA by -53.1% and -45.7% respectively and compared to the corresponding value in hyperthyroid group-II [Table (3) & Fig. (9)].

As regard the mean cGMP, T4 treated group-II showed 150% increment in comparison to the corresponding value of control group-I. Vitamin E therapy alone in group-III or with hyperthyroidism in group-IV improved the mean cGMP to be -28% and +51% respectively and compared to the corresponding value of control group-I. Furthermore, vitamin E in group-III and group-IV produced a significant reduction in the mean cGMP by -71.1% and -39.5% respectively and compared to the corresponding value in hyperthyroid group-II [Table (3) & Fig. (10)].

**Fig. (1):** Effect of i.m. T4 "200 µg/kg/day for 8 days" on the mean rabbits’ body weight compared to different studied groups (n=10).

† Insignificant difference between before and after therapy values within the same group (p>0.05).
‡ Insignificant difference between before and after therapy values within the same group (p<0.05).
§ Insignificant change compared to corresponding value in group-I (p<0.05).
¶ Significant change compared to corresponding value in group-II (p<0.05).

Vit-E oral dose was “200 µg/kg for 10 d.”.

**Fig. (2):** Effect of i.m. T4 "200 µg/kg/day for 8 days" on the mean rabbits’ heart weight/body weight ratio compared to different studied groups (n=10).

* Significant increase compared to group-I (p<0.05).
† Significant increase compared to group-I (p>0.05).
‡ Significant decrease compared to group-II (p<0.05).
¶ Significant increase compared to group-II (p>0.05).

Vit-E oral dose was “200 µg/kg for 10 d.”.
Fig. (3): Effect of oral vitamin E "200 µg/kg/day for 10 days" on the contractions of hearts isolated from hyperthyroid rabbits "injected i.m. with T 4 in a dose of 200 µg/kg/day for 8 days" before & after no-flow ischemia compared to different studied groups.

Fig. (4): Effect of oral vitamin E "200 µg/kg/day for 10 days" on the mean amplitude of contraction of isolated hyperthyroid rabbits' hearts compared to different studied groups (n=10).

# Significant difference compared to pre-ischemic value within the same group (p<0.05).
* Significant difference compared to corresponding value in group-I (p<0.05).
‡ Insignificant difference compared to corresponding value in group-I (p>0.05).
§ Significant difference compared to corresponding value in group-II (p<0.05).
‡ Insignificant difference compared to corresponding value in group-II (p>0.05).

Fig. (5): Effect of oral vitamin E "200 µg/kg/day for 10 days" on the mean coronary flow of isolated hyperthyroid rabbits' hearts compared to different studied groups (n=10).

# Significant difference compared to pre-ischemic value within the same group (p<0.05).
* Significant difference compared to corresponding value in group-I (p<0.05).
‡ Insignificant difference compared to corresponding value in group-I (p>0.05).
§ Significant difference compared to corresponding value in group-II (p<0.05).
‡ Insignificant difference compared to corresponding value in group-II (p>0.05).
Fig. (6): Effect of oral vitamin E “200 \( \mu \)g/kg/day for 10 days” on the mean heart rate of isolated hyperthyroid rabbits’ hearts compared to different studied groups (n=10).

# Significant difference compared to pre-ischemic value within the same group (\( p<0.05 \)).
* Significant difference compared to corresponding value in group-I (\( p<0.05 \)).
‡ Insignificant difference compared to corresponding value in group-I (\( p>0.05 \)).
* Significant difference compared to corresponding value in group-II (\( p<0.05 \)).
‡ Insignificant difference compared to corresponding value in group-II (\( p>0.05 \)).
§ Insignificant difference compared to corresponding value in group-II (\( p>0.05 \)).

Fig. (7): Effect of oral vitamin E “200 \( \mu \)g/kg/day for 10 days” on the mean left ventricular developed pressure (LVDP) of isolated hyperthyroid rabbits’ hearts compared to different studied groups (n=10).

