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Impact of Continuous Treatment with Propylthiouracil on Renal and Hepatic Functions in Rabbits

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ABSTRACT

This study was designed to investigate the effect of continuous treatment with the anti-thyroid drug, propylthiouracil (PTU), on renal and hepatic functions in rabbits as an experimental animal model. Animals were randomly divided into four different isolated groups (n = 10); Group I received normal saline. Group II, III, and IV were daily administrated with PTU in oral dosing of 50, 75, and 150 mg/kg, BWT, respectively, for three successive weeks. Serum T_3 and T_4 levels were measured in all groups. Increased serum creatinine and blood urea nitrogen levels (P<0.05) were also associated. Moreover, liver enzymes levels, aspartate aminotransferase, alanine aminotransferase, and total serum cholesterol levels showed a significant increase in a dose and time decedent manner. Thyroid glands of PTU-treated rabbits showed variable sized-follicles lined by multiple layers of follicular cells, which displayed signs of hyperactivity as the average follicular cell height. The diameter of its width was significantly increased compared with that in the control group. Besides, follicles were filled with a variable quantity of low-dense vacuolated colloids. Kidneys of such animals showed tubulointerstitial nephritis, glomerular atrophy, and multiple focal areas of mononuclear cell reaction. While the observed hepatic lesions were in the form of severe congestion in central vein and hepatic artery, hepatocellular necrosis, and granulocytic lymphoid cellular responses around portal areas associated with peri-portal fibrosis. Such lesions were dependent on doses of PTU. This study referred to that continuous treatment with an antithyroid drug PTU induced a hypothyroid state that was associated with impaired renal and hepatic functions in rabbits.

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INTRODUCTION

The thyroid gland plays a vital role in the tissue metabolism of different organs and systems (**Deepika et al., 2015**). The thyroid hormones control the basal metabolic rate, and so any altered functions of the thyroid are reflected on many body organs. The thyroid dysfunctions could be one of the most critical disorders in the rabbit (**Rodríguez-Castelán *et al.*, 2015, 2017**). The experimental studies on thyroid dysfunctions and the changes in body metabolic activities are based on the suppression of thyroid hormones' (tri-iodothyronine; T_3 and tetra-iodothyronine; T_4) production (**Silva *et al.*, 2004**). Failure of the thyroid to produce the requirement of thyroid hormones is known as hypothyroidism, and it may be mild, moderate, or severe (**Bushra *et al.*, 2016**).

Many drugs have been shown to alter thyroid functions in humans and animals and to affect more than one aspect of the thyroid hormones' physiology

(**Kaptein *et al.*, 1994**). Many compounds have the ability to inhibit the thyroid hormones' synthesis, irrespective of their mechanism of action, and they are collectively called the goitrogens. As a result of the decreased serum thyroid hormone levels, the thyroid-stimulating hormone (TSH) secretion is enhanced, which is responsible for the induction of goiter (**Sarne, 2016**).

The drugs used in the management of hyperthyroidism are chemically thionamides compounds (methimazole, carbimazole, and propylthiouracil). They are relatively simple thionamide molecules (**Awad *et al.*, 2018**). The propylthiouracil (6-propyl-2-thiouracil; PTU) is one of the main anti-thyroid drugs used in the United States (**Heidari *et al.*, 2015**). Treatment with PTU significantly increased the plasma lipoprotein T_3 and T_4 levels in normal rabbits (**Çelik *et al.*, 2000**) and markedly increased the TSH levels (**Bhanja and Chainy, 2010)**, that protected against the vascular injury in the hypercholesterolemic rabbit (**Freyschuss *et al.*, 1993**).

There is a well-known interaction between the thyroid and kidney functions. Thyroid hormones are involved in the growth and functions of the kidney, and the kidney normally plays a vital role in the metabolism, degradation, and excretion of thyroid hormones. In addition, the PTU-induced hypothyroidism can cause a kidney failure associated with vasculitis, lupus nephritis, or necrotizing glomerulonephritis with pulmonary hemorrhage (**Yu *et al.*, 2007**). Hypothyroidism affects the renal blood flow, glomerular filtration rate, water and electrolyte balance, and kidney structure (**Vargas *et al.*, 2006**). It also increased serum creatinine levels and induced hypernatremia (**Basu and Mohapatra, 2012**). Moreover, the elevation of serum creatinine levels is reversible in humans (**Karanikas *et al.*, 2004**). Additionally, some authors have reported that an elevation of the serum creatinine is associated with subclinical hypothyroidism (**Verhelst *et al.*, 1997**).

The thyroid hormones regulate the basal metabolic rate of hepatocytes and thus modulate the hepatic function, and the liver, in turn, metabolizes the thyroid hormones and regulates their actions. Therefore, the thyroid dysfunction may disturb the hepatic functions, and in turn, hepatic diseases adversely affect the thyroid function (**Malik and Hodgson 2002**). Consequently, it is not surprising that hepatic dysfunction was commonly observed in patients with thyroid disease (**Ibrahim *et al.*, 2016**). Previous studies suggest that the PTU-induced hepatotoxicity is likely to be hepatocellular at the level of mitochondrial injury rather than cholestasis (**Mete *et al.*, 1993**). Furthermore, PTU causes lipid peroxidation in the liver, which exerts oxidative stress on the liver tissue. On the other side, there is no specific antidote for such PTU-induced hepatic injury (**Karamikhah *et al.*, 2015**). There are some reports of PTU-induced liver failure and death (**Rivkees and Mattison, 2009**).

The New Zealand White rabbit has been previously used as an animal model to facilitate the pathological, physiological, and molecular studies of the hypothyroid-induced myxedema coma and evaluate its therapeutic interventions (**Ono *et al.*, 2016**). The present study aimed to investigate the possible correlations between the renal and hepatic functions in rabbits and the given drug (PTU), to assess the histo-morphometrical changes in thyroid glands, and to record the pathological changes in thyroid glands, kidneys, and liver induced by the different doses of PTU treatment in rabbits.

