

# Folic acid alleviates oxidative stress in thyroxine-induced spleen injury in hyperthyroid rat

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**Abstract— Objective:** Thyroid hormones are involved in the regulation of basal metabolic state and in oxidative metabolism. This study evaluated some biochemical alterations in post-pubertal hyperthyroidism. Additionally, the ameliorating role of folic acid supplementation was investigated. **Methods:** A total 60 male albino rats were equally divided into six groups; the first and second groups were the control and folic acid groups respectively while the 3<sup>rd</sup> group was the hyperthyroid rat group; the 4<sup>th</sup> and 5<sup>th</sup> groups were co- and post treated hyperthyroid rat with folic acid respectively and the 6<sup>th</sup> group was self treated hyperthyroid rat group. **Results:** Serum T<sub>3</sub> & T<sub>4</sub> levels were a significant increase while TSH levels were a significant decrease in rats receiving thyroxine indicating the induction of hyperthyroid state. Serum cholesterol, triglycerides, HDL and LDL levels (lipid profile) were a significant decrease in hyperthyroid rats when compared with control group. On the other hand; MDA, catalase and nitric oxide levels in spleen tissues were a significant increase in hyperthyroid rats when compare with control group, while glutathione and total protein levels were a significant decrease in hyperthyroid rats when compared with control group. Treatment of hyperthyroid with folic acid were improved this alterations. **Conclusions:** Our results revealed that, folic acid as a treatment was better if it is administered as an adjuvant after returning to the euthyroid state.

**.Index Terms—** Folic acid, Hyperthyroidism, Lipid profile, Oxidative stress, Rats, Spleen, Thyroxine.

## 1 INTRODUCTION

As it is known the endocrine system together with the nervous system enables other systems in the body to work in coordination with each other and protect homeostasis using hormones. Hormones secreted by the endocrine system are carried to target organs and cause affect through receptors [1-2]. The thyroid gland is among the most significant organs of the endocrine system and regulate several essential physiological processes such as energy metabolism, growth, formation of the central nervous system, tissue differentiation and reproduction [2-10]. It is well known that changes in the thyroid state are associated with marked alterations in immune and vascular system [11-13]. Thyroid hormones powers systemic vascular resistance, increases blood volume, and has inotropic and chronotropic effects on cardiac function [11,14].

Hyperthyroidism is characterized by an increase in serum T3 and T4 and a decrease in serum TSH. The most common cause of hyperthyroidism is Graves' disease which is the production of antibodies to TSH receptor [15]. Hyperthyroidism is a common metabolic disorder with prominent cardiovascular manifestations [16-18] It causes a hyper dynamic circulatory state because of a marked reduction in peripheral vascular resistance and an increased total blood volume and heart rate.

Oxidative stress is considered to play a pivotal role in the pathogenesis of aging and several degenerative diseases,

loads by increasing gene expression to up-regulate their reductive defense systems and restore the oxidant/antioxidant balance, but when this increased synthesis cannot be achieved due to damage to enzymes, or substrate limitations, or when the oxidative load is overwhelming, an imbalance persists and the result is oxidative stress [20]. It is well established that thyroid

hormone-induced oxidative stress in target tissues is responsible for some complications of experimental hyperthyroidism, include in thyrotoxic myopathy, liver injury and alterations of heart electrical activity [21].

Folic acid is water-soluble vitamins, which are essential in our life. Numerous clinical trials using folic acid for prevention of cardiovascular disease, stroke, cognitive decline, and neural tube defects have been completed or are underway [14]. Folic acid status is also affected by hypothyroidism [3-5,13,22,23]. Hyperthyroidism in man is associated with depletion of folate stores and sub clinical deficiency of this vitamin. This is attributed to an increased demand for folic acid in the hyper metabolic state [24].

The present study represents a contribution to declare the effect of high thyroid hormone status on blood and oxidative stress parameters. Additionally, the impact of these biomarkers on spleen in thyroxine-induced hyperthyroidism at the post-pubertal stage of male rats will be investigated. It also aims to elucidate the role of folic acid supplementation in enhancing spleen damage and building up the antioxidant status as a concurrent treatment with hyperthyroidism and as a post-treatment after restoration of the euthyroid state.

