

**EFFECT OF FOLIC ACID ON L-THYROXIN INDUCED NEPHROTOXICITY IN ALBINO RATS**Saber A. Sakr<sup>1\*</sup>, Ehab Tousson<sup>2</sup>, Somya Y. Shalaby<sup>1</sup>, Mohamed Rabea<sup>1</sup><sup>1</sup>Department of Zoology, Faculty of Science, Menoufia University, Egypt.<sup>2</sup>Department of Zoology, Faculty of Science, Tanta University, Egypt.**\*Correspondence for Author: Saber A. Sakr**

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**ABSTRACT**

The present study evaluated the biochemical, histological and immunohistochemical alterations in kidney of rats treated with L-thyroxin. Additionally, the ameliorating role of folic acid supplementation was investigated. The results showed that there was a significant increase in plasma T3, T4, creatinine, urea, MDA and nitric oxide in L-thyroxin treated rats as compared to control group. On the other hand, a significant decrease in TSH, glutathione (GSH) was recorded in L-thyroxin treated rats as compared to control group. Histological results revealed that kidney of L-thyroxin treated rats showed histopathological lesions such as leucocytic infiltrations, swelling and cytoplasmic vacuolation of the epithelial lining of the tubules and congestion of blood vessels. Most of the glomeruli were atrophied. Immunohistochemical results revealed that strong positive expression of PCNA was detected in the kidney tubules in L-thyroxin treated rats. Treating animals with L-thyroxin and folic acid leads to decrease in T3, T4, urea, MDA and nitric oxide. On the other hand, a significant increase in TSH and glutathione and improvements in histopathological alterations of the kidney was recorded. It is concluded that folic acid has antioxidant effects leading to amelioration of L-thyroxin toxicity.

**KEYWORDS:** Hyperthyroidism, L-thyroxin, kidney, histopathology, PCNA, oxidative stress.**INTRODUCTION**

Thyroid hormones (thyroxin T4 and triiodothyronine T3) are known to regulate the energy metabolism of most tissues including liver, kidney, heart and skeletal muscles. It is well established that thyroid hormones accelerate the basal metabolic rate and oxidative metabolism by causing an increase in the mitochondria mass, mitochondrial cytochrome content and respiratory rate.<sup>[1]</sup> Alterations in their normal levels cause some biochemical and clinical abnormalities such as hypothyroidism and hyperthyroidism. Extended exposure to the treatment with L-thyroxin may alter thyroid activity by interfering with thyroid hormones synthesis, which provokes the disruption of thyroid axis, resulting in numerous abnormalities.<sup>[2]</sup> Hypothyroidism and hyperthyroidism are a result of an imbalance of thyroid hormone. Hypothyroidism is simply not enough thyroid hormone and hyperthyroidism is too much. Either imbalance affects the metabolism in the body.

Kidney is a major target organ for thyroid hormone with important biological and medical implications.<sup>[3]</sup> Thyroidal status influences kidney function both during embryonic development and in the mature functioning of the kidney, directly by affecting glomerular function, the tubular secretory and absorptive capacities, electrolyte pumps and kidney structure, and indirectly by affecting

the cardiovascular system through its influence on renal blood flow which decrease in hypothyroidism.<sup>[4]</sup>

Thyroid hormones (TH) play an important role in growth, development, and physiology of the kidney.<sup>[5]</sup> Thyroid function also influences water and electrolyte balance on different compartments of the body.<sup>[6]</sup> The kidney also plays a role on the regulation of metabolism and elimination of TH and is an important target organ for TH actions.<sup>[4]</sup> TH has a hold upon tubular transport of sodium, via their actions on the sodium-potassium ATP pump (Na/K ATPase) and on the potassium permeability in the membrane of proximal tubules.<sup>[7]</sup> TH stimulates rennin release by the juxtaglomerular cells<sup>[8]</sup> and influence kidney angiotensinase activity.<sup>[9]</sup>

Folic acid is a water-soluble vitamin, which is essential in 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.<sup>[10]</sup> Folic acid and folate represent the synthetic form (folic acid) and the naturally occurring form (folate) of vitamin B9. Supplementation with folic acid has also been shown to reduce the risk of congenital heart defects, cleft lips<sup>[11]</sup>, limb defects and urinary tract anomalies.<sup>[12]</sup>

Bazzano,<sup>[13]</sup> reported that folic acid and vitamin B12 supplementation consumed before and during pregnancy may reduce the risk of heart defects in infants, taking folic acid does not reduce cardiovascular disease even though it reduces homocysteine levels. Also, it may reduce the risk for children to develop metabolic syndrome.<sup>[14]</sup> García-Miss *et al.*<sup>[15]</sup> showed that folate deficiency may increase the risk of schizophrenia because, by increasing homocysteine levels, folate also increases interleukin 6 and tumor necrosis factor alpha levels, and these two cytokines are involved in the development of schizophrenia. Ebaid *et al.*<sup>[16]</sup> reported that folic acid reduce oxidative stress, restore the normal concentrations of antioxidant enzymes, and exhibit antihyperlipidemic activities. The present study was designed to declare the effect of L-thyroxin on the kidney functions, oxidative stress parameters. Additionally, the ameliorating role of folic acid supplementation was investigated.

