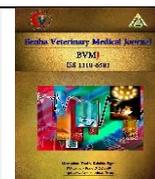




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Hemato-biochemical alterations and antioxidant status during drug-induced thyroid dysfunction in Wister rats

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ABSTRACT

This study was carried to determine the effect of induced disturbance in thyroid hormones (hyperthyroidism and hypothyroidism in animal model). In this study, sixty adult male Wister strain rats (180-200 gm) were divided into four groups represented by two control groups for both drug-induced hypothyroidism and drug-induced hyperthyroidism groups (15/each). Drug-induced hyperthyroidism group (received 0.4mg thyroxin/100g feed, with normal tap water daily), and drug-induced hypothyroidism group (received 0.2% lithium carbonate (Li₂CO₃) in drinking water with normal feed daily). Hematological parameters, some serum biochemical parameters, and assessment of oxidative stress represented by MDA levels in hepatic and renal tissues were assessed. Moreover, semi-quantitative scoring and histopathological findings of hepatic and renal tissues were investigated. Results revealed that any alterations in thyroid hormones levels either if hypothyroidism or hyperthyroidism, were significantly affect different clinic-pathological profile of various body function parameters, beside their indirect effect on tissue conditions that represented by histopathological tissue changes with increase in MDA levels. Referring to the obtained results, most of hematological indices, and sodium level appeared lower than normal in drug-induced hypothyroidism; beside that lipid profile and renal function were significantly raised. In contrast, most of hematological parameters, and sodium level were higher than control group findings, while kidney functions were lower than control group readings. Moreover, liver enzymes and MDA readings were significantly higher than control readings, accompanied with significant decrease in albumin levels in both drug-induced hypo- and hyperthyroidism groups assured microscopically findings of hepatic and renal histopathology

1. INTRODUCTION

Thyroid hormones (THs), thyroxine-T4 and triiodothyronine-T3, have a great role in regulating numerous body functions including carbohydrate and lipid metabolism, oxygen intake and many physiological functions such as renal and hepatic functions; in addition, they have indirect role in regulation of hematopoiesis processes. So, any alterations in their levels lead to significant biochemical and clinical alterations (Messarah et al., 2010).

These hormones regulate the basal metabolic rate of all cells, including hepatocytes; thereby they modulate hepatic function, and any alteration in thyroid function may perturb liver function (Malik, 2002). Besides, THs directly affect renal functions leading to significant modifications in creatinine and urea levels in blood (Dousdampanis et al., 2014). Renal and hepatic histopathological changes have been well-known in relation to significant alterations in THs and thyroid functions (Ben Saad et al., 2017).

Hyperthyroidism, excess thyroid hormone, enhances hyper-metabolic rate which characterized by increased resting energy expenditure, increased lipolysis, reduced cholesterol

levels and gluconeogenesis leading to weight loss (Brent, 2008).

On the other hand, hypothyroidism i.e. reduced thyroid hormone levels, is associated with diminishing metabolic rates marked by reduced resting energy expenditure, weight gain, increased cholesterol levels, reduced lipolysis, and reduced gluconeogenesis (Mullur et al., 2014).

Therefore, this study aimed to investigate the effects of experimentally induced hyperthyroidism and hypothyroidism on the body metabolism, liver and kidney functions in animal model through evaluation of hemato-biochemical parameters, and histopathological changes.

2. MATERIAL AND METHODS

2.1. Experimental animals:

Sixty male Wistar rats weighting about (200-250 g) were purchased from the United Company for Chemicals, Abu-Zabal, Egypt. These animals were subjected to a normal light/dark cycle and room temperature (23±3 °C) and allowed free access to chow and water. This work was approved by the institutional review board for animal experiments of the animal ethics committee, Faculty of

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Veterinary Medicine, Benha University, Egypt (BUFVTM 05-07-20). Rats were allowed one week to acclimatize before the commencement of the experiment. Animals were weighted weekly to adjust the dose of chemicals.