## 2 Materials and method

The experiments were performed on 40 male albino rats (*Rattus norvegicus*) weighing 125 ±10g and of 8 week's age. They were obtained from our laboratory farms, Zoology Depart-

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such as atherosclerosis, cardiovascular disease, type 2-diabetes and cancer [19]. Cells can tolerate moderate oxidative

ment, Faculty of Science, Tanta University, Egypt. The rats were kept in the laboratory for one week before the experimental work and maintained on a standard rodent diet (20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminized starch; Egyptian Company of Oils and Soap Kafr-Elzayat Egypt) and water available ad libitum. The temperature in the animal room was maintained at  $23\pm 2^{\circ}\text{C}$  with a relative humidity of  $55\pm 5\%$ . Light was on a 12:12 hr light-dark cycle. The experimental protocol was approved by Local Ethics Committee and Animals Research. The rats were randomly and equally divided into five groups (8 animals each).

G1: Control group in which animals did not received any treatment.

G2: Folic acid group in which animals received folic acid daily (El Nasr Pharmaceutical Chemicals Co.; at 8 mg/kg of body weight) for four weeks from 2nd week to 6th week [7].

G3: Hyperthyroid rats group in which rats received L-Thyroxin sodium administration (100  $\mu\text{g}/\text{Kg}$ , 4 weeks) as a chewable lab chow [25].

G4: Co-treated group in which animals received L-Thyroxin sodium daily in drinking water and folic acid simultaneously according to Tousson et al. [7].

G5: Post treated group in which animals received L-Thyroxin sodium daily in drinking water and folic acid simultaneously according to Tousson et al. [7].

G6: Self treated hyperthyroid group in which animals recovered without drugs.

At the end of the experimental period, rats were euthanized with intraperitoneal injection with sodium pentobarbital and subjected to a complete necropsy.

Blood samples were individually collected from the inferior vena cava of each rat in heparinized and non heparinized glass tubes to estimate biochemical parameters. The non heparinized blood used to obtain blood serum while the heparinized blood used to obtain blood plasma. Serum was separated from non heparinized blood by centrifugation at 3000 rpm for 15 minutes. The collected serum was stored at  $-18^{\circ}\text{C}$  until analysis.

## 2.1 Determination of thyroid hormones

Serum T<sub>3</sub>, T<sub>4</sub> and TSH were performed using Biocheck Kits Inc (Los Angeles, USA) catalog no BC-1005, BC-1008 and BC-1001 respectively (using monoclonal antibody).

## 2.2 Determination of Lipid profiles

Serum total cholesterol (TC) was determined according to the method of Allain et al. [26] and serum triglycerides (TG) were determined according to the method of Fossati and Prenciple [27] using kits supplied by Human. Serum high density lipoprotein cholesterol (HDL-c) was determined according to the method of Lopes-Virella et al. [28] using kits supplied by Human. Also low density lipoprotein cholesterol (LDL-c) were calculated according to Friedewald and Robert as following:  $\text{LDL-c} = \text{total cholesterol} - \text{HDL-c}$ .

Spleen homogenate: Spleen homogenate (10%; w/v) was prepared in ice-cold 0.067M phosphate buffer (pH=7) then, the homogenate was centrifuged at 3000 r.p.m for 10 min.

Total protein: Total protein content in tissue homogenate was measured according to the method of Lowry et al. [29]

## 2.3 Enzymatic and non-enzymatic antioxidant assays

### 2.3.1 MDA assay

Malondialdehyde (MDA), a noxious product of lipid peroxidation, was detected by TBARS analysis and measured as reported by Aebi [30].

### 2.3.2 Reduced GSH

GSH content was determined with dithionitrobenzoic acid using the method described by Beutler et al. [31] and was expressed in  $\mu\text{mol GSH}/\text{mg protein}$ . The method is based on the reduction of DTNB to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 412 nm.

### 2.3.3 Catalase

The catalase (CAT) activity was measured by monitoring H<sub>2</sub>O<sub>2</sub> (The substrate of the enzyme) decomposition at 240 nm according to the method described by Aebi [30].