MATERIALS AND METHODS

Forty male White New Zealand (*Oryctolagus cuniculus huxley*) rabbits, weighing (1200±127 g), and aged 2-3 months were used in this study. To preclude the fluctuating levels of sex hormones in females, male rabbits were selected. Animals were maintained in the experimental farm of the Department of Animal Behavior and Husbandry, Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt. Rabbits were caged singly and fed a standard diet and water *ad libitum.* Rabbits were provided with proper ventilation under natural light/dark photoperiod and were kept under a temperature of 20±2°C (***Seddik et al., 2019***).

Experimental design

Rabbits were maintained two weeks before experimentation for accommodation. Then rabbits were randomly divided into four groups, ten rabbits each as follows:

Groups/number	Daily treatment for 3-week with
First group: GI: (n =10)	Physiological saline solution, 5 ml per os /rabbit.
Second group: GII: (n =10)	PTU (50 mg/kg) in 5 ml saline per os /rabbit.
Third group: GIII: (n =10)	PTU (75 mg/kg) in 5 ml saline per os /rabbit.
Fourth group: GIV: (n =10)	PTU (150 mg/kg) in 5 ml saline per os /rabbit.

Animals were clinically examined for any signs during the experimental duration (**Celik *et al.*, 2000; Abdolhosseinipoor and Sadeghi-Dinani, 2018**).

Chemicals and reagents

Propylthiouracil (PTU) was purchased from Sigma-Aldrich, Co., Inc. (St. Louis, MO, USA).The T_3, T_4, and TSH kits were purchased from Vidas® (Human Gesellschaft für Biochemica und Dignostica mbH, Wiesbaden, Germany). The kits for estimating the serum creatinine, BUN, AST, ALT, and total cholesterol were purchased from Spectrum-diagnostics, Egypt.

Serum samples

Blood samples were obtained from the ear vein (n = 10/each group) at 0, 1st, 2nd, and 3rd weeks of the experiment. The serum was separated by centrifugation at 5000 ×g for 10 min and kept under -20 °C for analysis.

Serum biochemical analysis

Thyroid hormones, tri-iodothyronine (T_3), and tetra-iodothyronine (T_4), as well as thyroid-stimulating hormone (TSH), were determined according to (Carayon *et al.*, 2002). Serum creatinine and blood urea nitrogen (BUN) and were assayed spectrophotometrically, as previously described (Burtis, 1999). Activities of the serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT), as well as the concentration of total serum cholesterol, were assayed according to **Schumann and Klauke (2003)**.

Histopathological assessment

At the end of the experiment, all animals were anesthetized using isoflurane inhalation, sacrificed, and carefully dissected. The thyroid, kidneys, and liver from each animal were fixed in 10% formalin. All samples were embedded in the paraffin wax to prepare paraffin blocks according to **Bancroft and Stevens (1982)** to prepare tissue slides and stained with Hematoxylin and Eosin (H&E). The tissue slides were examined and observed under a microscope (Olympus CX 41 RF) connected to a computer through a digital camera (Olympus E-330). The photomicrographs were obtained and processed using cell B software.

Histo-morphometric assessment

Measurement of the thyroid epithelial cell height and the width of each follicle (Peksa *et al.*, 2011) were taken in 5 non-overlapping fields from 5 different H&E-stained sections/animal at a 400× magnification, using an image processing software (Image J versus 1.48, AD position, China) (**Abràmoff *et al.*, 2004**).

Histopathological scoring

Ordinal method for validating histo-pathological scoring was used to assign a score for each lesion observed in each PTU administrated group based on the tissue histopathological examination. Photomicrographs of histological preparations from the thyroid, kidney, and liver lesions were scored by 2 independent veterinary pathologists who were unaware of the treatment or its information. A mutual re-evaluation determined initial scoring inconsistencies. The score was based on the severity in the examined tissue; 0 = no lesions; 1 = mild (1 to 25% affected); 2 = moderate, (26 to 45% affected) and 3 = severe (> 45% affected) (**Gibson-Corley *et al.*, 2013**).

Statistical analysis

The data obtained were analyzed statistically using GraphPad Prism software. Two-way analysis of variance (ANOVA) followed by Tukey's post-test was performed for serum hormonal and biochemical analyses. Pearson's correlation analysis was used for association study if r = 0.2-0.4 a weak correlation, r = 0.4-0.7 a moderate correlation, and strong correlation if r =7-1. The data of histo-morphometrical and histopathological scoring were analyzed by one-way ANOVA followed by Tukey's post-test. Data were expressed as mean ± SD. *P*-value <0.05 was considered significant.

RESULTS

Clinical Findings

Clinical symptoms of hypothyroidism appeared within the third week of daily oral administration of PTU and included general weakness, inactivity, and animals were reluctant to move.

Effect of PTU on serum thyroid hormones level

Two-way ANOVA-repeated measures followed by Tukey's post hoc test revealed the mean values of T_3 (ng/ml) and T_4 (µg/ml) were significantly decreased in relation to increased duration of the treatment (Fig.1a). The highest values of the T_3 were reported in the control group (94.4± 5.01 ng/ml), while the lowest values of this hormone were observed in the group (IV) treated with the highest dose of PTU (61.0 ± 4.2 ng/ml). Similarly, the highest mean value of T_4 was reported in the control group (3.34 ± 0.96 µg/ml) while the lowest values of this hormone (0.95 ± 0.17 µg/ml) were reported in group II at the 3rd week of PTU treatment. Significant changes in TSH mean values were observed with an increased duration of time. The highest values of the hormone (0.06 µIU/ml) were recorded in group IV compared to the lowest mean value of the TSH was 0.01 (µIU/ml) in the control group (Fig.1a).