# Significant difference compared to pre-ischemic value within the same group (\( p<0.05 \)).
* Significant difference compared to corresponding value in group-I (\( p<0.05 \)).
‡ Insignificant difference compared to corresponding value in group-I (\( p>0.05 \)).
* Significant difference compared to corresponding value in group-II (\( p<0.05 \)).
‡ Insignificant difference compared to corresponding value in group-II (\( p>0.05 \)).
§ Insignificant difference compared to corresponding value in group-II (\( p>0.05 \)).

Fig. (8): Effect of oral vitamin E “200 \( \mu \)g/kg/day for 10 days” on the mean \( \frac{dp}{dt}_{max} \) of isolated hyperthyroid rabbits’ hearts compared to different studied groups (n=10).

# Significant difference compared to pre-ischemic value within the same group (\( p<0.05 \)).
* Significant difference compared to corresponding value in group-I (\( p<0.05 \)).
‡ Insignificant difference compared to corresponding value in group-I (\( p>0.05 \)).
§ Insignificant difference compared to corresponding value in group-II (\( p>0.05 \)).

Fig. (9): Effect of oral vitamin E “200 \( \mu \)g/kg/day for 10 days” on the mean Malondialdehyde (MDA) of isolated hyperthyroid rabbits’ hearts compared to different studied groups (n=10).

Data are mean \( \pm \) SD.
* Significant difference compared to corresponding value in group-I (\( p<0.05 \)).
‡ Insignificant difference compared to corresponding value in group-I (\( p>0.05 \)).
§ Insignificant difference compared to corresponding value in group-II (\( p>0.05 \)).

Fig. (10): Effect of oral vitamin E “200 \( \mu \)g/kg/day for 10 days” on the mean cGMP of isolated hyperthyroid rabbits’ hearts compared to different studied groups (n=10).

Data are mean \( \pm \) SD.
* Significant difference compared to corresponding value in group-I (\( p<0.05 \)).
‡ Significant difference compared to corresponding value in group-II (\( p<0.05 \)).
Table (1): Effect of I.M. T 4 in a dose of 200 µg/kg/day for 8 days on the mean rabbits’ body and heart weights compared to different studied groups (n=10).

<table>
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<tr>
<th>Parameters</th>
<th>Group-I &quot;Saline&quot;</th>
<th>Group-II &quot;T4&quot;</th>
<th>Group-III &quot;Vitamin E&quot;</th>
<th>Group-IV &quot;Vitamin E + T4&quot;</th>
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<tr>
<td>Mean body weight (BW) (kg):</td>
<td>After therapy</td>
<td>Before therapy</td>
<td>After therapy</td>
<td>Before therapy</td>
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<tr>
<td></td>
<td>2.24±0.1</td>
<td>2.15±0.17</td>
<td>2.25±0.3‡</td>
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<td>% change in the mean BW</td>
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<td></td>
<td></td>
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<td>(after therapy) to compared:</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Before therapy within the same group</td>
<td>+4.2%†</td>
<td>-13.4%#</td>
<td>+44.3%†</td>
<td>-13.1%#</td>
</tr>
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<td>Group-I</td>
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<td>-12.9%*</td>
<td>-1.8%‡</td>
<td>-14.7%*</td>
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<tr>
<td>Group-II</td>
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<td>+12.8%‡</td>
<td>-2.0%§</td>
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<tr>
<td>Mean heart weight (HW) (g)</td>
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<td>9.5±1*</td>
<td>8.7±0.9†</td>
<td>9.5±1.2*</td>
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<tr>
<td>% change in HW/BW ratio</td>
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<td>compared to group-II</td>
<td>+32.4%*</td>
<td>+46.7%‡</td>
<td>+35.1%*</td>
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<td>% change in HW-BW ratio</td>
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<td>-19.4%z</td>
<td>+2.04%§</td>
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<td>compared to group-II</td>
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</table>

Data are presented as mean ± SD and percent change. † Insignificant difference before and after therapy values within the same group (p>0.05). # Insignificant change compared to group-I (p<0.05). § Insignificant difference compared to the corresponding value in group-II (p<0.05). ¶ Insignificant change compared to group-II (p<0.05).