### 2.3.4 Nitric oxide

The Nitric oxide (NO) activity was measured by colorimetric method, Kits supplied by Bio-diagnostic, Egypt at 540 nm. by colorimetric method, Kits supplied by Bio-diagnostic [30] In acid medium and in the presence of nitrite the formed nitrous acid diazotize suphanilamide and the product is coupled with N-(1-naphthyl) ethylened iamine. The resulting azo dye has a bright reddish-purple color. Nitric oxide is expressed in sample as ( $\mu\text{mo} / \text{L}$ )

## 2.4 Statistical Analysis

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

## 3 Results

The animals under practice appeared healthy and did not show clinical signs of disease and no mortality was recorded during the experiment duration. The dose of folic acid did not initiate any side effects for the animals, whereas various side effects were observed in animals that received L-Thyroxin sodium (100  $\mu\text{g}/\text{Kg}$ , 4 weeks) such as loosing of body weight, weakness and yellowish body hair.

Our data summarized in Table (1) revealed the changes in thyroid hormones. Serum T<sub>3</sub> and T<sub>4</sub> levels were a significant increase in hyperthyroid rats when compared with the control group. Serum T<sub>3</sub> and T<sub>4</sub> levels were a significant decrease in treated hyperthyroid with folic acid when compared with hyperthyroid group. On the other hand; serum T<sub>3</sub> and T<sub>4</sub> levels were a significant increase in co treated hyperthyroid rats group when compared with post treated hyperthyroid one. Also, serum T<sub>3</sub> and T<sub>4</sub> levels were in significant decrease in self treated hyperthyroid rats when compared with hyperthyroid rats; and a significant increase when compared with treated hyperthyroid with folic acid (Table 1). Table (1) showed that the Serum TSH levels were a significant decrease in hyperthyroid rats when compared with the control group.

On the other hand; serum TSH levels were a significant increase in co treated hyperthyroid rats group when compared with post treated hyperthyroid one. Also Table (1) showed that; serum TSH levels were a significant increase in self treated hyperthyroid rats when compared with hyperthyroid rats; and a significant decrease when compared with treated hyperthyroid with folic acid.

**TABLE 1**  
CHANGES IN SERUM THYROID HORMONES T<sub>3</sub> (NG/DL), T<sub>4</sub> (NG/DL) AND TSH (NG/DL) LEVELS OF DIFFERENT GROUPS

Group	T3	T4	TSH
G1	41.03± 2.111	2.46±0.145	1.66±0.072
G2	39.50±2.255	2.41±0.262	1.63±0.089
G3	73.67±4.372**	5.06±0.291**	0.08±0.016***
G4	50.37±2.051*	4.13±0.207**	1.21±0.060**
G5	44.47±1.898	3.46±0.318*	1.43± 0.061
G6	70.00±4.041**	4.83±0.203***	0.29±0.045***

Data are expressed as mean ± SD of 10 observations. G1, Control; G2, Folic acid; G3, Hyperthyroid; G4, Co-treated; G5, Post treated groups and G6, self treated hyperthyroid group. (\*\*\*) P< 0.001 compared to G1 (\*\*) P< 0.01 compared to G1 (\*) p<0.05 compared to G1.

Our data summarized in Table (2) revealed the changes in lipid profiles in different groups under study. Table (2) showed that, cholesterol, triglycerides, HDL and LDL levels were a significant decrease in hyperthyroid group when compared with control group. On the other hand, cholesterol, triglycerides, HDL and LDL levels were a significant decrease in treated rats with folic acid when hyperthyroid and self treated hyperthyroid rats. Also; Table (2) showed that, cholesterol, triglycerides, HDL and LDL levels were a significant increase in co-treatment of hyperthyroid rats with folic acid when compared with post treatment of hyperthyroid rats with folic acid (Table 2).

**TABLE 2: CHANGES IN CHOLESTEROL (TC) (MG/DL), TRIGLYCERIDES (TG) (MG/DL), HDL (MG/DL) AND LDL (MG/DL) IN DIFFERENT GROUPS UNDER STUDY**

Gs	TC	TG	HDL	LDL
G1	70.6±3.4	109±7.7	39±1.7	39.6±1.7
G2	71.6±3.7	112±6.4	40.07±1.5	40.07±1.7
G3	39.3±3.4**	78.3±4.6*	23.3±2.02**	24.3±2.3**
G4	46±3.05**	88.3±4.9	28.6±2.02*	29.3±1.7*
G5	67.3±3.4	103.3±5.8	36±2.3	37.6±2.3
G6	43.6±3.1**	80±4.6*	26.6±1.4**	27±2.08**

Data are expressed as mean ± SD of 10 observations. G1, Control; G2, Folic acid; G3, Hyperthyroid; G4, Co-treated; G5, Post treated groups and G6, self treated hyperthyroid group. (\*\*\*) P< 0.001 compared to G1 (\*\*) P< 0.01 compared to G1 (\*) p<0.05 compared to G1.

