Evaluation of the Protective Role of Selenium on Oxidative Stress, some Physiological Parameters and Myocardial function In Thyroid Disturbance Adult Male Rats

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Abstract

This study is designed to evaluate the ameliorative effect of selenium on oxidative stress, some physiological parameters and myocardial function of adult male rats disturbance thyroid gland.

Hypothyroidism state was induced by administration of antithyroid drug propylthiouracil (PTU) (50mg/kg.B.W.) and hyperthyroid state was induced by administration L-Thyroxine(L-T_4) (50μg/kg.B.W.). Selenium (Se) treated rat group was given sodium selenite (10 μg/kg.B.W.).

Sixty adult male rats of (180-200 gm) body weight were used in this study and divided into six main groups. Control group, PTU treated group, L-T_4 treated group, Se treated group, Se+PTU treated group and L-T4 treated group. All these groups were drenched orally by cavage tube for two months.

At the end of the experiment period, the rats euthanized via chloroform blood was collected in order to serum lipid profile concentrations including Total cholesterol (T-Ch) triglyceride concentration (TG), High density lipoprotein (HDL), Low density lipoprotein (LDL), Very low density lipoprotein (VLDL) concentrations. Also PLT, PCT, MPV, P-LCR, prothrombine time, activated Partial thromboplastin time and fibrinogen of male rats.

The results showed a significant decrease in MPV and P-LCR of male rats treated with PTU and L-T_4. A significant increase in PLT and PCT of male rats treated with PTU and L-T_4. A significant decrease in prothrombine time, activated Partial thromboplastin time and fibrinogen of male rats treated with PTU compared with control group and Se,Se+PTU,Se+L-T_4 groups. A significant increase prothrombine time, activated Partial thromboplastin time and fibrinogen of male rats treated with L-T_4

compared with control group and Se, Se+PTU, Se+L-T_4 groups. In contrast, Lipid profile showed a significant ($P<0.05$) decrease of total cholesterol, low density lipoprotein and very low density lipoprotein while a significant increase of high density lipoprotein concentration in Se group. Whereas PTU group registered a significant increase in T.Chol, TG, LDL and VLDL concentrations and a significant ($P<0.05$) decrease of HDL concentration compared with control group. Also L-T_4 group registered a significant increase in LDL and VLDL concentrations and a significant ($P<0.05$) decrease of HDL concentration compared with control.

Different histological changes obtained in the heart of thyroid disturbant male rats oedema, RBCs and few neutrophils infiltration between cardiac muscle in addition to vascular degeneration of muscle cells, but normal architecture of heart was observed in Se-treated group.

Introduction

Thyroid hormones perform a wide metabolic functions including lipid regulation and the metabolism of carbohydrate, protein, electrolyte and mineral. Mobilization of triglyceride from the adipose tissue and increasing free fatty acids in the plasma is the most important effect on lipid metabolism.

Thyroid dysfunction has a great effect on lipids and other cardiovascular risk factors. Hypothyroidism is common and is accompanied with unfavorable effect on lipids(Rizos *et al*.,2011). Thyroid disorder can have obvious effect on lipid profile (Liberopoulos and Elisaf,2000).

Thyroid abnormalities are associated changes in the intermediate metabolism which include alteration in body weight and lipid profile. Marked decreases in TC, HDL, LDL-C, VLDL-C and triglyceride levels were observed in hyperthyroidism rats (Soliman,2013). Whereas Newairy *et al*.,(2007) showed that selenium administration to caused decrease in the concentration of most different lipid fractions, whereas the total mono and polyunsaturated fatty acids concentration showed increases in the lipid fractions. Serum concentrations were found to be inversely associated with coronary heart disease and dilated cardiomyopathy in observational studies (Flores-Mateo *et al*.,2006).

Material and Methods

This study was carried out on sixty adult male rats (*Rattus norvegicus*) weighing (180-200gm), at the beginning of the study The rats were housed in the animal house of College of Veterinary Medicine / University of Basrah.