2.2. Drugs and chemicals.

Lithium carbonate powder 99% was produced by VEB-LABORCHEMIE APOLDA Company, Germany; thyroxine tablets were manufactured by Aspen Bad Oldesloe GmbH-Germany, packed by GlaxoSmithKline-Egypt. Commercial diagnostic kits used for estimation of concentration of Triiodothyronine (T3) and total thyroxine (T4) were obtained from IBL International GMBH (Germany); thyroid stimulating hormone (TSH) was obtained from Atlas Medical Company (Cambridge), and malonaldehyde (MDA) was obtained from Biodiagnostic Company (Cairo, Egypt). Diagnostic kits for estimation of albumin, glucose, urea, creatinine, cholesterol, and triglycerides were obtained from BioMed diagnostic company (Cairo, Egypt); ALT and AST were obtained from Diamond Company (Germany); calcium and sodium were obtained from Reactivos GPL (Spain). Materials used for histopathological studies represented by 37% aqueous solution of formaldehyde, and Hematoxylin and Eosin (H & E) stain were obtained from El Gomhoria Co. (Cairo, Egypt).

2.3. Experimental design

In this study, 60 adult male Wister strain rats (200-250 gm) were divided into four equal groups as follow:

In the 1st control group (associated with drug-induced hyperthyroidism group), rats received normal tap water without any addition and normal feed without any addition. While, in the drug-induced hyperthyroidism induced group (2nd group), rats received standard feed with adding Thyroxin (0.4 mg/100 g feed) for 6 weeks with normal tap water. In addition, 2nd control group (associated with drug-induced hyperthyroidism group) rats received normal tap water without any addition and normal feed without any addition (the 3th group), while in the drug-induced hypothyroidism group (4th group), rats received 0.2% lithium carbonate in drinking water for 8 weeks with normal fodder.

2.4. Sampling

Six weeks after administration of lithium carbonate and thyroxin, after 12 hours of fasting, the blood samples were obtained from retro-orbital venous plexus using a fine-walled Pasteur pipette. Whole blood was collected in EDTA tubes for estimation of hematological parameters, and in plain clean centrifuge tubes for separation of serum to be used in estimation of biochemical parameters. Serum samples were used for quantitative determination of ALT, AST, urea, creatinine, sodium, and calcium were calculated. Hematological parameters (RBCs, WBCs, granulocytes, and lymphocytes counts, Hb, Hct, MCV, MCH, and MCHC findings), thyroid function tests, albumin, glucose, cholesterol, and triglycerides were estimated after administration of lithium carbonate for eight weeks.

Rats were sacrificed by cervical decapitation, then the liver and kidney tissues of rats of all groups were collected for MDA determination, and histopathological examination. Tissue paraffin sections were routinely prepared and stained with H&E stain according to Bancroft and Layton (2013).

Tissue homogenization for oxidative stress parameters was prepared as follow:

The liver specimens were quickly removed, then washed with cold saline then blotted on filter paper. The liver (1 gm) was suspended in 4 ml physiological saline (0.9 % NaCl) for homogenization (Teflon Homogenizer, India). The tissue homogenates were centrifuged 1500 ×g for 20 minutes at 4 °C. The supernatants were kept at -20 °C till determination of oxidative parameters according to Yang et al. (2010).

2.5. Statistical analysis.

Statistical analysis was performed using the statistical software package SPSS (Version 18.0; SPSS Inc., Chicago, IL, USA). Differences between groups were evaluated using a one-way ANOVA with a *post hoc* test (Duncan). For each test, all the data are expressed as the mean ± standard error (SE), and *P*-value <0.05 was considered significant.

3. RESULTS

3.1. Hematological findings (Table 1)

In drug-induced hypothyroidism group (group 2) RBCs and lymphocyte counts, Hb, Hct, and MCHC were significantly decreased; MCH and WBCs were significantly increased, while MCV and granulocytes were insignificantly slightly increased after eight weeks of the experiment. Regarding to drug-induced hyperthyroidism group (group 4), all the examined parameters showed significant increases, except total WBCs and lymphocytes counts, which showed significantly decreases after six weeks of the experiment.

3.2. Serum biochemical changes (Table 2)

As a consequence, in drug-induced hypothyroidism group, T₃ and T₄ showed significant reductions with significant high increase in TSH after eight weeks of the experiment; in contrary, in drug-induced hyperthyroidism group, T₃ and T₄ showed strong significant rush up, while TSH showed significant reduction after six weeks of the experiment.

In drug-induced hypothyroidism group, showed significant reduction in albumin and sodium concentrations were recorded after eight weeks of the experiment, respectively; while cholesterol, triglyceride, glucose, ALT, AST, urea, creatinine, and calcium levels were significantly increased after the period of the experiment.

Regarding to hyperthyroidism group, albumin, cholesterol, triglycerides, urea, and creatinine were significantly decreased; while glucose, ALT, AST, sodium, and calcium were significantly increased after six weeks of the experiment.