Effect of PTU on kidney and liver functions

Positive correlations existed between the mean values of blood urea nitrogen (BUN) and creatinine and the increased duration of experimental time [r=0.46]. The lowest values of BUN and creatinine were reported in the control group, while the highest values of both elements were seen in group IV at three weeks post-treatment with PTU (Fig.1b).

Total serum cholesterol level was assessed because hypercholesterolemia is a common finding among hypothyroid patients; the same pattern was found in the mean values of serum AST, ALT, and cholesterol levels; these values were significantly increased with prolonged duration of treatment (Fig. 2). The lowest mean value of them was recorded in the control group, and the highest values of all elements were seen in group IV (150 mg PTU/kg, BWT/day). The overall results indicated that PTU treatment for three weeks reduced the thyroid, renal and hepatic functions in terms of reduced serum thyroid hormones, BUN, creatinine, AST and ALT in rabbit (Fig. 1 & 2).

Thyroid histopathological changes induced by PTU

Thyroid glands, kidneys and liver from control and PTU-treated groups were free from any gross pathological changes. Follicles of thyroid glands from control rabbits revealed normal thyroid glandular architectures and thyroid follicles were lined with cuboidal epithelium with dense colloid which was rarely vacuolated (group I, Fig. 3a). The follicles of thyroid glands from PTU-treated groups showing histopathological changes as an apparent increase in follicular size (Fig. 3b-d, arrowheads), which exhibited peripheral low dense colloid with extensive vacuolations (Fig. 3b-d, stars), groups of follicles were lined with multiple layers of the vacuolated epithelium (Fig. 3b-d, red arrowheads), some pools of follicular cells were devoid of the lumen (interfollicular hyperplasia, Figs. 3b-d, white arrowheads). The presence of dilated blood vessels engorged with blood and congested infiltrating capillaries were also recorded (Fig. 3b-d, black arrows).

The mean follicular cell height of PTU-treated groups was significantly increased ($P<0.05$) compared with that of the control group (Fig. 4a). In addition it showed significant most extended linear dimensions (width of follicles) compared with the control group (Fig. 4b). Thyroid colloid vacuolation was significantly increased with the dose of PTU which indicates decreasing in its density (Fig. 4c). The severity of lesions varied from mild in the group II to moderate group III, till severe in the group IV. Such rigors were interrelated with the administrated dose of PTU.

Renal histopathological changes induced by PTU treatment

Microscopic examination of the kidney from control rabbits revealed more or less normal renal parenchyma (Figs. 5a and b). Histopathological examination of the kidneys from group II (50 mg PTU/kg, BWT/ day) showed that the lesions were present in renal tubules, glomeruli, and the renal vasculature.

Primary changes were present in renal tubules especially those in the cortical area. The renal tubular epithelium showed a series of degenerative changes up to necrosis; both proximal and distal convoluted tubules were affected. The tubular epithelium sometimes showed lysis of the cytoplasm and pyknosis of the nucleus. Sometimes the necrotic epithelium sloughed and the tubules were dilated. Changes in the tubules were also diffuse and involved a massive area of the cortex (Figs. 6a-d).

Changes of the glomeruli were focal and less severe than those of the tubules, consisted of atrophy, distortion, and hypocellularity of the glomerular tuft. Bowman's space was widened and the nuclei of the remaining capillary tuft were condensed (Figs. 6a-c). The interstitial capillaries were congested. In some cases, the blood vessels at the cortico-medullary junction were congested with a very mild perivascular edema (Figs. 6c-d). Focal small and multiple areas of lymphoid cell reactions were seen in the interstitial tissue (Figs. 6b-c). Pathological changes in the kidney of this group were less severe, less diffuse, and include a limited area of the renal tissue.

Microscopical examination of kidney sections stained with H&E from group III (75 mg PTU/kg, BWT/ day) revealed micro-morphological changes in the renal parenchyma, interstitial tissue, and renal vasculature. In the renal parenchyma, the tubular epithelium was vacuolated, cystically dilated and showed necrotic changes (Figs.7c and d). The glomerular were atrophied, distorted and showed hypocellularity with a prominent condensation of nuclei, mesangial and endothelial cells of its capillaries (Figs. 7a-c). Focal areas of lymphoid cell reactions were seen in the interstitial tissue. Severe hemorrhage and congestion of central vein (Figs.7a and b). Histopathological changes in this group were massive, diffuse and encompassed a large area of renal tissue compared to the preceding group.

The kidneys of the IV group (150 mg PTU/kg, BWT/ day) showed severe necrotic changes of renal tubular epithelium in both cortex and medulla (Fig. 8), the necrotic changes were severe and diffuse results in loss of the entire epithelium. They prominently atrophied, hypocellular and were associated with widening of the bowman's space (Figs. 8a-c). Inter-tubular blood vessels were congested and hemorrhage occurs at the cortico-medullary junction (Fig. 8b-d).

Histopathological scoring of renal lesions revealed a significant increase in the severity of glomerular atrophy (Table 1), and renal tubular damage (i.e., tubular dilatation, epithelium vacuolation and necrosis) (Table 1) were dependent the dose of PTU treatment.

Table 1: Scoring of the renal histopathological changes induced by PTU-treatment				
Lesions	**Control**	**Group II**	**Group III**	**Group IV**
Renal glomeruli:				
Atrophy, distortion, hypocellularity of glomerular tuft,	0	2	3	3
Bowman's space widening,	0	2	2	3
Nuclei condensation	0	1	2	3
Renal tubules:				
Epithelium vacuolation, degeneration, necrosis, sloughing.	0	2	3	3
Cytoplasmic lysis, nuclear pyknosis,	0	1	2	3
Tubular dilatation.	0	1	3	3
Renal vasculature				
Interstitial capillaries congestion.	0	1	2	3
Haemorrhage.	0	1	3	3
Perivascular oedema.	0	1	2	2
Interstitial tissue areas of lymphoid cell reactions.	**0**	2	3	3

The score was based on the severity in the examined tissue; 0 = no lesions; 1 = mild (1 to 25% affected); 2 = moderate, (26 to 45% affected) and 3 = severe (> 45% affected).