## MATERIALS AND METHODS

The experiments were performed on male albino rats (*Rattus norvegicus*) weighing (130 g ±10g). They were obtained from Helwan laboratory farms, Egyptian Organization for Vaccine and Biologic Preparations. 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 was available *ad libitum*. The temperature in the animal room was maintained at 23±2°C with a relative humidity of 55±5%. Light was on a 12:12 hr light-dark cycle. The experimental protocol was approved by Local Ethics Committee and Animals Research approved by Menoufia University. The rats were randomly and equally divided into five groups (10 rats each).

**Group 1 (G1):** Rats of this group were considered as control.

**Group 2 (G2):** Animals received folic acid (El Nasr Pharmaceutical Chemicals Co.) at a dose level of 8 mg/kg of body weight/ day) for three weeks from 3rd week to 6th week.<sup>[17]</sup>

**Group 3 (G3):** Rats of this group received of L-thyroxin sodium at a dose of 100 µg/kg / body weight daily for three weeks to induce hyperthyroidism.<sup>[18]</sup>

**Group 4 (G4):** Rats of this group received L-thyroxin sodium (100 µg/kg/ body weight/day) for three weeks, followed by folic acid (8 mg/kg / body weight/day) for another three weeks.

**Group 5 (G5):** Rats of this group were received L-thyroxin sodium (100 µg/kg / body weight/day) for three weeks and left without treatment for another three weeks.

## Biochemical analysis

At the end of the experiment period, blood samples were collected from each rat in heparinized glass tubes to obtain plasma. Another part of blood were centrifuged at 3000 rpm for 15 minutes to obtain serum, the collected

serum was stored at -18 °C until analysis. Serum was analyzed to determine the T3 hormone concentration in serum was assayed by using commercial kit that was supplied by Biocheck, Inc (USA). T3 was estimated according to the method of.<sup>[19]</sup> TSH hormone concentration in serum was assayed by using commercial kit supplied by Biocheck, Inc (USA). TSH was estimated according to the method of.<sup>[20]</sup> kidney was quickly removed, weighed and stored at -20°C then 10%W/V homogenate was prepared by grand 0.3 g of tissue in 3ml of saline, homogenate was used to estimate oxidative stress parameters as malondialdehyde (MDA) was estimated by the method of<sup>[21]</sup>, nitric oxide (NO) was estimated by the method of<sup>[22]</sup> and glutathione (GSH) in tissue homogenate following the procedure was estimated by the method of.<sup>[23]</sup>

## Histological study

Kidneys were immediately removed from dissected rats and were immediately fixed by immersion in 10% buffered formalin solution and left for 24-48 hours. The specimens were then dehydrated, cleared and embedded in paraffin. Sections of 5 µm thick were cut by mean of rotary microtome and stained with haematoxylin and eosin and examined under light microscope.

## Immunohistochemical study

The immunostaining was performed using the avidinbiotin complex (ABC) method and an automatic autostainer (CODE-ON immune/DNA slide stainer: Biotek solution, Santa Barbara, CA). Formalin-fixed slides were deparaffinized and blocked for endogenous peroxidase with 1.75% hydrogen peroxide in methanol for 20 minutes, antigen retrieval for 15 minutes using Biogenex Antigen Retrieval Citra solution in 90°C water bath for 30 minutes. The slides were allowed to cool for 20 minutes before continuing. Slides were then blocked by normal horse serum for 5 minutes at 37°C. The monoclonal antibody was applied overnight in humid medium at room temperature followed by the biotinylated secondary antibody for 15 minutes at 37°C and the ABC complex for 15 minutes at 37°C (Vectastain Elite ABC kit; Vector laboratories, Burlingame, CA). Diaminobenzidine (DAB) was applied for 20 minutes at room temperature as chromogenic slides was counterstained with haematoxylin, dehydrated, and covered by cover slips. In negative control slides, the same system was applied with replacement of the monoclonal antibody by diluted normal bovine serum. PCNA- immunostaining was performed using polyclonal rabbit-anti-human (A3533 Ig fraction; DAKO, Glostrup, Denmark).

## Statistical analysis

Data were presented as the mean ± standard error of mean (SEM). The analysis was done using the Statistical Package for the Social Sciences (SPSS software version 16). Student t- test was performed to assess the significance of differences between groups. Significance at P<0.05 was considered statistically significant.