Table (2): Effect of oral vitamin E "200 µg/kg/day for 10 days" on the mean cardiac function parameters of hearts isolated from hyperthyroid rabbits 'injected I.M. with T 4 in a dose of 200 µg/kg/day for 8 days' before & after 30min. no-flow ischemia compared to different studied groups (n=10).

<table>
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<th>Parameters</th>
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<th>25min after repertusion</th>
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<td>5±0.8</td>
<td>3.9±0.5</td>
<td>2.8±0.4</td>
</tr>
<tr>
<td>Coronary flow (ml/min)</td>
<td>Mean</td>
<td>12.7±1.1</td>
<td>3.4±0.5</td>
<td>2.7±0.4</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>Mean</td>
<td>10.2±1.8</td>
<td>5.3±1.0</td>
<td>5.9±1.0</td>
</tr>
<tr>
<td>LVDP (mmHg)</td>
<td>Mean</td>
<td>12.3±1.3</td>
<td>4.8±0.7</td>
<td>4.6±0.8</td>
</tr>
<tr>
<td>dP/dt (mmHg/sec) x 10^2</td>
<td>Mean</td>
<td>40±3.0</td>
<td>21.9±2.4</td>
<td>45.2%±2.0</td>
</tr>
</tbody>
</table>

Data are mean ± SD. * Significant difference compared to pre-ischemic value within the same group (p<0.05). ** Significant difference compared to the corresponding value in group-I (p<0.05). † Insignificant difference compared to the corresponding value in group-I (p<0.05). § Insignificant difference compared to the corresponding value in group-II (p<0.05). ¶ Insignificant change compared to group-II (p<0.05). HR = Heart rate. LVDP = Left ventricular developed pressure. dP/dt = Δ Pressure/Δ tension.
Discussion

The present work was conducted to study the effect of vitamin E on the hyperthyroid rabbits’ isolated hearts’ response to ischemia-reperfusion. The hyperthyroid state of T4-treated animals was reflected in their higher heart/body weight ratios. This result was supported by Takeuchi et al. [29] who stated that thyroid hormone causes hypertrophy of the myocardium. The present study showed that hearts from hyperthyroid rabbits in group-II had a significant increase in the mean heart rate (HR), either before or after 25 min. ischemia-reperfusion (I/R) in comparison to that of control group-I, or treated with vitamin E alone in groups-III. Administration of vitamin E in hyperthyroid rabbits in groups-IV significantly reduced the mean HR either before or after 25 min. ischemia-reperfusion compared to hyperthyroid rabbits in group-II. These results were in agreement with studies of Venditti et al. [30] and Masullo et al. [15] who found that hyperthyroid hearts displayed significant tachycardia during reperfusion. The fact that the tachycardic response during reperfusion of hyperthyroid hearts was due to a major oxidative stress. These findings could confirm the present work reduction in the mean HR induced in vitamin E treated hyperthyroid group-IV, in comparison to hyperthyroid groups-II, and this reduction might be due the antioxidant property of vitamin E. There was strong evidence that the higher oxidative stress inducing tachycardia during reperfusion in the hyperthyroid rats was associated with nitric oxide synthase (NOS) activity with possible overproduction of nitric oxide. The perfusion of hearts with the NOS inhibitor L-NNA, N (omega)-nitro-L-arginine, allowed preventing the tachycardic response [31]. These findings might explain the significant increase in the mean HR after 25 min. reperfusion compared to the pre-ischemic value within hyperthyroid group-II of the present study that was associated with a significant increase in the mean c.GMP reflecting the increase in NOS. Also, the present study revealed significant reduction in c.GMP level in vitamin E treated hyperthyroid group-IV compared to hyperthyroid group-II that might explain the significant reduction in HR induced by vitamin E in hyperthyroid group-IV compared to hyperthyroid group-II.