MDA, Catalase and NOx levels in spleen tissues were a significant increase in hyperthyroid group when compared with control, while GSH and Total protein levels were a signifi-

cant decrease in hyperthyroid group when compared with control group (Figures 1-5). MDA, catalase and NOx levels in spleen tissues were a significant decrease in treated hyperthyroid with folic acid when compared with hyperthyroid group. On the other hand; spleen MDA, catalase and NOx levels were a significant increase in co treated hyperthyroid rats group when compared with post treated hyperthyroid one. Also, Figures (1-3) showed that; MDA, catalase and NOx levels in spleen tissues were a significant decrease in self treated hyperthyroid rats when compared with hyperthyroid rats; and a significant increase when compared with treated hyperthyroid with folic acid (Figures 1-3).

On the other hand; Figures (4&5) showed that; total protein and GSH levels in spleen tissues were a significant increase in treated hyperthyroid with folic acid when compared with hyperthyroid group. GSH levels were a significant decrease in co treated hyperthyroid rats group when compared with post treated hyperthyroid one; while the total protein levels were in significant decrease in co treated hyperthyroid rats group when compared with post treated hyperthyroid group (Figures 4&5). Also, total protein and GSH levels in spleen tissues were a significant increase in self treated hyperthyroid rats when compared with hyperthyroid rats; and a significant decrease when compared with treated hyperthyroid with folic acid (Figures 4&5).