They housed with meta covers measuring (15× 35×50) and had a bedding of fine wood which was changed twice a week. Rats were housed in these plastic cages with food and drinking *ad libitum* under 20-25°C controlled temperature condition and alternate 12 hours light/dark period.

Animals were divided into six main groups, 10 rats in each group. Control group treated with normal saline, second group treated 50 mg/Kg B.W. of PTU dissolve in 1ml of normal saline, third group treated50 µg/Kg B.W. of L-T_4 dissolve in 1ml of normal saline , fourth group treated 10 µg/Kg B.W. of sodium selenite dissolve in 1ml of normal saline, fifth group treated with both PTU+Se and the sixth group treated with both L-T_4 +Se and all these treatments were drenched to rats orally by agavage for two months.

Blood was collected from each rat by heart(cardiac puncture). Then blood samples were deposited into the tube without anticoagulant and after that the blood samples were centrifuged at (3000 rpm) for 15 minutes and serum samples were divided into two parts and stored in polyethylene eppendroff tubes at (-20ºC) in order to measure lipid profile. Also plasma was taken after deposition of blood samples in sodium citrated tubes and blood centrifugation at 3000 rpm for 15 minute in order to measure prothrombin time, activated partial thromoboplastin time and fibrinogen concentration. The remaining (2ml) of blood was deposited into the tube with anticoagulant used for hematological analysis (PLT,MPV, PCT and P-LCR).

Total cholesterol concentration estimated according to method described by (Allain *et al*., 1974). Estimation of triglyceride was done by method described by Bucolo and David(1973). Also serum LDL-C concentration calculated according to (Ram,1996). Whereas, very low-density lipoprotein concentration was calculated by dividing serum triglyceride by five (Bruits and Ashwood, 1999) and all these methods was done by using special kits.

Histology examination.

After removing the heart were immediately fixed in Bouin's fluid for 12 hours and the Bouin's fixative was washed from the samples with 70% alcohol. The tissues were then cut in slabs of about 0.5cm transversely and the tissues were dehydrated by passing through different grades of alcohol: 70% alcohol for 2 hours, 95% alcohol for 2 hours, 100% alcohol for 2 hours, 100% alcohol for 2 hours and finally 100% alcohol for 2 hours. The tissues were then cleared to remove the alcohol; the clearing was done for 6 hours using xylene. The tissues were then filtrated in molten Paraffin wax for 2 hours in an oven at 57°C, thereafter the tissues were embedded. Serial sections were cut using rotary microtone at 5 microns (5μm). The satisfactory ribbons were picked up from a water bath (50 -55°C) with microscope slides that had been coated on one side with egg albumin as an adhesive and the slides were dried in an oven. Each section was deparaffinized in xylene for 1minute before immersed in absolute alcohol for 1 minute and later in descending grades of alcohol for about 30seconds each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 minutes. The slides were rinsed in water, then differentiated in 1% acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 seconds and rinsed in water for a few seconds, before being immersed in 70%, 90% and twice in absolute alcohol for 30 seconds each to dehydrate the preparations. The preparations were cleared of alcohol by dipping them in xylene for 1 minute. Each slide was then cleaned, blotted and mounted with DPX and cover slip, and examined under the microscope.Photomicrographs were taken at40X, 100X and 400Xmagnifications(Bancroft *et al*.,1990 and Luna,1993).

Stastistical Analysis:- It was performed by one-way covariance (ANOVA) test. Data were expressed as mean ± SD. Statistical significance was set at ($P \leq 0.05$).The SPSS (statistical packages for the social sciences) program V. 21 were used.(SPSS,2017)

Result

١-Effect of Propylthiouracil, Thyroxine, Selenium alone, Co-treatment with PTU+Se and L-T_4+Se on Lipid Profile in Male Rats.