3.3. Antioxidative parameter (MDA changes) (Table 3)

Referring to the stress factor and lipid peroxidation associated with thyroid dysfunction, renal and hepatic tissue homogenates showed significant increases in both hypo- and hyperthyroidism along the experimental period, which were more prominent in hyperthyroidism rather than hypothyroidism. Furthermore, liver tissue samples showed higher MDA levels than kidney samples.

3.4. Histopathological finding in liver and kidney tissues:

The microscopic examination of liver of rats in control non-treated group showed normal hepatocytes arranged in cords (Fig. 1 A), while the examined liver of rat in hypothyroidism revealed granular hepatocytes associated with hypertrophy of Kupffer's cells (Fig. 1 B). and mononuclear cells infiltration (Fig. 1 C).

Table 1 Hematological parameters in control, hyperthyroidism at 6th week, hypothyroidism at 8th week from the beginning of the experiment.

Parameters	Control*	Hypothyroidism	Control**	Hyperthyroidism
RBCs (×10 ⁶ /μl)	7.74±0.27 ^a	4.87±0.37 ^b	9.07±0.36 ^b	11.37±0.69 ^a
Hb (g/dl)	14.78±0.31 ^a	12.12±0.72 ^b	14.90±0.71 ^b	18.95±0.75 ^a
Hct (%)	41.23±1.86 ^a	33.23±1.34 ^b	45.69±0.32 ^b	52.55±0.15 ^a
MCV (fl)	59.33±1.20 ^a	57.87±0.65 ^a	55.25±1.65 ^b	59.83±0.33 ^a
MCH (pg)	15.04±0.30 ^b	15.84±0.49 ^a	15.22±0.72 ^b	17.93±0.33 ^a
MCHC (%)	26.75±0.70 ^a	24.30±0.87 ^b	25.90±1.08 ^b	30.20±1.00 ^a
WBCs (×10 ³ /μl)	10.03±0.19 ^b	12.90±1.50 ^a	9.30±0.99 ^a	7.75±0.80 ^b
Granulocytes (×10 ³ /μl)	1.94±0.48 ^a	2.02±0.30 ^a	2.02±0.40 ^b	2.34±0.12 ^a
Lymphocytes (×10 ³ /μl)	6.19±0.29 ^a	4.37±0.83 ^b	5.98±1.08 ^a	4.69±0.80 ^b

Results are expressed as mean ± S.E. a, b & c: There is significant difference (P≤0.05) between any two means, within the same row (of each parameter) have the different superscript letter. Control*: Control regarding with hypothyroidism induced group. Control**: Control regarding with hyperthyroidism induced group.

Table 2 Serum biochemical changes and MDA level in tissue in control, hyperthyroidism at 6th week, hypothyroidism at 8th week from the beginning of the experiment

Parameters	Control*	Hypothyroidism	Control**	Hyperthyroidism
T3 (pg/ml)	86.35±2.49 ^a	31.15±3.27 ^b	86.56±3.38 ^b	335.4±15.66 ^a
T4 (μg/dl)	6.52±0.23 ^a	1.03±0.03 ^b	6.58±0.09 ^b	15.24±1.42 ^a
TSH (μIU/ml)	1.83±0.21 ^b	8.08±2.18 ^a	1.96±0.08 ^a	0.23±0.07 ^b
Albumin (g/dl)	4.02±0.11 ^a	2.15±0.06 ^b	4.15±0.13 ^a	1.81±0.15 ^b
Cholesterol (mg/dl)	67.75±4.31 ^b	93.75±8.26 ^a	66.33±3.93 ^a	42.60±4.34 ^b
Triglyceride (mg/dl)	74.33±4.06 ^b	94.25±7.6 ^a	75.50±5.62 ^a	48.90±4.20 ^b
Glucose (mg/dl)	105.75±7.34 ^b	145.25±6.80 ^a	104.40±5.24 ^b	173.40±6.66 ^a
ALT (SGPT) (U/L)	31.41±5.01 ^b	82.35±16.28 ^a	30.61±8.31 ^b	78.99±20.18 ^a
AST (SGOT) (U/L)	115.43±19.91 ^b	130.26±11.88 ^a	110.23±10.91 ^b	160.58±19.33 ^a
Urea (mg/dl)	22.51±2.22 ^b	53.14±113.89 ^a	21.75±2.22 ^a	14.12±4.11 ^b
Creatinine (mg/dl)	0.47±0.03 ^b	1.29±0.31 ^a	0.48±0.03 ^a	0.31±0.05 ^b
Sodium (mmol/L)	145.21±2.08 ^a	133.00±4.58 ^b	145.00±2.3 ^b	157.00±2.55 ^a
Calcium (mg/dl)	11.05±0.91 ^b	13.67±0.80 ^a	11.70±0.81 ^b	14.40±0.51 ^a