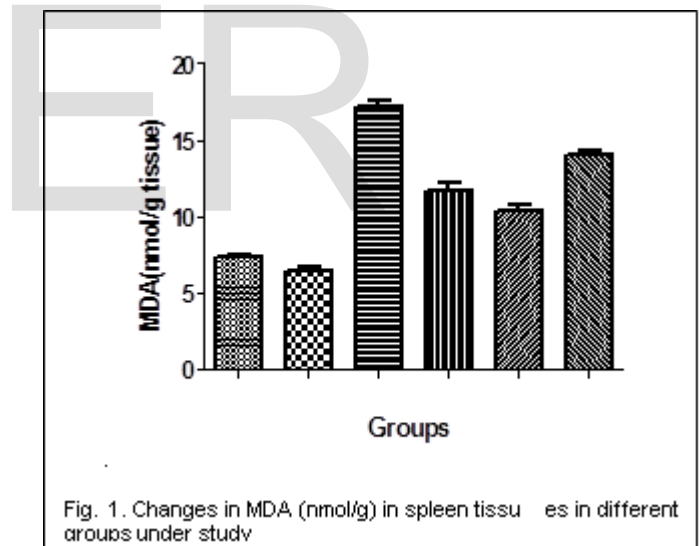
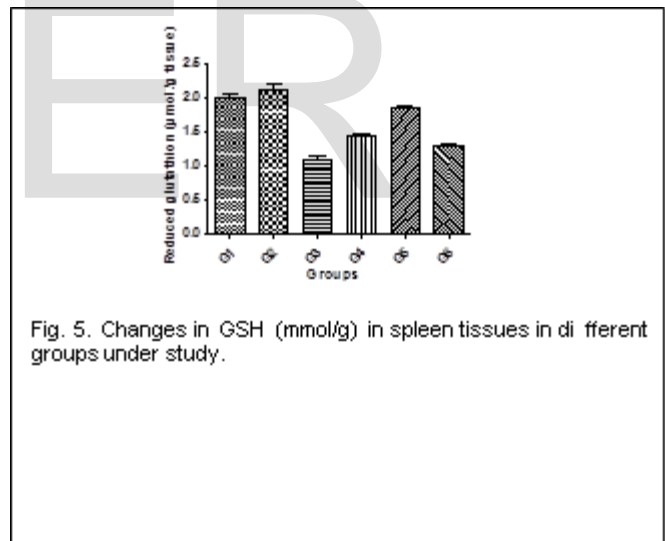
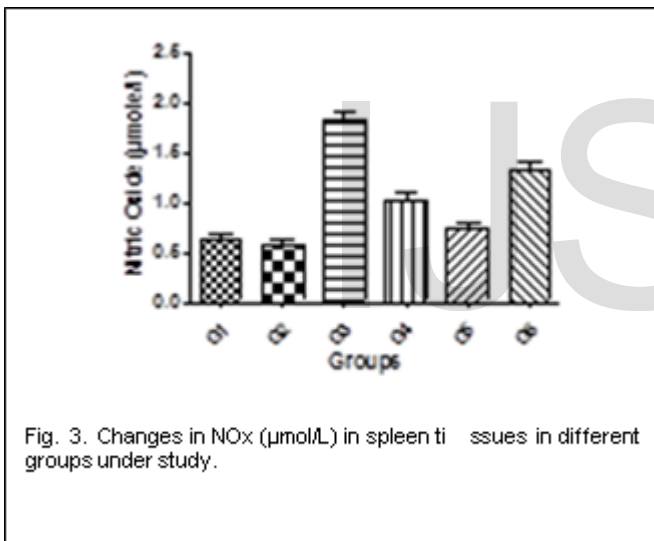
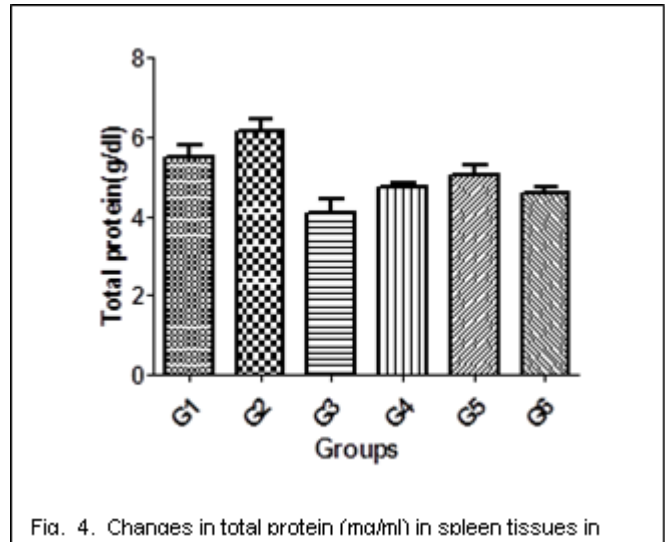
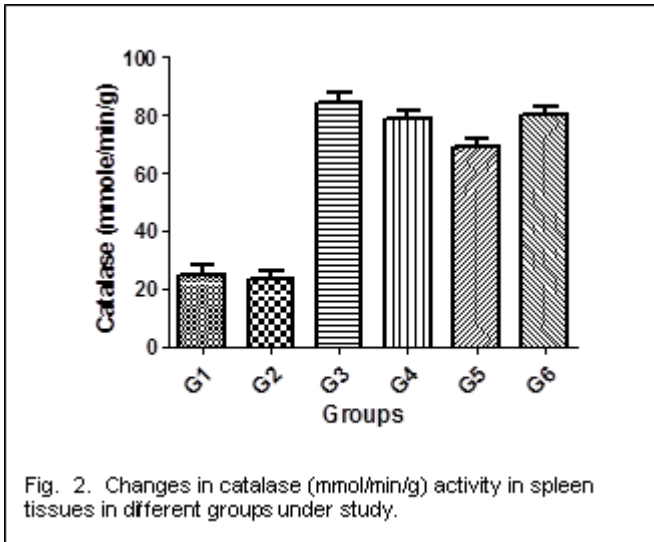


Fig. 1. Changes in MDA (nmol/g) in spleen tissues in different groups under study



#### 4 DISCUSSION

In order to ensure the hyperthyroid state we regularly determined the serum T3, T4 and TSH through the dose period where serum T3 and T4 levels is a significant increase and serum TSH levels is depressed in rats receiving thyroxine indicating the induction of hyperthyroid state. In the present study, the effect of thyroxine on T3, T4 and TSH seems to be reversed in adult rats when the treatment was withdrawn after 4 weeks as the levels of the T3, T4 and TSH that tends to be nearing normal levels. This increase in TSH can be explained by decreased production of T3 from the thyroid gland that minimizes TSH feedback inhibition resulting in an increase in

its secretion by the anterior pituitary gland; this result coincides with studies of Shibutani et al. [32]. Also; treatment of hyperthyroid rats with folic acid were improved the thyroid hormones.