The obtained results in Table (1) revealed significant (P≤0.05) increase of total cholesterol, triglyceride, LDL and VLDL in serum of males rats treated with PTU compared with the control group and another groups while HDL showed no significant(P≤0.05) differences in serum of males rats treated with PTU compared with the control group and another groups.

Table(١):Effect of PTU, L-thyroxine, Selenium alone, Co-treatment with PTU+Se and L-T4+Se on Lipid Profile in Male Rats. Mean±SD N=10

Parameters / Treatment	Total Cholesterol mg/dl	Triglyceride mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Control (Normal saline 0.9%NaCl)	61.62±13.23 bc	48.81±3.00 a	39.00±1.82 bc	28.47±4.95 b	12.40±2.21 b
Propylthiouracil	154.33±59.11 a	55.55±17.46 a	33.66±13.77 bc	47.66±8.77 a	21.66±2.33 a
L-Thyroxin Sodium	47.47±12.61 c	36.68±10.85 ab	30.14±8.83 c	52.00±11.88 a	23.33±2.42 a
Sodium Selenite	80.71±6.06 b	48.35±7.06 a	48.66±3.82 ab	23.32±5.29 b	9.97±1.21 b
Selenium+Propylthiouracil	85.39±7.10 b	30.20±8.93 b	42.08±2.38 b	30.50±3.93 b	11.16±3.43 b
Sellenium+ Thyroxine	75.20±12.22 b	47.81±20.67 a	55.36±11.46 a	24.76±11.72 b	11.62±2.94 b

N=number of animals., small letters denote differences between groups,P≤0.05 vs. control, NS=non-significant.

2-Effect of Propylthiouracil, Thyroxine, Selenium alone, Co-treatment with PTU+Se and L-T_4+Se on Platelet Morphological Parameters in Male Rats.

The obtained results in Table (2) revealed significant(P≤0.05) increase in platelets count and PCT in blood of males rats treated with PTU and L-T_4 compared with control group and another treated groups while no significant (P≤0.05) difference in platelets count and PCT in blood of males rats treated with Se compared with the control group.

The results of MPC showed significant(P≤0.05) decrease in male rats treated with PTU and L-T_4 compared with the control group.

The results of P-LCR showed significant($P \leq 0.05$) increase in male rats treated with L-T_4 compared with the control group while P-LCR showed no significant ($P \leq 0.05$) difference in males rats treated with PTU compared with the control group.

Table (2): Effect of PTU, L-T_4, Selenium,Co-treatment with PTU+Se and Co-treatmentwith L-T4+Se on Platelet Morphological Parameters in Male Rats. Mean±SD N=10

Parameters / Treatment	PLT $10^3/\mu L$	MPV fl	PCT %	P-LCR
Control (Normal saline 0.9%NaCl)	412.5±20.45 b	8.71±1.02 a	5.87±1.20 b	14.68±0.47 c
Propylthiouracil	544.0±51.28 a	7.08±0.11 b	6.98±0.15 a	16.90±0.52 bc
L-Thyroxin Sodium	528.0±34.95 a	7.73±0.58 b	6.89±0.49 a	18.48±1.71 b
Sodium Selenite	293.67±15.37 c	6.61±0.30 b	4.55±0.87 c	18.20±1.26 b
Selenium+Propylthiouracil	454.67±37.38 b	7.56±0.54 b	5.76±0.81 b	19.10±4.52 ab
Sellenium+ Thyroxine	308.50±23.78 c	6.88±0.30 b	3.83±0.80 c	20.95±0.51 a

N=number of animals., small letters denote differences between groups,P≤0.05 vs. control, NS=non-significant.

3-Effect of Propylthiouracil, Thyroxine, Selenium alone, Co-treatment with PTU+Se and L-T_4+Se on Prothrombin Time, Activated Partial Thromboplastine and Fibrinogen in Male Rats.