Results are expressed as mean ± S.E. a, b & c: There is significant difference (P≤0.05) between any two means, within the same row (of each parameter) have the different superscript letter. Control*: Control regarding with hypothyroidism induced group. Control**: Control regarding with hyperthyroidism induced group

Table 3 MDA (nmol/g) changes in tissue homogenate in control, hyperthyroidism at 6th week, hypothyroidism at 8th week from the beginning of the experiment

Parameters	Organ	Control*	Hypothyroidism	Control**	Hyperthyroidism
MDA (nmol/g)	Liver	7.45±0.92 ^b	12.50±1.17 ^a	9.36±0.12 ^b	29.21±3.02 ^a
	Kidney	11.45±0.92 ^b	17.50±2.07 ^a	12.02±0.02 ^b	31.21±2.92 ^a

Results are expressed as mean ± S.E. a, b & c: There is significant difference (P≤0.05) between any two means, within the same row (of each parameter) have the different superscript letter. Control*: Control regarding with hypothyroidism induced group. Control**: Control regarding with hyperthyroidism induced group.

Moreover, in hyperthyroidism group the examined liver showed marked granular hepatocytes associated with diffuse vacuolar degeneration associated Kupffer's cells activation.

The histopathological examination of kidneys of rats in control group showed normal renal glomeruli and tubules, while the examined kidneys in hypothyroidism group revealed mild tubular epithelial degeneration (Fig. 2B). Moreover, the examined kidney of rats in hyperthyroidism group showed severe degenerative changes within the peri-glomerular renal tubules and papillary necrosis of the brush borders of the renal tubules' normal renal glomeruli and tubules, with congestion of the renal blood capillaries and coagulative necrosis of the renal tubules (Fig. 2C).

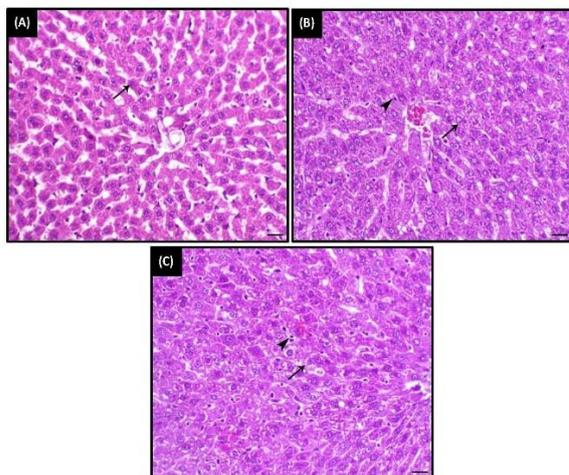


Figure 1 Liver of rat (A) control animal showing normal hepatocytes arranged in cords (arrow), (B) Hypothyroidism group showing granular hepatocytes (arrow) associated with hypertrophy of kuffer's cells (arrowhead), and (C) hyperthyroidism group showing marked granular hepatocytes associated kuffer's cells activation, H&E, bar= 50 μm.

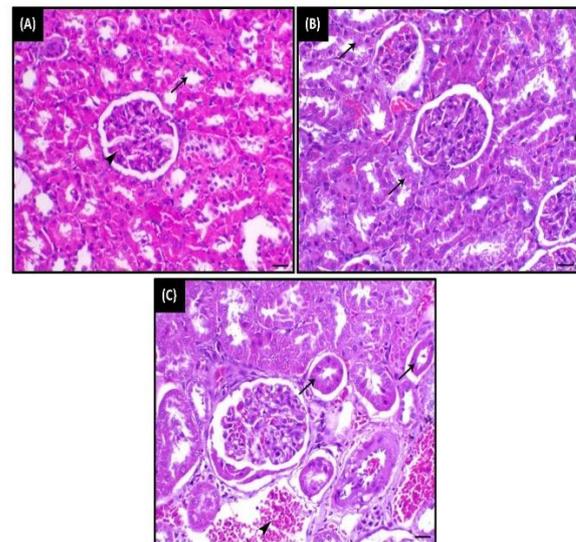


Figure 2 Kidney of rat (A) control animal showing normal renal glomeruli and tubules (arrowhead and arrow respectively), (B) hypothyroid animal showing mild tubular epithelial degeneration (arrows) and (C) hyperthyroid animal show severe degeneration changes within the peri-glomerular renal tubules and necrosis of brush borders of the renal tubules (arrowhead), with congestion of the renal blood capillaries and coagulative necrosis of the renal tubules, H&E, bar= 50 μm.

