

Effect of alcoholic leaf extract of *Heliotropium europium* on the levels of Testosterone , Prolactin ,TSH ,T3 and T4 hormones in Blood of male albino rats exposed to oxidative stress by H2O2

Sahib, J. Abdoul Rahman

drsahib60@gmail.com

Department of Biology - College of Science – Tikrit University- Iraq

Abstract

The current study aimed to compare the effects of alcoholic leaf extract of *Heliotropium europium* and Vit. C as antioxidants on levels of hormones Testosterone, Prolactin, TSH, T3 and T4 in blood of adult albino male rats exposed to oxidative stress by hydrogen peroxide H₂O₂ (0.5%) in the drinking water throughout 30 days. For the purpose of the study preliminary experiments were carried out to find the most effective dose of plant extracts and it was found that (300mg / kg / b.w). Rats (3-4 aged months) and weight (250-285g) were distributed into four groups: H₂O₂+ *Heliotropium europium* alcoholic leaf extract group, H₂O₂+ Vit. C group, H₂O₂+ *Heliotropium europium* alcoholic leaf extract+Vit.C group. The plant extract of leaves as (300) mg / kg and Vit.C (250) mg / kg of body weight in a single daily dose. The results showed that the animals exposed to oxidative stress induced by hydrogen peroxide led to a significant decrease ($p \leq 0.05$) in the levels of serum Testosterone, Prolactin and T3 and non significant difference in the TSH and T4 levels when compared with control. The treatment with H₂O₂+ plant extract showed a significant increase in the Testosterone level and a significant decrease in T3 and T4 levels, and lack of significant difference in the levels of Prolactin and TSH, while the treatment of group H₂O₂+Vit.C led to a significant increase in testosterone, PRL and TSH and a significant decrease in T3 and T4. While the group exposed to stress and treated with plant extract and Vit.C showed a significant increase in testosterone, prolactin levels and significant decrease in TSH and T4 levels with non significant difference in the T3 level when compared with hydrogen peroxide group.

Key Words : *Heliotropium europium*, H₂O₂, Free radicals, oxidative stress , Testosterone , Prolactin ,TSH.

hormones: Testosterone , Prolactin , Triiodothyronine (T₃), Tetraiodothyronine (T₄) and Thyroid Stimulating Hormone (TSH) in the blood of male adult albino rats exposed to oxidative stress.

Materials and Methods

This study used male albino rats weights (250 - 285)g and age ranges between (12-16)weeks obtained from Faculty Medicine / University of Mosul and housed in animal house of Biology Department in Tikrit University where placed in metal cages at (3±22) C⁰ with normal light cycle (12 h light and 12 h dark), Animals were fed with standard laboratory diet[15] and water was given ad libitum during the experiment period from November 2011 to February 2012.

Determination the effect dose of plant extract :

For the purpose of the study preliminary experiments were carried out to find the perfect effective dose of plant extracts and it was found that (300mg / kg) the most effectous.

Experimental Design :

Rats were distributed randomly into six groups (each group contain 5) animals as follows:

1-Control group (untreated group): were given food and water continuously

throughout the duration of the experiment.

2-Hydrogen Peroxide group : Given (0.5%) of hydrogen peroxide in drinking water for 30 days.

3- Hydrogen Peroxide and plant extract group : given hydrogen peroxide as in the second group with dosage with alcoholic extract of *Heliotropium europium* leaves (300mg/kg)for 30 days.

4- Hydrogen Peroxide and vitamin C :this group was given hydrogen peroxide as in the second treatment with the dosage of vitamin C (250mg/kg)for 30 days .

5-Hydrogen Peroxide and plant extract with Vitamin C :this group were given 0.5% H₂O₂ as in the second treatment with dosage by alcoholic extract of *Heliotropium europium* leaves (300mg/kg) and Vitamin C (250mg/kg)for 30 days .