## RESULTS

### i. Histological results

Examination of sections of control kidney cortex revealed normal appearance of renal tubules and glomeruli **Fig. (1)**. Also, Examination of kidney section prepared from animals treated with folic acid is identical to that observed in normal control **Fig. (2)**. kidney sections of L-thyroxin group showed histopathological alterations such as leucocytic infiltrations, damage of renal tubules, swelling and cytoplasmic vacuolation of the epithelial lining of the tubules and congestion of blood vessels. Most of the glomeruli were atrophied **Figs. (3-7)**. Kidney section of L-thyroxin treated with folic acid group showed a degree of improvement. Organized tubular and glomerular structures with well-established epithelia which resembled that of the control group except mild inflammatory infiltration was observed **Figs. (8, 9)**. Finally, Kidney section of recovery group showed lose of kidney architecture, degeneration of some glomeruli and others were atrophied with dilatation in the sub capsular space (**Figs. 10, 11**).

### ii. Immunohistochemical results

Examination of kidney sections in control and folic acid groups showed few expression of PCNA (**Fig. 12**). Strong positive expression of PCNA was detected in the kidney section in L-thyroxin treated rats (**Fig. 13**). However; mild positive expressions of PCNA was observed in rat treated with L-thyroxin and folic acid (**Fig. 14**). Strong positive expression of PCNA was detected in the kidney section of recovered group (**Fig. 15**).

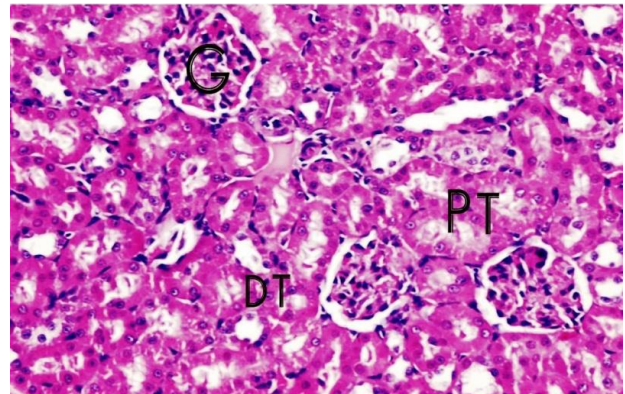
### iii. Biochemical results

Data in **figures (16-18)** revealed that T3, T4 significantly increased in L-thyroxin treated group compared with control one, while TSH decreased. On the other hand, animals treated with L-thyroxin and folic acid showed significant decrease in T3 and T4 levels, and increase in TSH. Similarly, a significant decrease in T3 and T4 levels and increase in TSH was recorded in recovery group as compared to L-thyroxin treated group.

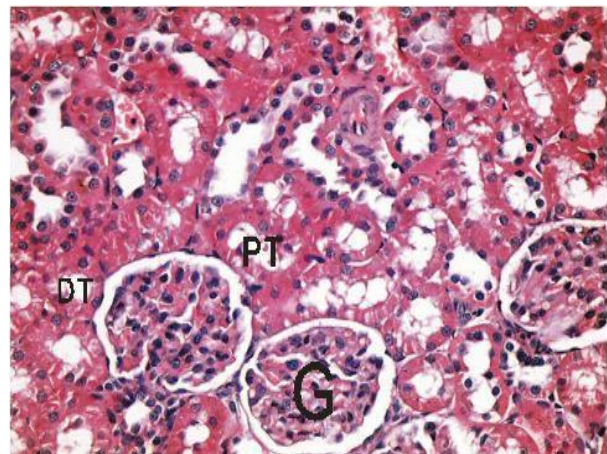
### iv. Oxidative stress parameters

Data in **figure 19** revealed that there was a significant increase in MDA values in the kidney of L-thyroxin treated group rats as compared to the control group. Animals treated with L-thyroxin and folic acid reduced the MDA levels as compared to L-thyroxin treated animals. A significant increase in MDA levels was recorded in animals of recovery group. Data in **figure 20** revealed that a significant increase in nitric oxide was observed in L-thyroxin treated rats as compared to control group. Animals treated with L-thyroxin and folic acid reduced the nitric oxide levels as compared to L-thyroxin treated animals. On the contrary, the recovery group showed significant increase in nitric oxide. Data in **figure 21** revealed that reduced glutathione level was decreased in L-thyroxin treated rats as compared to the control group and increased after treatment with folic

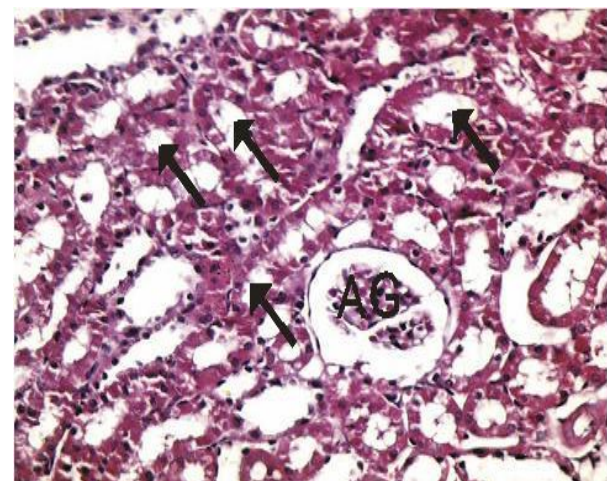
acid. Recovery group showed a significant increase in reduced glutathione levels as compared to L-thyroxin treated animals.