The obtained results in Table (3) revealed significant($P \leq 0.05$) decrease in prothrombin time, activated partial thromboplastin and fibrinogen of males rats treated with PTU compared with the control group and another treated groups while no significant difference in prothrombin time, activated partial thromboplastin and fibrinogen of males rats treated with Se compared with control group. While the result of prothrombin time, activated partial thromboplastin and fibrinogen in males rats treated with L-T_4 revealed significant($P \leq 0.05$) increase compared with control group, Se group alone and another treated groups.

Table (3):Effect of PTU, L-T_4, Selenium,Co-treatment with PTU+Se and Co-treatmentwith L-T4+Se on Prothrombin Time, Actived Partial Thromboplastine Time and Fibrinogen in Male Rats. Mean±SD N=10

Parameters / Treatment	Prothrombine Time (Second)	Activated Partial ThromboplastinTime (Second)	Fibrinogen (mg/dl)
Control (Normal saline 0.9%NaCl)	23.13±6.07 b	60.00±11.83 b	260.43±24.75 b
Propylthiouracil	15.70±1.19 c	32.71±4.58 c	191.00±17.66 c
L-Thyroxin Sodium	39.10±0.58 a	96.63±7.42 a	350.17±14.89 a
Sodium Selenite	20.78±0.11 b	77.50±54.43 b	250.77±14.01 b
Selenium+Propylthiouracil	22.45±1.76 b	72.13±53.63 b	220.77±12.20 b
Sellenium+ Thyroxine	21.58±0.33 b	83.21±14.45 ab	240.75±4.70 b

N=number of animals., small letters denote differences between groups,P≤0.05 vs. control, NS=non-significant.

4- Histological examination

4.1-Heart:-

The heart of **control** group rats appeared normal heart tissue with normal architecture and normal cardiac fiber as shown in figure (1). Also heart of male rats treated with **Se** revealed normal architecture of cardiac muscle as shown in figure (2). While heart of male rats treated with **PTU** appeared histopathological changes. The changes included oedema as shown in figure(3). In addition, heart of rats treated with **L-T_4** revealed histopathological changes such as RBCs and few neutrophils infiltration between cardiac muscle bundle, in addition to vacuolar degeneration of muscle cells as shown in figure (4). Moreover the heart of rats treated with **PUT+Se** showed normal architecture of cardiac muscle and this section is ameliorative than heart treated with **PTU** alone as shown in figure (5). Also heart of rats treated with **L-T_4+Se** revealed normal architecture of cardiac muscle which can be distinguished, this section is ameliorative than heart treated with **L-T_4** alone as shown in figure (6).

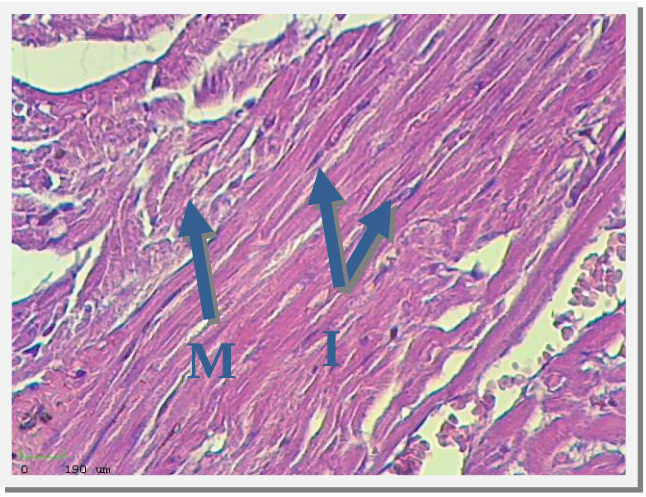

Fig.(1):- Section of the heart of control group male rats. Showing normal myocardial muscle cells, normal architecture, normal cardiac fiber and present intercalated disc(I), stain (H&E) 400X.