3.5. Semi-quantitative scoring of liver and kidney tissues: Hepatic lesions in hypothyroidism group showed mild vascular lesions, degeneration, necrosis, inflammation, and moderate extent of lesions. On the other hand, in case of hyperthyroidism severe focal vascular lesions, degeneration, and extent of lesions were recorded, moreover, moderate necrosis and inflammation lesions were noticed as mentioned in Table (4). While renal lesions in hypothyroidism group showed mild vascular lesions, necrosis and inflammation with moderate degeneration and

extent of lesions; while severe focal vascular lesions and necrosis were noticed with severe diffuse degeneration, and inflammation with moderate extension of lesions as mentioned in Table (5).

4. DISCUSSION

In this study, drug-induced hypothyroidism causes significant decreases in almost all the examined hematological parameters, except MCH and WBCs which were significantly increased, which came in agreement with Saad et al. (2017) and Malhotra and Dhawan (2008). In addition, experimentally induced hyperthyroidism causes significant increases in all the examined parameters, except WBCs and lymphocytes, which showed significantly decrease findings which came in agree with Kandir and Keskin (2016). Alteration in hematological parameters in hypothyroidism and hyperthyroidism could be attributed to the major role of thyroid hormones that enhance the process of erythropoiesis as concluded by Zahediasl et al. (2010).

These alterations in hypothyroidism and hyperthyroidism came in line with Yousif (2012), Katta (2015), and Kandir and Keskin (2016). Thyroid dysfunction is known to induce liver injury and affected albumin metabolism (Bhalla et al., 2007 and Hsieh et al., 2019). The current study supported this conclusion where albumin levels significantly decreased, while ALT, AST and MDA levels significantly increased in both hypothyroidism and hyperthyroidism which came in line with Kowska et al. (2014), and Ben Saad et al. (2017), who reported a significant increase in MDA levels in both hypothyroidism and hyperthyroidism indicating oxidative stress and increasing lipid peroxidation processes.

Highly significant increase in cholesterol and triglycerides values in case of hypothyroidism may be attributed to the direct effect of thyroid hormones which regulate on synthesis and degradation of cholesterol (Paradis, 2009). These findings are in agree with those recorded by Nelson and Couto (2009), and Vijaimohan et al. (2010), who said that the increase of these parameters is a sign of liver damage; while, hypo-cholesterolaemia and hypo-triglyceridaemia in case of hyperthyroidism affected group were previously recorded by Rizos et al. (2011) who attributed to that despite the increased activity of the HMG-CoA reductase in case of hyperthyroidism, levels of total cholesterol, LDL-C, lipoproteins and TG tend to decrease in patients with clinical or subclinical hyperthyroidism. This is due to increased LDL receptor gene expression resulting in enhanced LDL receptor-mediated catabolism of LDL particles as recorded by Kung et al. (1995).

The obtained significant increases in glucose levels in both hypothyroidism and hyperthyroidism were in agree with the recorded results of Messarah et al. (2010) and Ben Saad et al. (2017); hyperglycemia associated with thyroid dysfunction may be attributed to that any alteration in

thyroid functions, leading to perturbed genetic expression of a constellation of genes along with physiological aberrations leading to impaired glucose utilization and disposal in muscles, overproduction of hepatic glucose output, and enhanced absorption of splanchnic glucose; these factors contribute to insulin resistance (Wang, 2013). Thyroid hormones have pre-renal and intrinsic renal effects by which they increase the renal blood flow and the glomerular filtration rate (GFR). Hypothyroidism is associated with reduced GFR and hyperthyroidism results in increased GFR as well as increased renin – angiotensin – aldosterone activation (Basu and Mohapatra, 2012). The obtained results agreed with those recorded by Ali-Marwa (2014) and Katta (2015); significant raised and reduced values of renal functions in case of hypothyroidism and hyperthyroidism, respectively may be attributed to the secondary decrease and increase in the GFR affected by reduction and increase in thyroid hormone leading to alteration of serum urea and creatinine as mentioned by Schaefer (2010). However, significant destruction of renal tissue which was supported by histo-pathological findings, significant reduction in serum urea and creatinine can be attributed to that, it is well known that serum creatinine, an inverse marker of GFR, is significantly decreased in hyperthyroid patients due to the reduction in overall muscle mass (Manetti et al., 2005; Den Hollander et al., 2005) and impaired conversion of creatine to creatinine are regularly found in untreated hyperthyroid patients (Shirota et al., 1992; Kuhlback 1957), and these results are consonant with our data.