Blood sampling :

At the end of 30 days of treatment and administration period, the animals were fasted overnight after which they were painlessly sacrificed by decapitation under chloroform anaesthesia and the blood (6-8mls) collected by cardiac puncture ,the blood put in tubes without anticoagulant and left in the laboratory temperature for (15) minutes then it was separated by centrifuge and sera save at (-20) C⁰.

Hormonal assay:

Introduction

Free radicals (FR) are atoms or molecules which contain a single electron or more than one electron in their external orbit , they were produced in living cells especially in mitochondria and they are either reactive oxygen species or reactive nitrogen species[1]), while oxidative stress represents as situation that increases the production of free radicals and oxidants in body so that exceeds the ability of antioxidants to remove or minimize their effects, leading to the formation of oxidative damage to cells and tissues and thus lead to initiation of various diseases in humans, such as cancer , diabetes , cardiovascular diseases, arthritis[2]. and disorder in hormone levels lead to male infertility[3]. For reducing to free radicals activity and damages caused by them there are complex systems of multiple models for antioxidants maintained by man and animals ,some of them are exogenous and another are endogenous and they are a reducing substances. Their activity represent by inhibition or ending reactions that electrons moved from a certain material to oxidant factor and that leading to production of intermediate free radicals[4].

Human have used plants and medicinal herbs during the thousands of years on earth in search of food and heal their pains, that's where the use of herbs and medicinal plants are an important tool in the treatment of many diseases as most currently known drugs are from plant sources[5] Recently, The interest has increased by using some plant extracts in the treatment of many diseases such as cancer, diabetes, arthritis, hormonal and nervous system disorders

because they give a better results than manufactured drugs and lacking of their harmful side effects[6].

The Plants of Boraginaceae family such as *Heliotropium europium* which have a great biological importance because they contain a many of active compounds, include alkaloids as pyrrolizidine and Acetyl lasiocarpine in the entire plant [7] and Alkaloids in leafs as lasiocarpine, heliotrine [8,9] and indicine-N-Oxide wich acts as anticancer and antioxidants [10] and contain types of Glycosides as Qurencetin , Isorhamntine, heliotrope and Isorhamntin [11]in addition to Flavonoids as Narigrnin[12].

The most important materials induced oxidative stress and cause damage in living cells is Hydrogen Peroxide which attack many living systems as polyunsaturated fatty acids of cellular membranes due to lipid peroxidation which cause initiation of many diseases as Diabetes , Cancer and Cardiovascular diseases [13]in addition to its role in breaking peptide bonds , denaturation of proteins and damage of DNA leading to intiation of genetic mutation[14]. Therefore, the current study aimed to determine the effect of alcoholic extract of *Heliotropium europium* and Vitamin C as well known antioxidant on the level of

leydig cells to produce of Testosterone . We note from figure (1) that the treatment of rats exposed to hydrogen peroxide with alcoholic extract of plant with vitamin C was better than the treatment of alcoholic extract of plant alone and this result may be due to synergism and cooperation of Vitamin C with compounds found in plant and thus leading to best results .

It is noted from Figure (2) a significant decreasing ($p \leq 0.05$) in the concentration of serum prolactin in the group treated with hydrogen peroxide as compared with control group and the reason may be due to the effect of oxidative stress on the axis of the hypothalamus - pituitary which leads to inhibition of this axis and thus reduce the hormones secreted from pituitary gland, including the prolactin and the treatment of animals exposed to oxidative stress with vitamin C and plant extract with vitamin C together have led to a significant increasing in prolactin concentration as compared to hydrogen peroxide group. The reason may be due to the presence of Quercetin in plant which consider as a powerful antioxidant and reduce from free radicals generation thus leading to prevent or reduce their effect on hypothalamus-pituitary axis and thus return pituitary gland to secrete their hormone , including prolactin[11], As for Vitamin C as antioxidant , it reduced from free radicals effects on this axis and stimulated the secretion of pituitary gland .