Fig.1

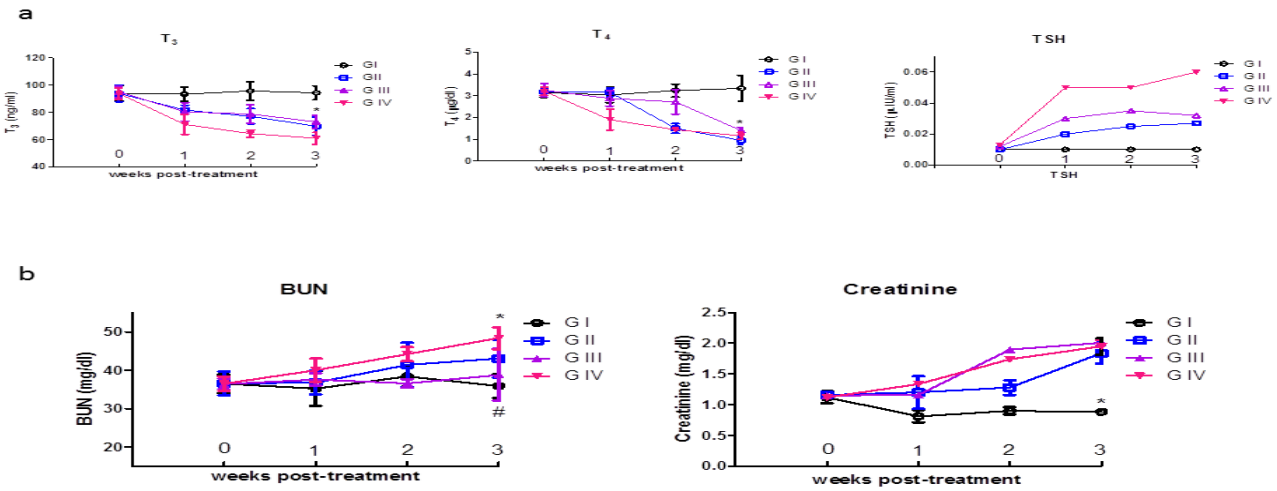

Fig. 1. Serum T_3 T_4 and TSH levels (a) and blood urea nitrogen and creatinine levels (b) at the 0, 1st, 2nd, 3rd weeks post-treatment with propylthiouracil.

Hepatic histopathological changes induced by PTU-treatment

Histopathological examination of the liver from control rabbits (group I) revealed normal hepatic architectures which comprised hepatic lobular size, normal central vein, hepatic cords, hepatocytes and portal area contents (bile duct, hepatic artery and vein) (Figs. 9 a and b).Liver sections of PTU-treated rabbits showing variable hepatic injury differed in severity according to the administrated dose. In Group II (50 mg PTU/kg, BWT/ day), liver sections showed vascular congestion (Figs. 10a-d). Hepatocellular necrotic changes, as well, losing of central veins entire endothelial lining (Figs. 10a-c). Granulocytic cellular reactions were observed around portal areas. Also, dissociation of hepatic cords arrangements perhaps affected hepatic architectures (Fig. 11d). Hepatic changes observed in group III (75 mg PTU/kg, BWT/ day) revealed a critical hepatic injury in the form of severe dilatation in the central vein which engorged with thrombotic blood due to the desquamated lining endothelium (Figs. 11b-d). Hepatocellular necrosis, loss of hepatic architectures, and early periportal fibrosis were also noted (Fig. 12a and c).

Liver sections from group IV (150 mg PTU/kg, BWT/ day) rabbits showed massive histopathological changes compared with the preceding groups (Figs 12). Based on histopathology scoring, the severity of hepatocellular necrosis and damage, periportal fibrosis with or without lymphoid cellular reaction, and vascular congestion with or without endothelial damage were significantly increased with the increased dose of PTU compared with the control group (Table 2).

Table 2: Scoring of the hepatic histopathological changes induced by PTU-treatment

Lesions	**Control**	**Group II**	**Group III**	**Group IV**
Hepatocellular necrosis.	0	2	3	3
Dissociation of the hepatic cords arrangements.	0	1	2	3
Vascular congestion + / - endothelial damage	0	2	3	3
Losing of the central veins entire endothelial lining.	0	2	2	3
Severe central veins dilatation.	0	2	3	3
Central veins engorgement with thrombotic blood.	0	2	2	3
Peri-portal fibrosis + / - lymphoid cellular reaction	0	1	2	3

The score was based on the severity in the examined tissue; 0 = no lesions; 1 = mild (1 to 25% affected); 2 = moderate, (26 to 45% affected) and 3 = severe (> 45% affected).

Fig. 2

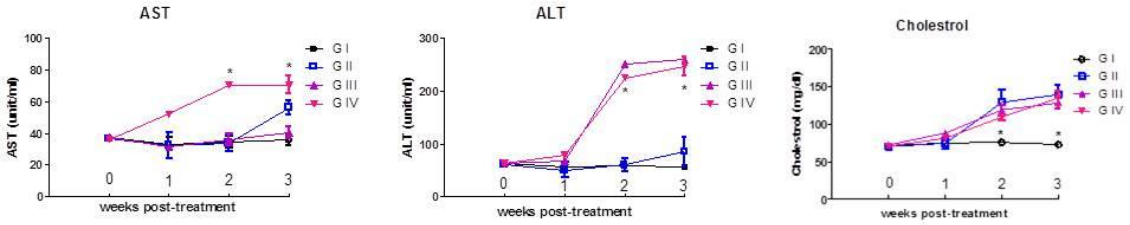

Fig. 2. Serum AST, ALT and total cholesterol levels rabbits at the 1st, 2nd, 3rd-week post-treatment with propylthiouracil.