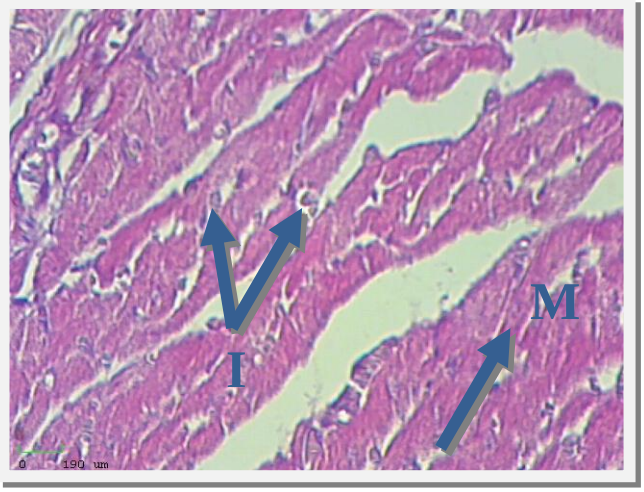

Fig.(2):- Section of the heart of rats treated with Se. Showing normal myocardial muscle cells(M), normal architecture, normal cardiac fiber, and present intercalated disc(I), stain (H&E) 400X.

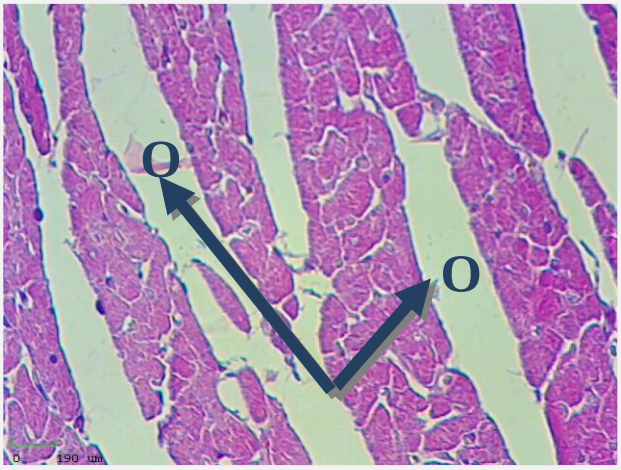

Fig.(3):- Section of the heart of rats treated with PTU. Showing interstitial oedema(O), occasional vacuolated myocardial muscle cells and subpericardial fibrosis, stain (H&E) 400X.

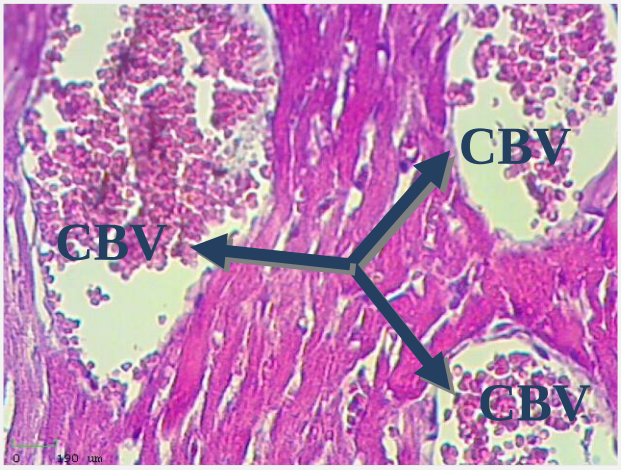

Fig.(4):- Section of the heart of rats treated with L-T_4. Showing congested small blood vessels between myocardial muscle cells (CBV) in some pericardial region and minimal fibrosis in subpericardial region, stain (H&E) 400X.