**Fig. 1:** Section of kidney of control rat showing normal structure of the cortex, glomeruli (G), proximal convoluted tubules (PT) and distal convoluted tubules (DT), (H&E., X400).



**Fig. 2:** Section of kidney of folic acid rat showing normal structure of the glomeruli (G), proximal (PT) and distal convoluted tubules (DT), (H&E., X400).



**Fig. 3:** Section of kidney of L-thyroxin treated rat showing atrophied glomeruli and the renal tubules showed cytoplasmic vacuolation (arrows), (H&E., X400).

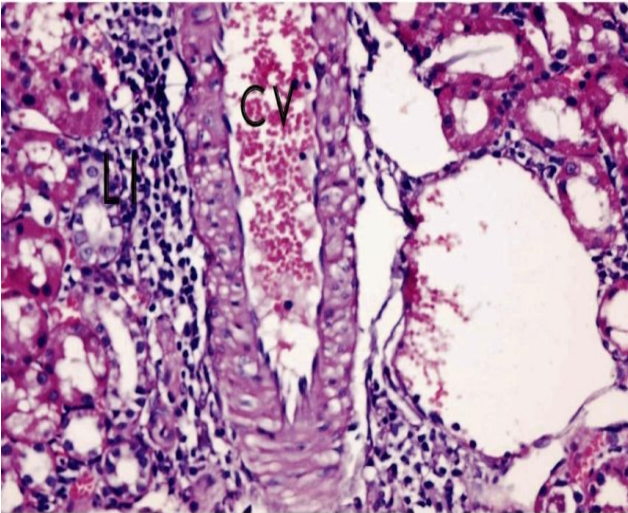


Fig. 4: Section of kidney of L-thyroxin treated rat showing variable pathological changes leucocytic infiltration and congested blood vessels (CV), (H&E., X400).

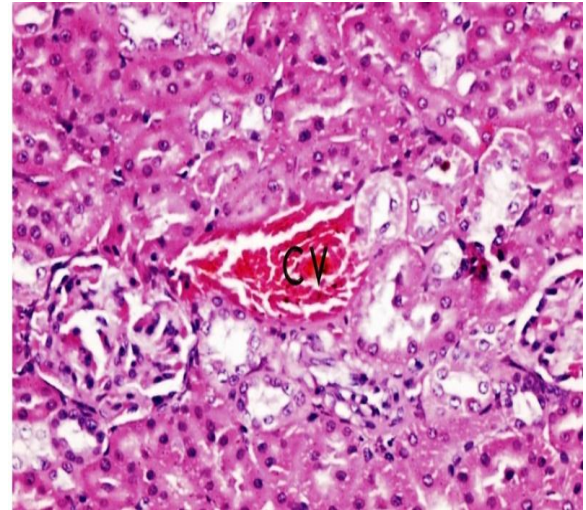


Fig. 7: Section of kidney of L-thyroxin treated rat showing congestion of blood vessels, damage in proximal and distal convoluted tubules, where their brush borders were detached, (H&E., X400).

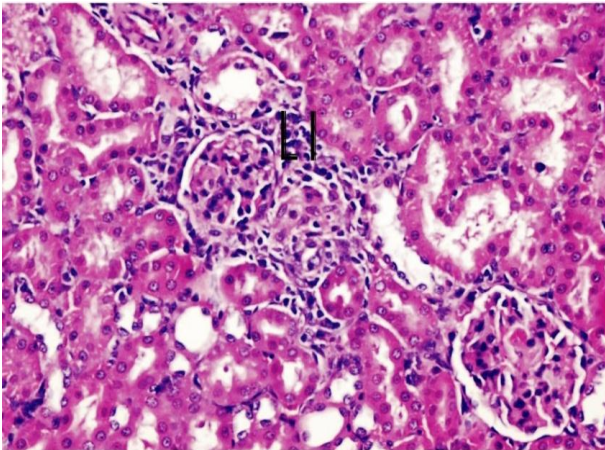


Fig. 5: Section of kidney of L-thyroxin rat showing leucocytic infiltrations, with damage in proximal and distal convoluted tubules (H&E., X400).