It was Shown from (Figure 3)that there was no significant difference in the concentration of the Thyroid Stimulating Hormone (TSH) in hydrogen peroxide group as compared with the control group, These findings were compatible with the results of, [25] in male rabbits exposed to oxidative stress that did not find any significant difference in TSH concentration between the group exposed to oxidative stress and the control group when rabbits treated for 30 days and studies also shown that there was nonsignificant differences in TSH concentration between control groups and oxidative stress resulted from diabetes[26,27]. It is clear from Figure (3)

presence of a significant increasing in group exposed to oxidative stress by hydrogen peroxide and treated with vitamin C as compared to hydrogen peroxide group only and this is attributable to stimulate the vitamin C content of the pituitary gland to secrete TSH as a result of the low concentration of T3 (Figure 4)by negative feed back mechanism, or may be attributed to the depletion of iodine from thyroid gland thereby increasing the responsiveness to TSH[25] or to the inhibition of Thyroperoxidase .As for group exposed to oxidative stress and treated with alcoholic leaves extract of plant did not lead to a significant difference in the concentration of TSH compared with theH2O2 group, these findings were compatible with the results of findings [28] wich have there is no change in the concentration of TSH in the group of oxidative stress, which in turn is reflected on the levels of thyroid hormones circulating in the blood.

It was showed from figures (4and 5) that group exposed tro oxidative stress has led to a significant increasing in T3 and non significant difference in T4 concentrations when compared with control group and this reason may related to inhibition of 5-monodeiodinase which controls of the transfer of T4 to T3 [27] .

According to hydrogen peroxide with Vitamin C group there was a significant decreasing in concentrations of T3 and T4 as compared with control group and this reflected on the significant increasing in the concentration of TSH in this group as compared with control group and this reason in decreasing of the levels of two hormones may be due to roles of Vitamin C as an antioxidant which works on the inhibition of antithyroid compounds and these compounds metabolize in the body to Thyocyanate and Isothyocynate and finally to sulfur containing compounds [29] . While[30] noted that Cyanogenic glycosides convert in the body to Thyocyanate by Clucosidase or Salfer transferase found in tissues of plants or animals .

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1- Determination of Testosterone concentration.: the concentration of testosterone was estimated depending on the principle steps in the kit imported from American Monobind company and as instruction of the manufacturer's own technology ELISA [16].

2- Determination of Prolactin (PRL) concentration : the concentration of Prolactin was estimated depending on principle the steps came with kits of American Monobind company and as instruction of the manufacturer's own technology ELISA[16].

3- Determination of TSH concentration : the concentration of TSH was estimated depending on the principle steps in kit imported from American Monobind company and as instruction of the manufacturer's own technology ELISA[17].

4- Determination of T3 concentration: the concentration of T3 was estimated depending on the steps in kit imported from of American Monobind company and as instruction of the manufacturer's own technology ELISA[18]

5-Determination of T4 concentration : the concentration of T4 was estimated depending on the principle steps in kit imported from of American Monobind company and as instruction of the manufacturer's own technology ELISA[19]

Statistical analysis:

The significant differences between the studied groups were found by using ready statistical software (SAS,2001), and statistical means was tested using polynomial Duncan test at significance level ($p \leq 0.05$) to determine the significant differences between groups [20]

Results and Discussion

Figure (1) illustrated that there was a significant decreasing ($p \leq 0.05$) in Testosterone concentrations in Hydrogen Peroxide group in comparison with control group , this result corresponding with results of [21] who noted to a significant decreasing in hormone concentrations in treatment of male albino rats with hydrogen peroxide in drinking water for 30 days. The reasons may be related to the oxidative stress which lead to inhibition of hypothalamus-pituitary axis [22] or due to reactive oxygen species effects in leydig cells which it works on the inhibition of steroidogenic acute regulatory protein replication found in mitochondrial membrane in it cells (represent the main center of testosterone synthesis) and this protein simplify the entry of cholesterol into the cell , it also work on inhibition cytochrome P450 side chain cleavage enzyme which has a major role in hormone biosynthesis in leydig cells [23].

the treatment of hydrogen peroxide group with alcoholic extract or vitamin C or alcoholic extract with vitamin C led to a significant increasing of testosterone concentration in comparison with hydrogen peroxide group.