The mean pre-ischemic contractility parameters (amplitude of contractions, LVDP and dP/dt max) of hearts isolated from hyperthyroid groups II and IV significantly increased in comparison to control group-I, this result might be due to the hyperdynamic state of hearts with hyperthyroidism and this effect is related to the positive inotropic effects of thyroxin on the heart [9]. Also, the present study resulted in increase heart weight/BW ratio indicating increased cardiac mass in hyperthyroid groups II and IV that certainly [32] played a role in the increased pre-ischemic cardiac contractility [32]. Moreover, the increase in pre-ischemic contractility parameters in hyperthyroid groups II and IV might be due to the inhibitory effect of thyroid hormone on adult rabbits’ ventricles expression of Na +,Ca 2+ exchanger (NCX), an antiporter membrane protein which removes calcium from cells and sarco (endo) plasmic reticulum "SR" calcium ATPase (SERCA2a), which transfers Ca 2+ from the cytosol of the cell to the lumen of the SR. Hypothyroidism resulted in sustained high levels of NCX expression [33, 34]. Takeuchi et al. [29] found inhibited SERCA2a function in hyperthyroid heart during the ischemia-reperfusion process.

On the other hand, the mean amplitude of contractions, LVDP and dP/dt max after 25 min. (I/R) significantly reduced in hyperthyroid group-II compared to control group-I or vitamin E treated groups either alone in group-III or with hyperthyroid rabbits in group-II. This result was supported by Takeuchi et al. [29] who found that thyroid hormone causes hypertrophy of the myocardium. The present study showed that hearts from hyperthyroid rabbits in group-II had a significant increase in the mean heart rate (HR), either before or after 25 min. ischemia-reperfusion (I/R) in comparison to that of control group-I, or treated with vitamin E alone in groups-III. Administration of vitamin E in hyperthyroid rabbits in groups-IV significantly reduced the mean HR either before or after 25 min. ischemia-reperfusion compared to hyperthyroid rabbits in group-II. These results were in agreement with studies of Venditti et al. [30] and Masullo et al. [15] who found that hyperthyroid hearts displayed significant tachycardia during reperfusion. The fact that the tachycardic response during reperfusion of hyperthyroid hearts was due to a major oxidative stress. These findings could confirm the present work reduction in the mean HR induced in vitamin E treated hyperthyroid group-IV, in comparison to hyperthyroid groups-II, and this reduction might be due the antioxidant property of vitamin E. There was strong evidence that the higher oxidative stress inducing tachycardia during reperfusion in the hyperthyroid rats was associated with nitric oxide synthase (NOS) activity with possible overproduction of nitric oxide. The perfusion of hearts with the NOS inhibitor L-NNA, N (omega)-nitro-L-arginine, allowed preventing the tachycardic response [31]. These findings might explain the significant increase in the mean HR after 25 min. reperfusion compared to the pre-ischemic value within hyperthyroid group-II of the present study that was associated with a significant increase in the mean c.GMP reflecting the increase in NOS. Also, the present study revealed significant reduction in c.GMP level in vitamin E treated hyperthyroid group-IV compared to hyperthyroid group-II that might explain the significant reduction in HR induced by vitamin E in hyperthyroid group-IV compared to hyperthyroid group-II.
In the present work, we observed a significant increase in cGMP, which reflected increased NO production, that was associated with a significant reduction in isolated cardiac contractility parameters in hyperthyroid group-II compared to control group-I. It has been proposed that many of the deleterious effects of NO with free radical on myocardial contractility parameters are mediated by the formation of peroxynitrite (ONOO). Levrand et al. [40] postulated that ONOO triggers apoptosis in cardiomyocytes in vitro and in the myocardium in vivo. ONOO is a strong biological oxidant and nitrating species formed from the near diffusion-limited reaction of the free radicals with nitric oxide and superoxide anion [41]. It has been documented that ONOO formation represents a major mechanism of myocardial injury in various cardiac pathologies including myocardial infarction and chronic heart failure [42]. ONOO may cause myocardial cytotoxicity through direct oxidative damage to lipids, proteins and DNA [43], activation of metalloproteinases [44], and the nitration of tyrosine residues within proteins [45]. One major pathway of ONOO dependent myocardial cytotoxicity relies on oxidative DNA damage and activation of the nuclear enzyme poly (ADP-ribose) polymerase (PARP), which consumes cellular nicotinamide dinucleotide (NAD) and adenosine triphosphate (ATP), leading to cell necrosis [46]. So that in the present work, administration of vitamin E in hyperthyroid group-IV showed a significant increase in isolated cardiac contractility parameters compared to hyperthyroid group-II might be due to vitamin E antioxidant effect that decrease free radical and hence ONOO production.