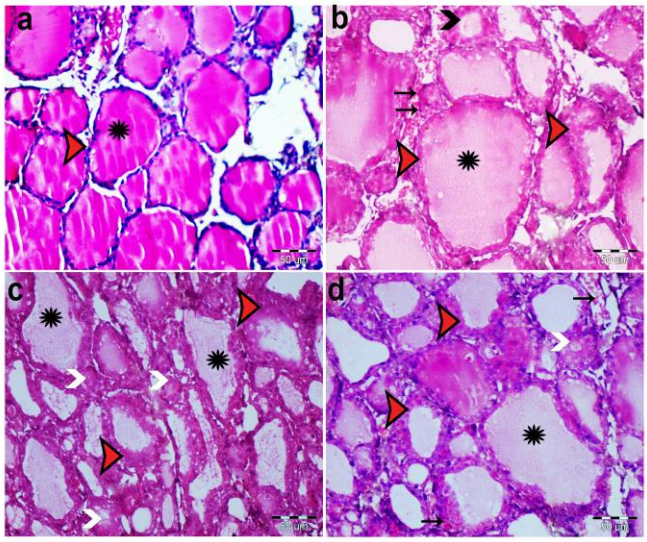

Fig. 3. Photomicrographs of thyroid glands stained by Hematoxylin and Eosin (H&E), showing (a) group I (Control) demonstrating thyroid follicles lined by cubical follicular cells exhibiting rounded nuclei (red arrow) filled with dense colloid (star).

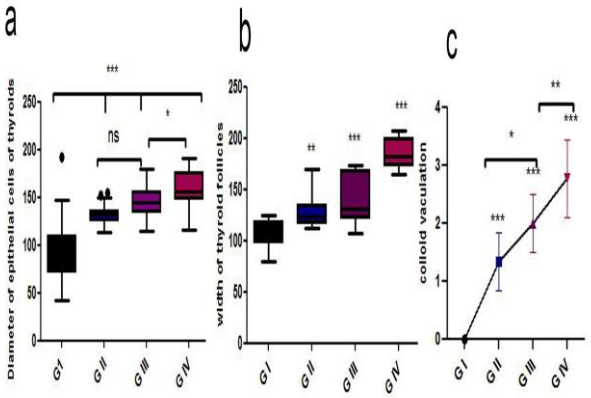

Fig.4. Histomorphometry graph showing the effects of propylthiouracil (PTU) on epithelial cell heights in thyroid glands. (b) Plot showing quantitative measurements of thyroid follicular width. (c) Qualitative observations, data shown density and vacuolation of follicular colloid.

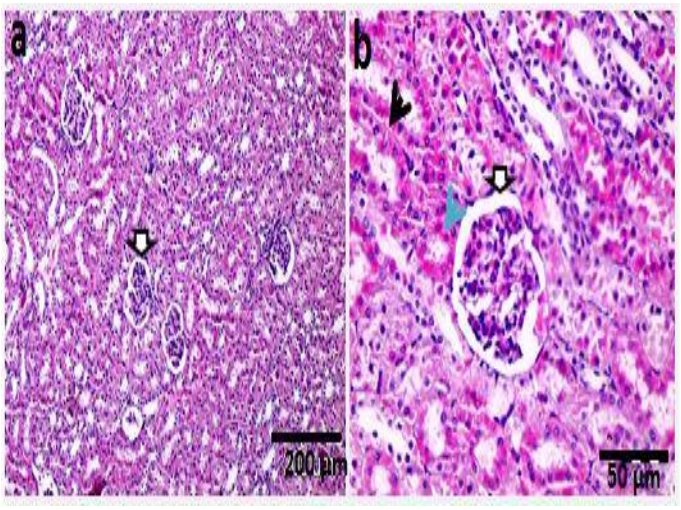

Fig. 5. Photomicrographs of control rabbits' kidneys (group I), stained by H&E (a & b), renal cortex (white arrow), proximal (blue arrows) and distal convoluted tubules (black arrow).

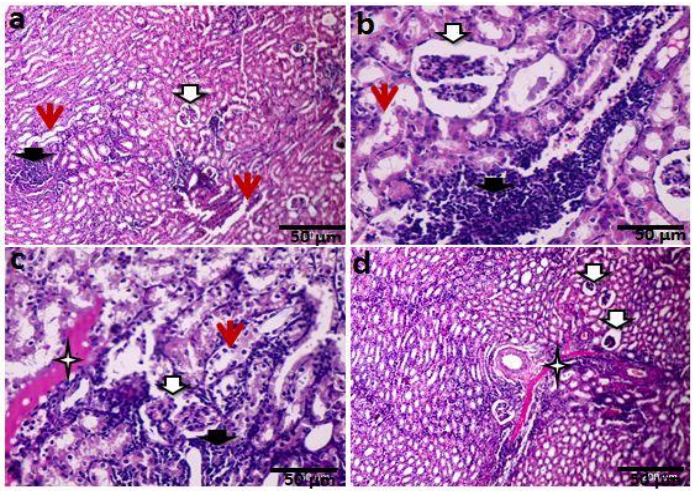

Fig. 6. Photomicrographs of hypothyroid rabbits kidney group II, stained by H&E, changes in renal corpuscles (white arrows) and urinary tubules, atrophied glomeruli (white arrows) and degenerated renal tubular epithelium (red arrows). Blood vessels at the cortico-medullary junction were congested with a very mild perivascular edema (c, d stars). Aggregations of lymphocytes (black arrows).