The reason of this increasing due to the containing of alcoholic extract of Heliotropium europium leaves on many of antioxidant compounds as Glycosides and Flavonoids which act to reduce or prevent the damage of free radicals and thus reduced oxidative stress which induced by hydrogen peroxide and these molecules listed on work on stimulating selenoprotein enzyme which contain selenocysteine and the last one has a major role in the transport of Selenium and protection tissues from Reactive Oxygen Species (ROS) effects and his work is similar to Glutathione Peroxidase and has a relationship with Testosterone secretion in leydig cells[24]. As for the role of Vitamin C as antioxidant is known to protect cells from oxidative damage and also has an important role in stimulating the pituitary gland to secrete Interstitial cell stimulating hormone(ICSH),Which stimulates the

The high concentrations of Thiocyanate inhibit the combination of iodine with insoluble thyroglobuline [31], While the treatment of hydrogen peroxide exposed rats of alcoholic extract and vitamin C didn't led to any significant differences in the concentration of T4 but led to a significant increasing in T3 concentration as compared with hydrogen peroxide group and the reason may be due to reducing the binding of T3 with its carrying proteins and finally leading to decreasing the concentration of T3 in blood[29].

Conclution

There are a lot of data wich show that free radicals and reactive oxygen species increased damage done the compounds of living cells the effect of alcoholic leaf extract of *Heliotropium eapropiam* acts as antioxidant supplement wich is decrease harmful effect of the free radicals through some improvement was observed in the levels of studed hormones .

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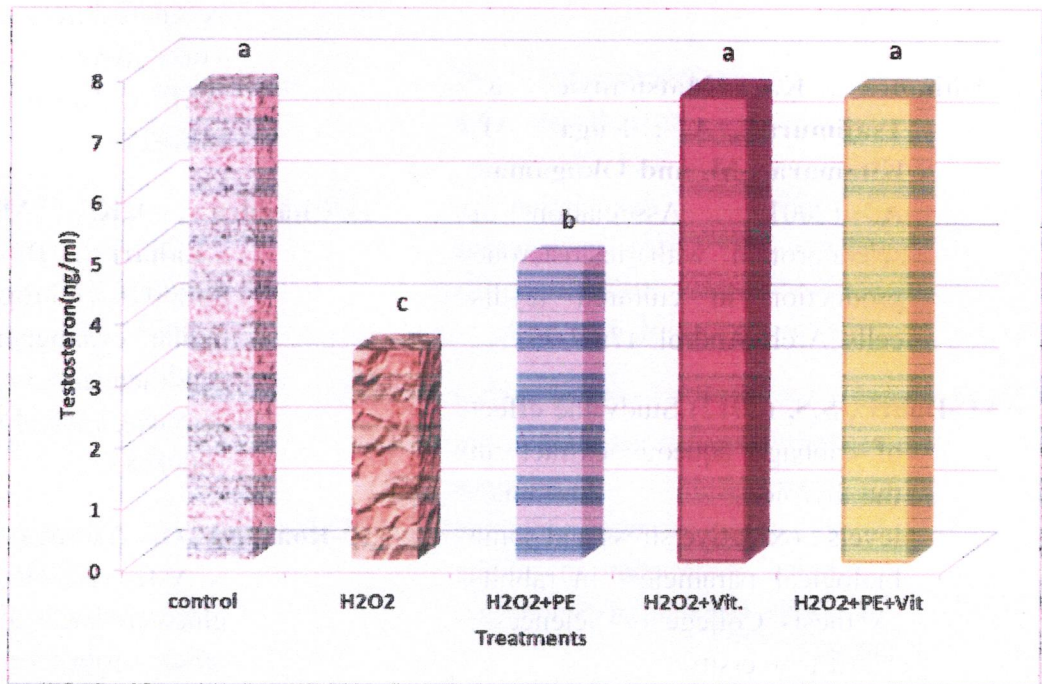