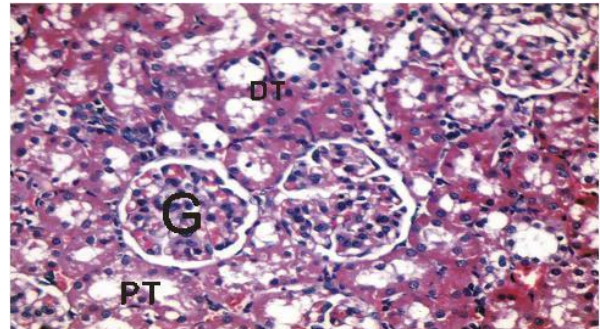


Fig. 8: Section of kidney of rat treated with L-thyroxin and folic acid showing normal tubules and glomerular structures with well-established epithelia, (H&E., X400).

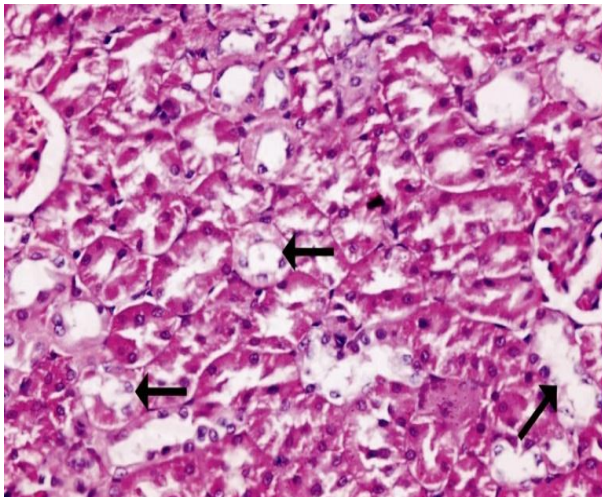


Fig. 6: Section of kidney of L-thyroxin rat showing swelling and cytoplasmic vacuolation of the epithelial lining of the renal tubules, (H&E., X400).

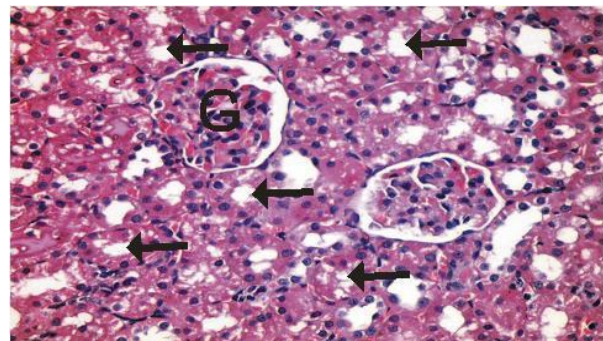


Fig. 9: Section of kidney of rat treated with L-thyroxin and folic acid showing normal proximal and distal convoluted tubules, (H&E., X400).

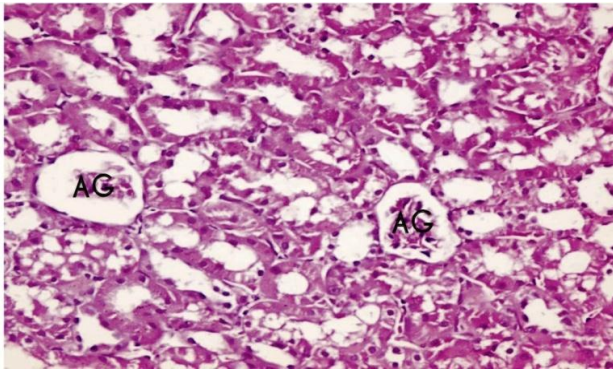


Fig. 10: Section of kidney of recovered rat showing atrophied glomeruli, (H&E., X400).

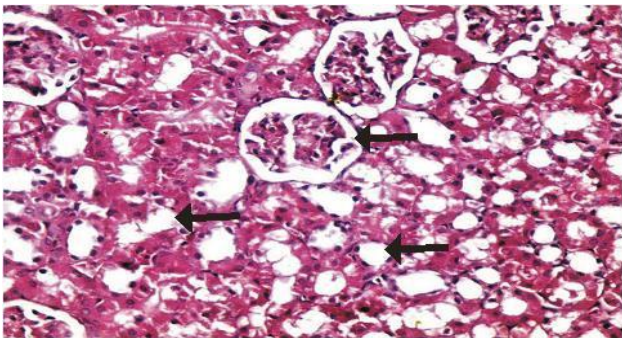


Fig. 11: Section of kidney of recovered rat showing damage in proximal and distal convoluted tubules which surrounded cytoplasmic vacuolation (arrows), (H&E., X400).