A further explanation for reduced isolated cardiac contractility parameters in hyperthyroid group-II is due to increase in lipid peroxidation which was reflected by a significant increase in cardiac MDA level in hyperthyroid group-II compared to control group-I. This result is in agreement with Chehade et al. [47] who found that MDA was increased in hearts isolated from hyperthyroid rats compared to euthyroid ones. The reduction of the free radical scavenger system and the increase in lipid peroxidation activities might induce myocardial dysfunction [48]. Masullo et al. [15] stated that the higher reperfusion-induced injury occurring in hyperthyroid animals is due to increase in lipid peroxidation. Furthermore, Venditti et al. [30] found that lipid peroxidation and the susceptibility to in vitro oxidative stress were higher in the T3 treated than in euthyroid rats. Also, Araujo et al. [49] observed increased lipid peroxidation in isolated hyperthyroid rats’ hearts. So that, the significant improvement in isolated cardiac contractility after...
vitamin E administration in hyperthyroid group-IV compared to hyperthyroid group-II, as vitamin E is the most important non-enzymatic natural lipid-soluble chain breaking antioxidant in tissue [50,51]. It protects against lipid peroxidation by acting directly with a variety of oxygen radicals to form a relatively innocuous tocopherol radical [52]. A further protective effect of vitamin E against oxidative stress in isolated hyperthyroid hearts might be via prevention H2O2 induced apoptosis in non-infarcted myocardial cells with prevention of mitochondrial apoptosis initiators (cytochrome c “Cyto c” and caspase-3) [53] release and activation [54]. These findings indicated that antioxidant vitamins reduce myocyte apoptosis mediated via inhibition of mitochondrial pathway [55].

Moreover, vitamin E induced improvement in isolated cardiac contractility of hyperthyroid group-IV may be due to its vasodilator effect which appeared in the significant increase in coronary effluent in vitamin E treated hyperthyroid group-IV compared to hyperthyroid group-II. This in agreement with Plantinga et al. [56] who proved that vitamin E has beneficial effects on endothelium-dependent vasodilation and arterial stiffness in untreated, essential hypertensive patients. Kugiyama et al. [57] stated that the increase in oxidative stress may at least partly contribute to endothelial vasomotor dysfunction. So that increase in coronary effluent in the present work might be due to vitamin E ability to restore normal endothelial function and to reduce vascular superoxide anion formation [58] and hence improved endothelium-dependent vasodilation [59].

Conclusion:

These results suggested that after exposure of isolated hyperthyroid rabbits’ hearts to ischemia-reperfusion, as a model for oxidative stress, oral vitamin E could improve the contractility parameters, coronary flow as well as tachycardic response to reperfusion in addition to the improvement of the cardiac capability to face oxidative stresses in hyperthyroidism. This might need a further clinical study to prove the role of vitamin E in preventing ischemic cardiac dysfunction in patients with thyrotoxicosis.

References

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