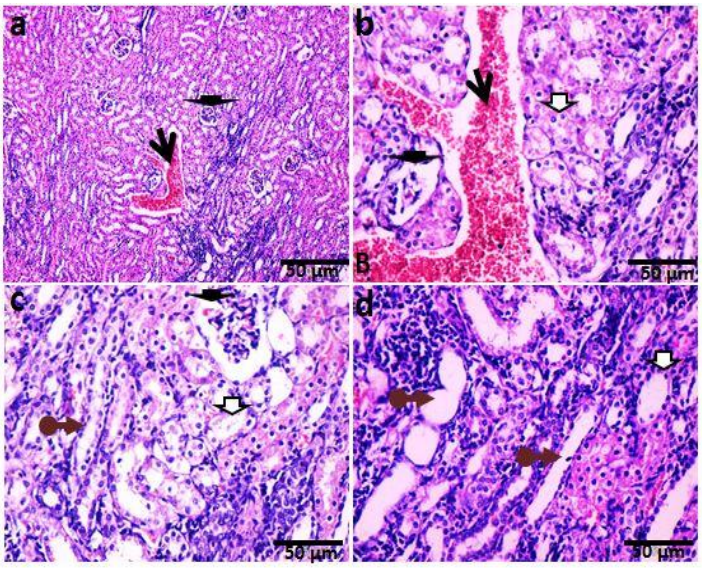

Fig. 7. Photomicrographs of hypothyroid rabbits kidney group III showing tubule-interstitial nephritis, with atrophied renal corpuscles (black arrowheads), degenerated epithelium in proximal urinary tubules (white arrows), dilatation and losing of lining epithelium in distal tubules (brown arrows). And congested and thrombosis vessels (black arrows).

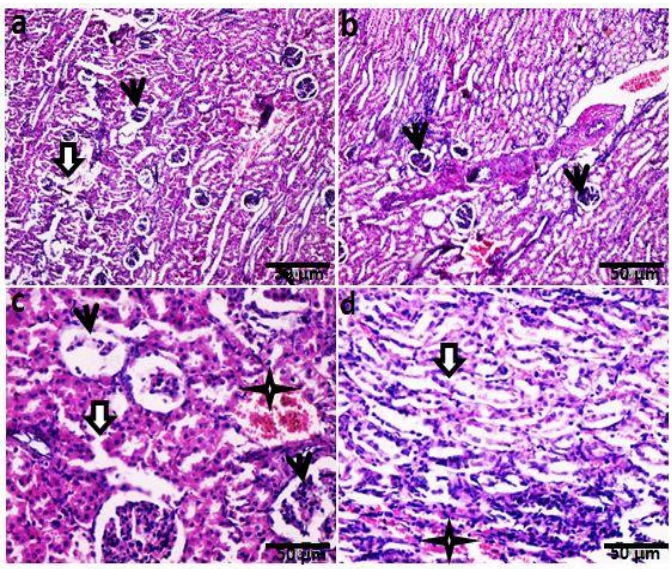

Fig. 8. Photomicrographs of kidney group IV, showing severe nephritis in the form of atrophied renal corpuscles (black arrows), urinary tubules showing cystic dilatation and losing of the epithelium (white arrows). Congestion and thrombosis and lymphoid aggregation (star).

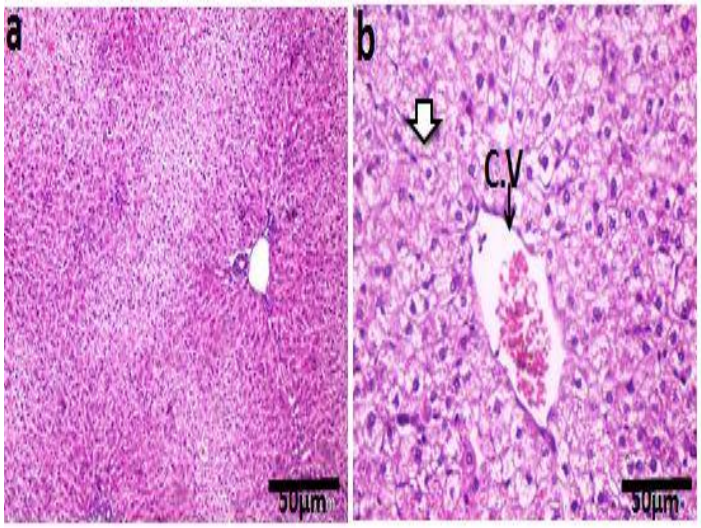

Fig. 9. Photomicrographs of rabbits liver group I (Control), stained by H&E. (A and B), showing normal hepatic architecture, central vein (CV), hepatocyte (h), sinusoid (S).

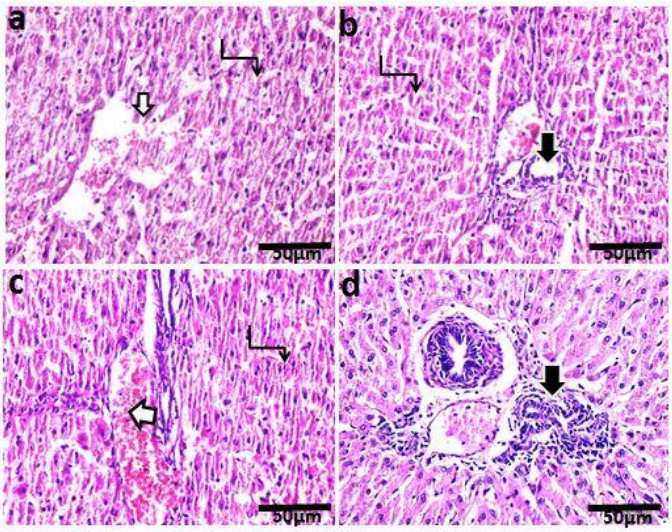

Fig. 10. Photomicrographs of rabbits liver group II, showing severe congestion in central vein and hepatic artery (white arrows). Hepatocellular necrosis, desquamation of necrotic tissue around the central vein and pre-portal lymphocytic reaction (black arrows) dissociation of hepatic cords architectures (black elbow arrows).