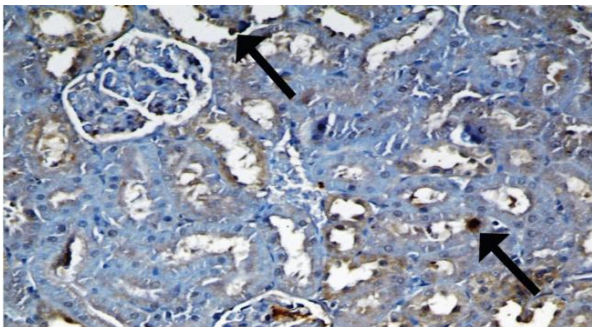


Fig. 12: Section of kidney of control rat showing few expression of PCNA, (PCNA immunohistochemical stain, X400).

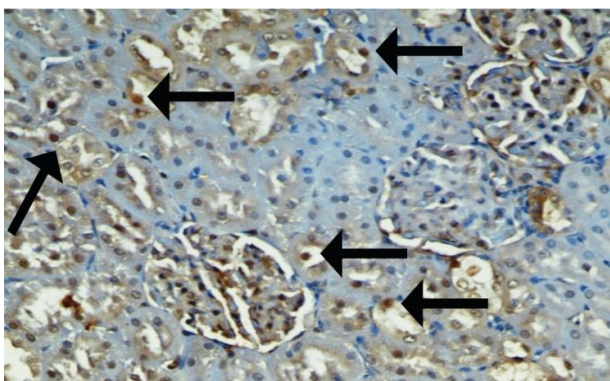


Fig. 13: Section of kidney of L-thyroxin rat showing Strong expression of PCNA, (PCNA immunohistochemical stain, X400).

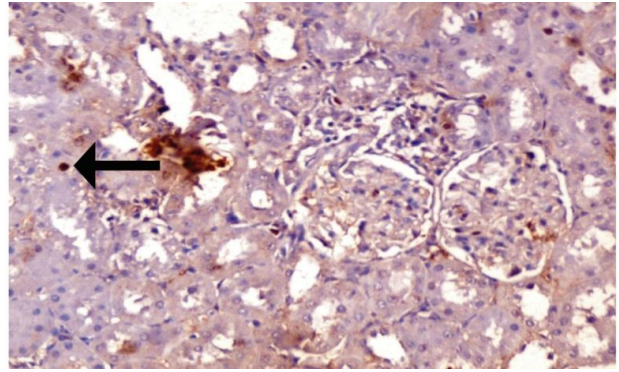


Fig. 14: Section of kidney of post treated rat showing mild positive expression of PCNA, (PCNA immunohistochemical stain, X400).

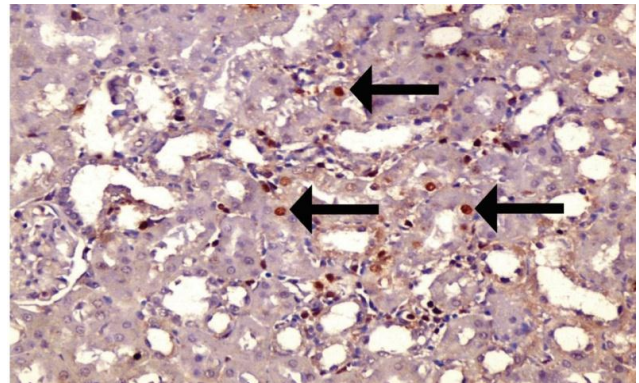


Fig. 15: Section of kidney of recovered rat showing Strong expression of PCNA, (PCNA immunohistochemical stain, X400).

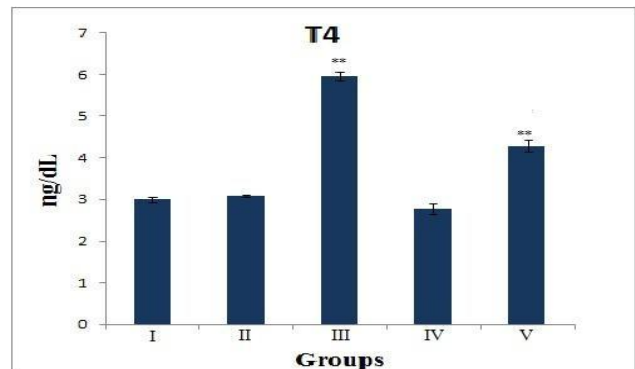


Fig. 16: Serum triiodothyronine (T4 ng/dl) levels in different groups.