On the other hand, untreated hyperthyroid cases have an elevated BUN: creatinine ratio, because of elevated BUN and decreased creatinine (Shirota et al., 1992). There is an increased production of urea from liver due to increased body turnover of protein, which is qualitatively greater than the increase in renal excretion, leading to an over-all increase in BUN (DiBartola and Brown, 2000). Nevertheless, results of our experiment revealed that there is severe liver injury indicated by high levels of serum ALT and AST as well as histopathological alterations of liver tissue. This hepatic damage has reduced urea synthesis capacity resulting in reduced hepatic power to detoxify ammonia (Aldridge et al., 2015). This make decreased serum urea level in case of hyperthyroidism is plausible even in the presence of renal functions.

In the present work, hypothyroidism causes moderate decreases in sodium levels which could be attributed to that the pathogenesis of “hypothyroid hyponatremia” is secondary to the inability to excrete a free water load, caused by both a decrease in the delivery of water to the distal nephron, as well as excess vasopressin secretion (Abuzaid and Birch, 2015). Besides, the impaired water excretion in the setting of hypothyroidism is likely to be related to a reduction in renal perfusion secondary to the systemic effects of thyroid hormone deficiency on cardiac output and peripheral vascular resistance.

Table 4 Semi-quantitative scoring of hepatic lesions in control, hyperthyroidism, and hypothyroidism groups.

Groups	Vascular lesions	degeneration	necrosis	Inflammation	Extent of lesions
Control	-	-	-	-	-
Hypothyroidism	+	+	+	+	++
Hyperthyroidism	+++	+++	++	++	+++

(-) means no detectable lesions; (+) indicates mild lesions; (++) indicates moderate lesions; (+++) indicates severe focal lesions; (++++) indicates severe diffuse lesions.

Table 5 Semi-quantitative scoring of renal lesions in control, hyperthyroidism, and hypothyroidism groups

Groups	Vascular lesions	Renal degeneration	Renal necrosis	Inflammation	Extent of lesions
Control	-	-	-	-	-
Hypothyroidism	+	++	+	+	++
Hyperthyroidism	+++	++++	+++	++	++

(-) means no detectable lesions; (+) indicates mild lesions; (++) indicates moderate lesions; (+++) indicates severe focal lesions; (++++) indicates severe diffuse lesions

Otherwise, sodium levels were significantly increased in hyperthyroidism group which agreed with the results recorded by Abebe et al. (2016), who attributed hypernatremia to the effect of hyperthyroidism that can result in or accelerate chronic kidney disease (CKD) by the mechanisms of intra-glomerular hypotension (increased filtration pressure) and consequent hyper-filtration.

Investigation of calcium levels showed significant increases in hypothyroidism which may be attributed to metabolic derangement induced by thyroid hormone deficiency such as altered calcium homeostasis. The obtained results were concise with those recorded by kowska et al. (2014). Furthermore, calcium levels showed significant increase in hyperthyroidism group; the obtained results came in agree with those recorded by DeGroot (2015) and Chen et al. (2017), who attributed that to high degree of exchangeability of calcium in the bones of cases suffered from thyrotoxicosis and the high rate of loss of calcium in the urine.

Concerning with semi-quantitative scoring of lesions, and microscopical findings of the examined liver and kidneys in control non-treated and treated groups, it was cleared that thyroid dysfunction has harmful direct effect on different organs integrity as previously reported by Ali-Marwa (2014), Katta (2015), and Ben Saad et al. (2017), who reported different pathological alterations of both liver and kidney hypo- and hyperthyroidism.

5. CONCLUSIONS

Drug-induced hypothyroidism and hyperthyroidism could greatly alter clinicopathological image of hematology (WBCs, RBCs, and HB) and serum biomarkers of hepatic and renal functions with significant histopathological alterations in the liver and kidneys.

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