Thyroid hormone is reported to increase HMG-CoA reductase which is an integral membrane protein of the smooth ER, is the major point of regulation on the pathway to cholesterol synthesis, much of the cholesterol synthesis in vertebrates takes place in the liver. A small fraction of the cholesterol made there is incorporated into the membranes of hepatocytes, but most of it is exported in one of three forms: biliary cholesterol, bile acids, or cholesteryl esters. Cholesterol and cholesteryl esters, like triacylglycerols and phospholipids, are essentially insoluble in water, yet must be moved from the tissue of origin to the tissues in which they will be stored or consumed. They are carried in the blood plasma as plasma lipoproteins [13]. The total cholesterol level in the hyperthyroid group is lower than the negative control group or the folic acid group we may suppose that the thyroid hormone may increase both cholesterol synthesis and cholesterol degradation as a result of the high total body catabolic rate of the hyperthyroidism. Hyperthyroidism is reported to decrease the HDL level and after treatment the HDL level increases and these findings is consistent with our results.

Antioxidants are molecules that are capable of slowing or preventing the oxidation of other molecules, thereby protecting cells from damages caused by exposure to free radicals, including reactive oxygen species, which are produced during oxidation reactions in biological cells, antioxidants can be either phytochemicals or vitamins and other nutrients, they range from micro molecules such as glutathione, vitamins, to macromolecules such as catalase, glutathione and peroxidase. The spleen is the largest secondary immune lymphoid organ in the body, where it contains large population of lymphocytes. It is composed of two functionally and morphologically distinct compartments, the red and the white pulps. The red pulp is the site of blood filtering, iron metabolism and removal of bacteria from the blood by macrophages. The thyroid hormones affect the effectiveness of antioxidants in the rat spleen tissues. Lipid peroxidation is an autocatalytic mechanism leading to oxidative destruction of cellular membrane [33]. Such destruction can lead to cell death and to the production of toxic and reactive aldehyde metabolite called malonaldehyde (MDA). In the present study, MDA, catalase and NOx levels were significantly ( $P < 0.05$ ) increased in hyperthyroid rats as compare with control and folic acid groups. While Glutathione and total protein levels were significantly ( $P < 0.05$ ) decreased in hyperthyroid rats as compared with control group. Also; treatment of hyperthyroid rats with folic acid improved this changes in oxidative stress parameters. This finding is in line with Venditti et al. [34]. Also, these findings are confirmed by positive correlation between T3 and heart MDA. Joanta et al. [35] revealed an increase of the lipid peroxides content and carbonyl proteins level in blood, liver, thyroid, heart and skeletal muscle in experimental hyperthyroidism, suggesting that thyroid hyperfunction is accompanied by oxidative stress. Our results also agree with Zaiton et al. [36] who revealed increased concentration of lipid peroxidation products in the myocardium and solear muscle in hyperthy-

roid rat, but not in liver or tissue. Cell damage occurs when there is an excess of reactive species derived from oxygen and nitrogen, or a defect of antioxidant molecules. This observed increase in both reactive oxygen and nitrogen species after L-Thyroxin sodium treatment was parallel to the increase in NO, MDA and the decrease in GSH. These results was in line with Quesada et al [36] study that can be summarized as increased NO production may contribute to the hyperdynamic circulation in hyperthyroidism due to NOS (nitric oxide synthetase) activity was up regulated in tissues of hyperthyroid rats [37]. Reduced glutathione (GSH) is one of the most important molecules in the cellular defense mechanism against chemically reactive toxic compounds or oxidative stress [38]. On the other hand, GSH as an oxidative stress marker showed a significant decrease in hyperthyroid rats. This decrease was improved to nearly normal values by treatment with folic acid. The current results agree with Arikan et al [39] determined that the level of lipid peroxidation products was higher in the hyperthyroidism group than control levels; however GSH and GSH-PX were significantly lower than control levels.

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