Figure (1): Effect of different treatment on the concentration of testosterone in male albino rats exposed to oxidative stress

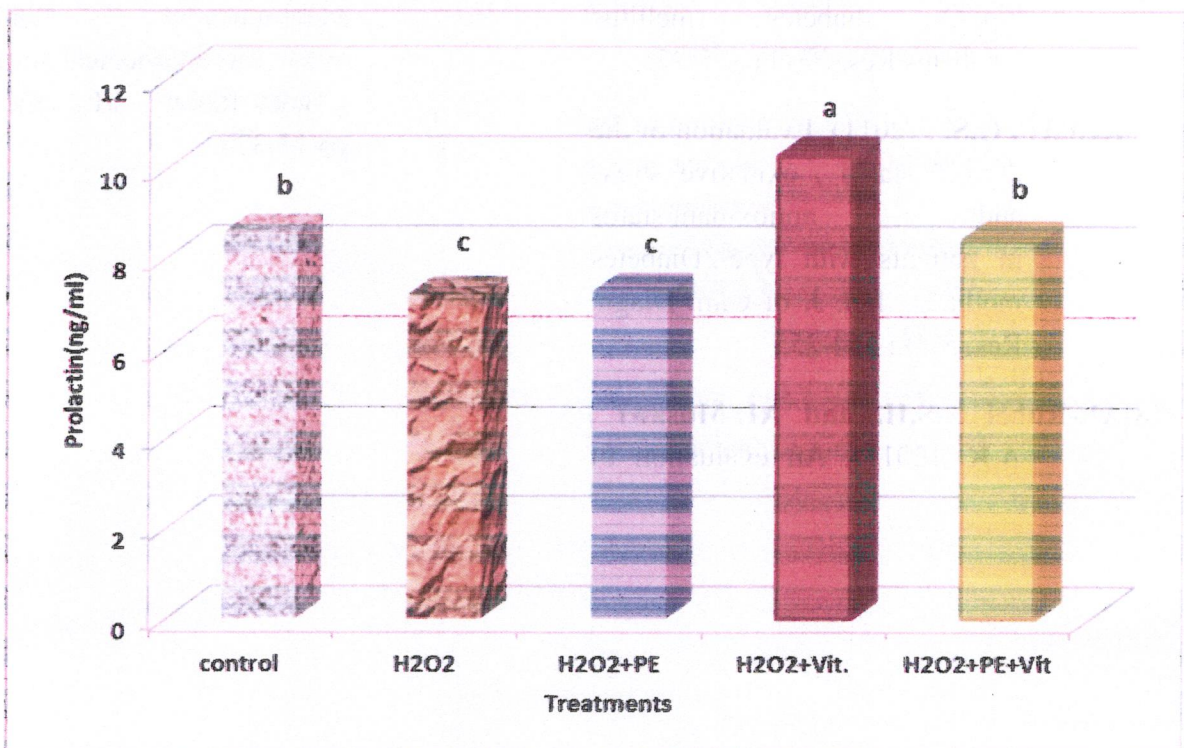


Figure (2): Effects of different treatment on the concentration of prolactin in male albino rats exposed to oxidative stress.