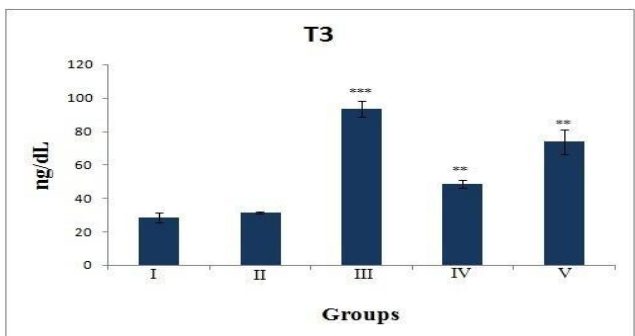


Fig. 17: Serum triiodothyronine (T3ng/dl) levels in different groups.

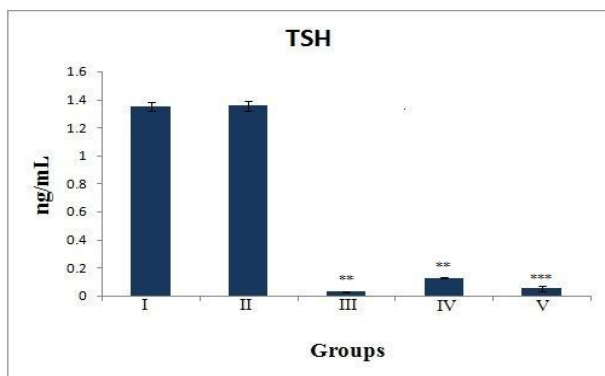


Fig. 18: Serum triiodothyronine (TSH ng/dl) levels in different groups.

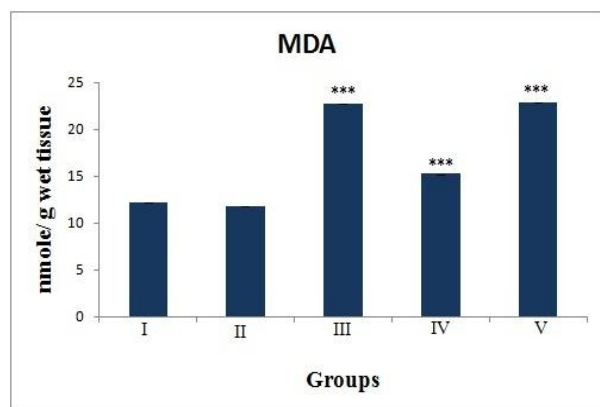


Fig. 19: Changes in kidney homogenate MDA level in different groups.

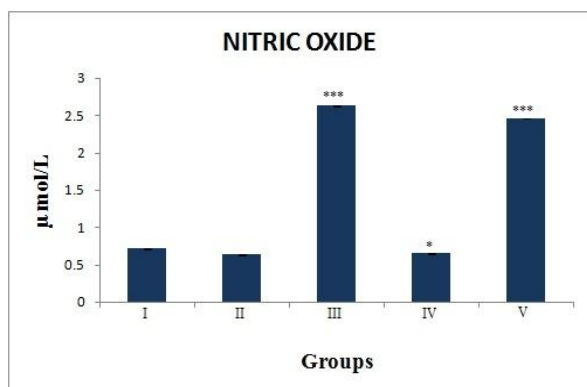


Fig. 20: Changes in kidney homogenate nitric oxide level in different groups.

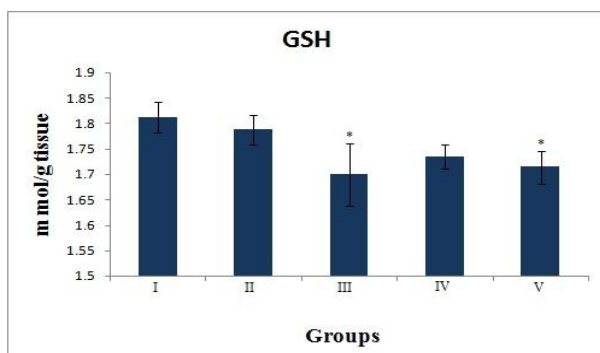


Fig. 21: Changes in kidney homogenate reduced glutathione level in different groups.

## DISCUSSION

Thyroidal status influences kidney function both during embryonic development and in the mature functioning of the kidney, directly by affecting glomerular function, the tubular secretory and absorptive capacities, electrolyte pumps and kidney structure and indirectly by affecting the cardiovascular system through its influence on renal blood flow which decrease in hypothyroidism. Also, kidney is involved in the metabolism and elimination of thyroid hormone.<sup>[4]</sup>

The current study showed that there was a significant increase in serum  $T_3$  level and significant decrease in serum TSH level in rats treated with L-thyroxin when compared to the control and folic acid groups. This indicates that L-thyroxin was suitable for induction of hyperthyroidism. The increased in production of  $T_3$  explained as L-thyroxin appears to exert its primary effect on the synthesis of the thyroid hormones, thyroxin ( $T_4$ ) and triiodothyronine ( $T_3$ ), by blocking oxidative iodination within the thyroid gland itself. In addition L-thyroxin alter the metabolism of thyroid hormones outside of the thyroid gland by interfering with the peripheral deiodination of  $T_4$ . The decrease in TSH secretion by the anterior pituitary gland as  $T_4$  and/or  $T_3$  exert a negative feedback effect on pituitary secretion of TSH.<sup>[24]</sup>