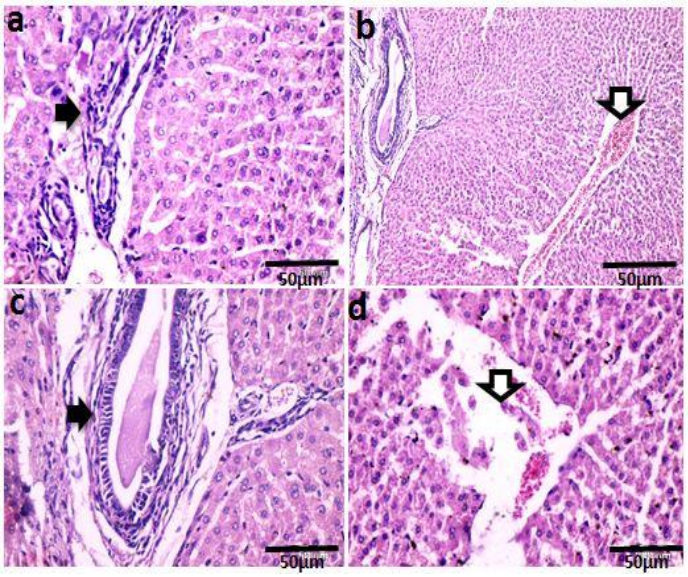

Fig. 11. Photomicrographs of rabbits liver group III showing severe hepatic injury in the form of severe dilatation in the central vein, CV was engorged with blood which makes thrombus with the desquamated endothelium (b, d), (white arrows) and hepatocellular necrosis, losing of hepatic architectures and early pre-portal fibrosis (a, c) (black arrows).

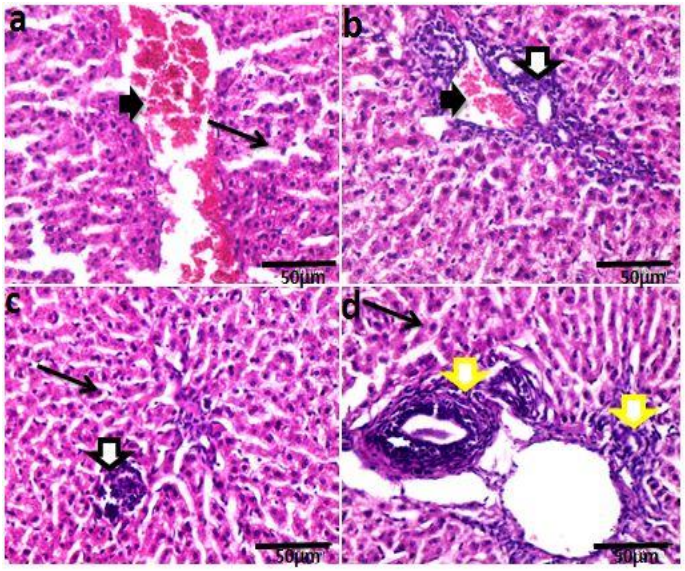

Fig. 12. Photomicrographs of rabbits liver group IV showing severe hepatic damage in the form of severe congestion in central vein and hepatic artery (a and b) (blackhead arrows), hepatocellular necrosis, sinusoid dilatation (black arrows), and focal lymphocytic aggregations (b, c) (white arrows) and peri-portal fibrosis (d) (yellow arrows).

DISCUSSION

A hyperplastic goiter was induced in rabbits using different doses of PTU. Thyroid dysfunction (hypothyroidism) was diagnosed using some biochemical markers as the determination of T_3, T_4, and TSH in the serum of experimental animals which were significantly decreased when correlated with those of the control animals (**Bhanja and Chainy, 2010**). It had been postulated that PTU act on thyroid peroxidase, so it inhibits the production of thyroid hormones by interfering with thyroid peroxidase-mediated iodination of tyrosine residues in thyroglobulin. PTU also inhibits coupling steps in thyroxin production, as well as the peripheral conversion of T_4 to T_3 (**Mechanick and Davies, 1997; Cooper, 2005**).

We observed clinical signs consist of lethargy, reluctant to move, muscular weakness, emaciation and slow reflex appeared on rabbits after treatment with PTU. A low basal metabolic rate was indicated by trails of animals huddling together and keeping their bodies near the sources of heat, which may be due to their intolerance to cold (**Mahmoud and Nishikawa, 2016**). Previous studies also reported similar signs in rabbits (**Çelik *et al.,* 2000**). In our opinion, the observed symptoms were due to the effect of given drugs on the production of thyroid gland hormones (T_3 and T_4).

Hypothyroidism induced experimentally using different doses of PTU was associated with prominent pathophysiology and patho-morphology of the kidneys. Impairment of renal function was documented by an increased level of biochemical markers of renal function mainly urea and creatinine (Fig. 1b). The serum level of both elements was significantly increased in rabbits after treatment with PTU when compared to those of normal control group. These findings may be because in PTU-induced hypothyroidism, there is a reduction in renal blood flow and glomerular filtration rate and hence reduced the clearance of creatinine and urea from the blood (**Ingbar and Braverman, 1986**).

Moreover, hypothyroidism, associated with kidney dysfunction, seems to be more related directly to a reduction in thyroid hormone levels (**Suher *et al.,* 2005; Lippi *et al.,* 2008**). Confirming our results, hyperuricemia due to hypothyroidism is associated with increased serum creatinine and decreased creatinine clearance; it was also proposed that hypothyroid hyperuricemia results from the reduction in the renal plasma flow and decreased glomerular filtration which originated from deficiency of thyroid hormones (**Mclanghlin and Mactier, 1994**).