Effect of alcoholic leaf extract of *Heliotropium europium* on the levels of Testosterone , Prolactin ,TSH ,T3 and T4 hormones in Blood of male albino rats exposed to oxidative stress by H2O2

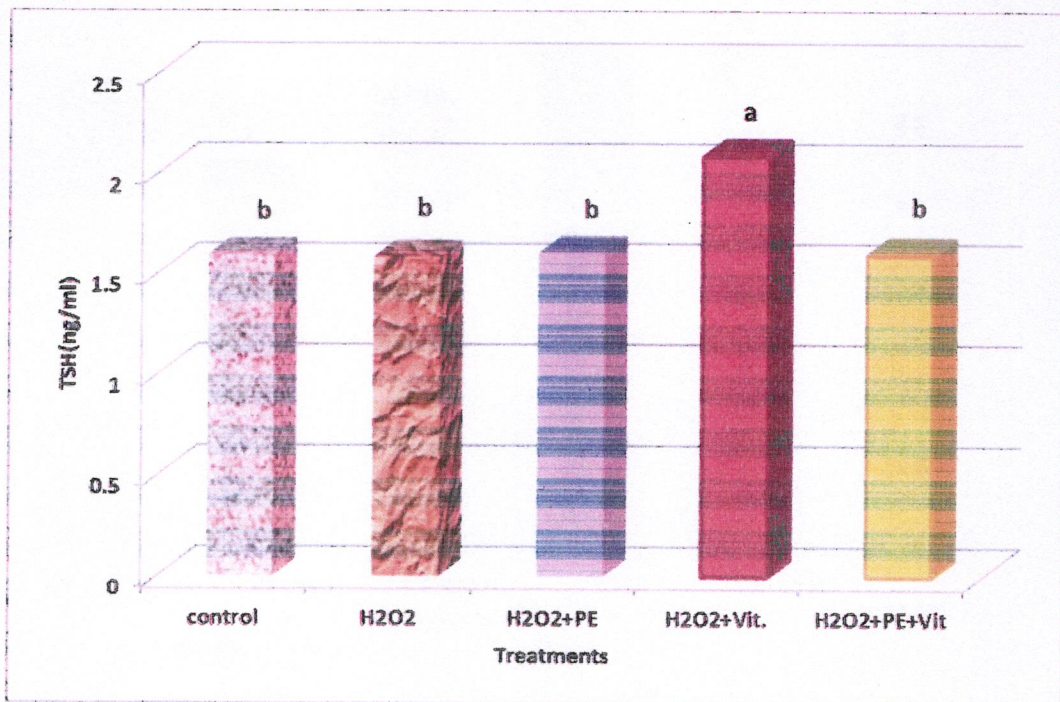


Figure (3):Effect of different treatment on the concentration of TSH in male albino rats exposed to oxidative stress