The obtained results revealed that treated rats with L-thyroxin induced many histopathological alterations in rat kidney. These alterations include leucocytic infiltrations, congestion of blood vessels and damage in proximal and distal convoluted tubules and glomeruli. In agreement with these results, Paydas et al.<sup>[25]</sup> reported that hyperthyroidism was accompanied with tubulointerstitial nephritis. Also, hyperthyroidism was found to cause ultrastructural changes in the kidney such as irregularity of microvilli and basal folding in the proximal tubules. The glomeruli showed basal lamina thickening, irregularity of pedicels and increase of mesangium.<sup>[26]</sup> Tashkandi et al.<sup>[27]</sup> reported that methimazole- hypothyroidism caused degenerative changes in glomeruli and in convoluted tubules of the kidney of rats.

Proliferating cell nuclear antigen (PCNA) is a stable cell cycle- related nuclear protein and represents a reliable marker for the proliferative activity. Immunohistochemical results showed an increase in the number of PCNA positive expressions in kidney following L-thyroxin administration in comparison to the control and folic acid. Similarly, Heron and Rakusan<sup>[28]</sup> demonstrated that hypothyroid/hyperthyroid endothelial cells of heart showed significantly higher PCNA.

Results of oxidative parameters revealed that there is a significant increase in MDA and NO in kidney homogenate of L-thyroxin group as compared to control group, while there is a significant decrease in GSH. These results are consistent with the results reported by

Asayama and Kato<sup>[29]</sup> who demonstrated increased malondialdehyde levels in liver, heart and muscles of rats. Similarly, malondialdehyde contents in liver, heart, and muscles rats were largely increased when treated with thyroxin.<sup>[30]</sup> Under these circumstances the free radicals react with lipids, protein, and DNA often causing irreparable damage that can lead to cell death. One of the deleterious consequences of oxidative damage is lipid peroxidation, which involves hydrogen abstraction from fatty acids by free radicals such as  $\cdot\text{OH}$  and once initiated is a self propagating process.<sup>[31]</sup> It has been suggested by Seven *et al.*,<sup>[32]</sup> that free radical scavenging enzyme activity can be induced by excessive formation of ROS in experimental hyperthyroidism. Previous studies have suggested that hyperthyroidism increased free radical production and lipid peroxidation levels and it has been documented that thyroid dysfunctions increases lipid peroxidation reactions and reactive oxygen species.<sup>[33,34]</sup> Lipid peroxidation is an autocatalytic mechanism leading to oxidative destruction of cellular membranes.<sup>[35]</sup> In the present study, tissues homogenate glutathione levels were decreased in L-thyroxin treated rats as compared to control rats, possibly secondary to increased ROS generation. These results are in agreement with the results obtained by Araujo *et al.*,<sup>[2]</sup> who state that the administration of L-thyroxin to rats resulted in decrease of glutathione concentration in the heart. The cellular glutathione plays an important role as biological antioxidant defense systems, which act as protective mechanisms against oxidative damage. Therefore, the decreased level of glutathione may be due to overproduction of free radicals and increased lipid peroxidation in hyperthyroidism.<sup>[28]</sup>

Treatment of hyperthyroid rats with folic acid showed an improvement in the biochemical, histological and immunohistochemical alterations that induced by L-thyroxin. These results are in agreement with a number of studies that declare the adjuvant role of folic acid. Mohamed *et al.*<sup>[36]</sup> reported that individually or combined folic acid and vitamin c caused relative enhancement in the thyroid hormones with a normal lipid profile in hyperthyroid rats. They added that folic acid and /or vitamin C reduced the oxidative stress. Folic acid proved to have remarkable protective effect against toxicity of methotrexate in albino rats in by minimizing the degenerative changes.<sup>[37]</sup> Experimental evidence has been proved that folic acid significantly decreased the levels of free radicals in the liver of rabbits treated with chromium.<sup>[38]</sup> Tousson *et al.*<sup>[39]</sup> mentioned that folic acid alleviates oxidative stress and hyperhomocysteinemia involved in liver dysfunction of hypothyroid rats. Mohammadian *et al.*<sup>[40]</sup> suggested that folic acid protects the liver against cholestasis by reducing serum Hcy and by its antioxidant properties. In conclusion, the current study indicated that hyperthyroidism in male rats was associated with oxidative stress and other alterations in kidney of albino rats. Treatment of hyperthyroid rats with folic acid leads to an improvement in biochemical

and histopathological alterations caused by l-thyroxine via its antioxidative action.

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