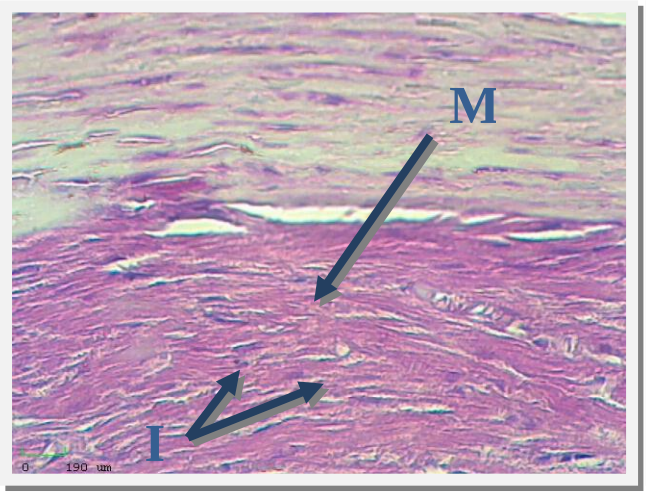

Fig.(5):- Section of the heart of male rats treated with PTU+Se. Showing normal myocardial muscle cells(M), normal architecture, normal cardiac fiber and present intercalated disc(I), stain (H&E) 400X.

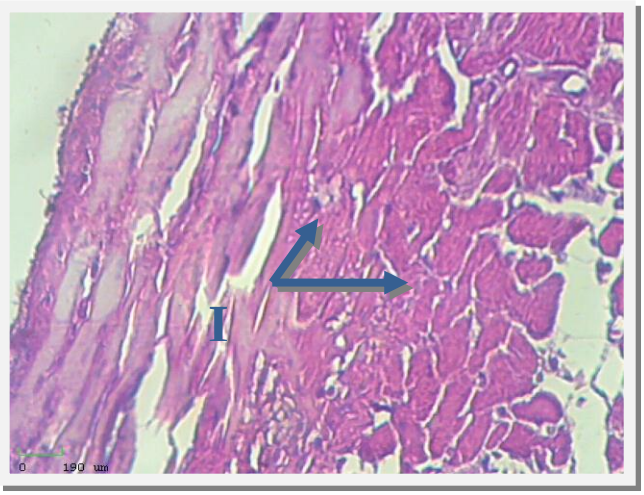

Fig.(6):- Section of the heart of male rats treated with L-T_4+Se. Showing normal heart tissue normal architecture, normal cardiac fiber and present intercalated disc(I), stain (H&E) 400X.

Discussion

This study indicate the thyroid disturbance affect on lipid profile, platelet count, anticoagulant and cardiovascular system. Thyroid disturbance also affected on selenium concentration. When selenium deficiency lead to increases in serum total cholesterol, low density lipoprotein, apolipoprotein A, apolipoprotein B concentrations and possibly triglyceride concentrations were observed in patients with hypothyroidism (Pearce,2004) which confirm our result. Also Murgod and Soans(2012) had found that TC, TG, LDL-C concentrations were increased in hypothyroidic patients than control group.

Marked decrease in HDL-C level was observed in hyperthyroid rats (Saliman,2013). In addition, Khan *et al*.,(2014) concluded that HDL-C reduced in hyperthyroid patient and these results are coordinated with our present study. Whereas (Saliman,2013) and Rizos *et al*.,(2011) found that TG, LDL-C, VLDL-C were decreased in hyperthyroidism which are disagree with our results. Also selenium deficiency lead to increased platelet aggregation and an altered arachidonic acid metabolism.

Latheef *et al*.,(2014) showed that 1mg of selenium supplementation elevated serum lipid profile in rabbit and this disagree with our present study except for HDL-C level which increased in our study. Lower tissue selenium status has been linked to an

increased risk of several aging – related diseases, including cardiovascular disease (Rijin *et al*.,2014). The essential trace element selenium (Se) is crucial for many biological functions including thyroid hormone metabolism, the body's antioxidant defense systems, the adaptive and acquired immune system and prevention of certain cancers. Accumulating evidence suggests that selenium is also of importance for optimal functioning of the cardiovascular system.

Animal studies have also demonstrated that cardiovascular benefits of selenium, where dietary Se supplementation prior to ischemia- reperfusion injury resulted in improved cardiac functional recovery, reduced incidence of reperfusion arrhythmias, and preservation of ventricular ultra-structure (venardos *et al*.,2004)

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