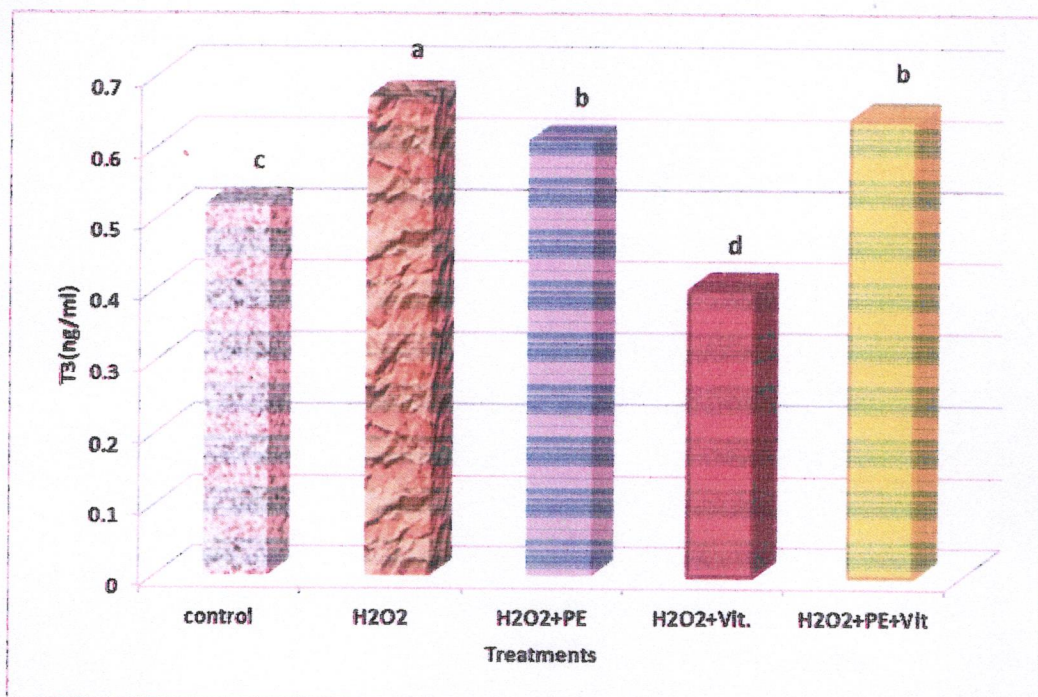


Figure (4): Effect of different treatment on the concentration of T3 in male albino rats exposed to oxidative stress

Effect of alcoholic leaf extract of *Heliotropium europium* on the levels of Testosterone , Prolactin ,TSH ,T3 and T4 hormones in Blood of male albino rats exposed to oxidative stress by H2O2

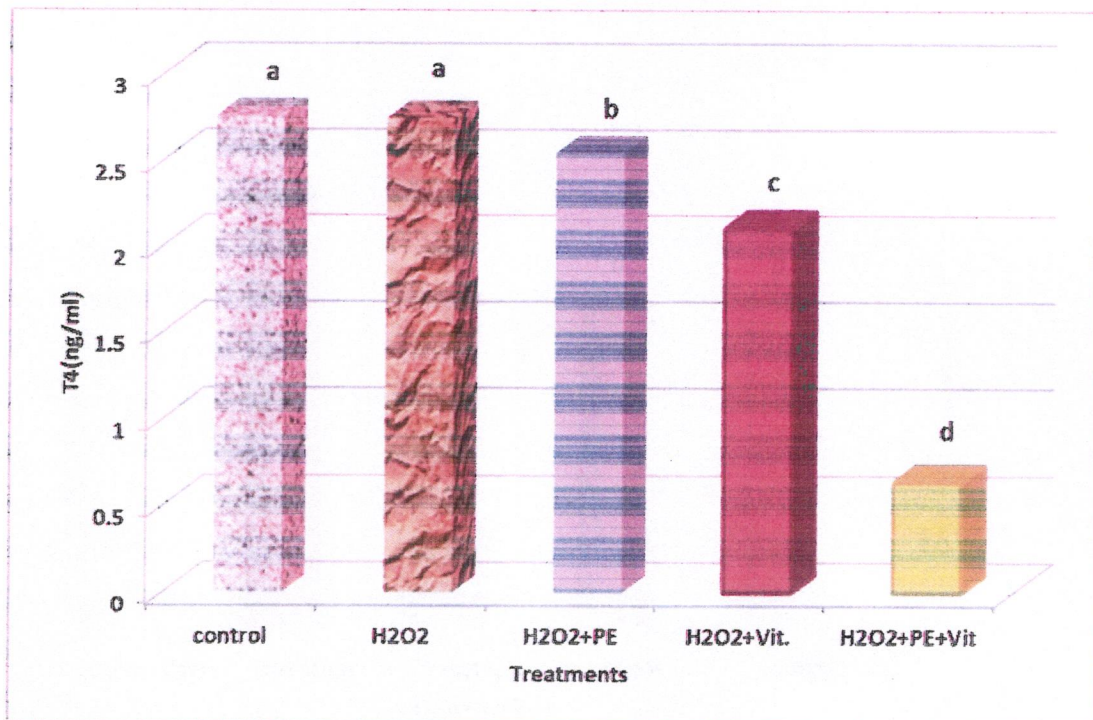


Figure (5): ffect of different treatment on the concentration of T4 in male albino rats exposed to oxidative stress