Thyroid disorders in patients often accompany disturbances in liver functions (**Ibrahim *et al.,* 2016**). In this study mean serum AST values were significantly higher when compared with control groups (Fig. 2). The increase in AST and ALT after treatment with PTU may not be direct effects of the anti-thyroid drug per se but maybe resultant from thyroid dysfunction. Our results were in agreement with those previously reported (**Kubota *et al.,* 2008**). **Oren *et al*.** (**1998**) considered the reduction in total T_3 level as an adaptive change in the hypothyroid state that opt to decrease the basal metabolic rate within hepatic cells and keep hepatic function and total body proteins stores. Bushra and his colleagues reported that any alteration of thyroid action in women would affect the system of many organs and change many enzymes level, including liver enzymes, AST and ALT (**Bushra *et al.,* 2016**), that confirmed our results.

Thyroid hormones are potent to influence multiple aspects of lipid, carbohydrate, protein, and mineral metabolism (**Baxter and Webb, 2009**). It has been shown that hypothyroidism was associated with metabolic syndrome and disturbance in lipid metabolism (**Hanirex and Kaliyamurthie, 2013**). In this report, we found the serum cholesterol mean values showed a significant increase in a duration-dependent manner in the PTU-treated groups (Fig. 2), our observation was similar to those reported in goats (**Ibrahim *et al.,* 1984**).

Past trails in that way were shown by Hoogerbrusge *et al.* (**Hoogerbrusge v.d Liaden *et al.,* 1990**), they indicated that total cholesterol (TC) was higher in hypothyroid animals and human (**Oribe, 1989**). Also, **Lanol *et al.* (1986)** found a higher TC, but it was normal in clinically hypothyroid subjects. The plasma concentration of TC was also increased in primary hypothyroid states (**Muis *et al.,* 1985**), which support our results. These results are in line with our experimental observations in which serum TC was elevated in PTU-treated rabbits which succeed in inducing hypothyroidism after three weeks of treatment.

In these studies, we also used histopathological examination to confirm the induction of hypothyroidism; thyroid sections of PTU-treated groups showed an increased follicular epithelium length, in terms of higher follicular cell height than the control group. This was in parallel with other researchers' results (**Ferreira *et al.,* 2007**). They approved an increased follicular epithelium height in the induced hypothyroid cases. Furthermore, in the present study, the increases in follicular sizes, which looked to be lined by multiple layers of follicular cells. This could be backed to the low level of T_4 that consequently may raise TSH levels, which could be perpetuated in the

follicular cell proliferation. The previous studies in farm and experimental animals (**Ferreira *et al.*, 2007; Mostaghni *et al.*, 2008**) confirmed the other suggestion and illustrating additionally, intrafollicular adenomatosis consisted of an increase in the follicular epithelial cell layers, forming papillary projections into the lumen of follicles, which in some instances divided the follicle or even completely occluded its lumen. In the present study, some thyroid follicles displayed lower dense vacuolated colloid that in PTU-treated than the control group. It was considering the fact that PTU has no effect on the iodinated thyroglobulin previously gathered in the gland and that clinical implications of such drug may be hindered until thyroglobulin stores are exhausted (**Howland *et al.*, 2006**).

It could be hypothetically accepted that follicular cells increase the uptake and release activity of thyroid hormones into the circulation to counterbalance the increased demand (**Elkalawy *et al.*, 2013**). Further, the hypothyroid group showed extensive congested capillaries profoundly infiltrating the thyroid follicles upon PTU treatment, which could be assigned to the elevated level of TSH. This finding was in concordant with previous researchers (**Čakić-Milošević *et al.*, 2004**). The degree of severity of hypothyroidism was a PTU dose-related (**Salama *et al.*, 2016**). Similar results were observed in induced hypothyroidism in zebrafish and female mice (**Bouaziz *et al.*, 2005; Schmidt and Braunbeck, 2011**).

Histopathological studies ruled out the effect of thyroid hormone deficiency on the renal cortex and outer renal medulla. The proximal and distal convoluted tubules and medullary part of the ascending lobe of Henle were primary and severely affected in rats (**Canavan *et al.*, 1994**). PTU, which found to induce a direct effect on the thyroid gland causing hypothyroidism, was indirectly affected the renal function and structure such effect may be mediated by an associated deficiency of thyroid hormone (T_3 and T_4). Tubulointerstitial nephritis was also diagnosed in rabbits suffering from experimental hypothyroidism induced by PTU; our results were also supported by data found in rats (**Cano-Europa *et al.*, 2008; Tousson *et al.*, 2011**).

Besides, the present study illustrated that PTU-induced severe hepatic injury was in the form of severe congestion in central vein and hepatic artery, hepatocellular necrosis and sinusoid dilatation that correlated with previous studies in mice (**Karamikhah *et al.*, 2015**). In this recent study, the intimate relationship between hypothyroidism with kidney and liver dysfunction have established this notion that may be proved by a significant decrease in T_3 and T_4 levels and pathomorphological alterations of the thyroid (hyperplastic goiter) with subsequent significant increase the concentration of serum urea and creatinine, and renal pathological changes represented by tubule-interstitial nephritis. Besides the significant increase in serum AST, ALT and cholesterol levels and the observed alterations in hepatic tissue architectures described by congested vessels and hepatocellular necrosis.

CONCLUSION

This study illustrated that the administration of variable doses of PTU to rabbits resulted in hypothyroidism with different degrees of severity (mild, moderate and severe), and these severities were dose and duration dependent. Hypothyroidism, renal dysfunction, and hepatic damage were proved physiologically, morphometrically and histopathologically. It could be plausibly accepted that PTU dose must be strictly adjusted; otherwise, it will induce adverse effects manifested by hypothyroidism associated with renal and hepatic dysfunctions.

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COMPLIANCE WITH ETHICAL STANDARDS

All experimental procedures were performed according to ethical guidelines for the Laboratory Animal Care and Use Committee of Sohag University.

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