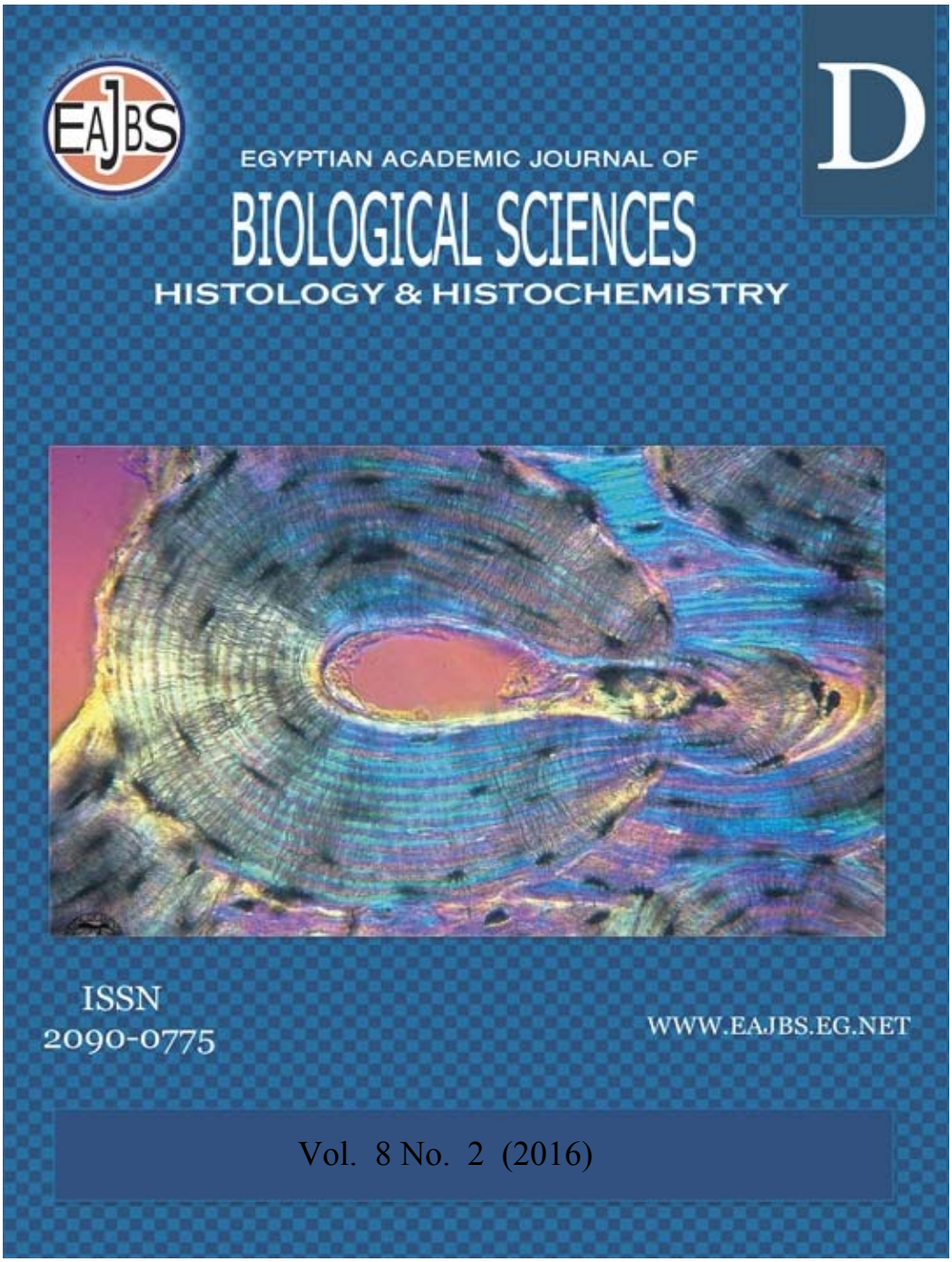

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Impact of Induced Thyroxine and Carbimazole Vacillation on Liver of Female Rats

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ABSTRACT

The present study was designed to investigate the effects of levothyroxine and carbimazole on body weight gain, liver functions, and histology of female rats. Rats were divided into three groups (12 rats/group), with each group divided into two sub-groups (6 rats/group) based on the period of treatment for three and six weeks At the end of the study, the rate of body weight gain of hyperthyroid rats exhibited a significant decrease after three and six weeks. The rate of body weight gain of hypothyroid female rats displayed a significant decrease after three weeks only. Liver to body weight ratio (L/BW) of hyperthyroid rats displayed a significant increase after three and six weeks. However, (L/BW) of hypothyroid rats manifested no significant change and a significant decrease, respectively after three and six weeks of study versus control. The rate of food consumption was different in the three groups and even in the same group at different times. The liver function tests were found changed in both hyper- and hypothyroid female rats. The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels of both hyper- and hypothyroid rats displayed a significant increase after three and six weeks. Reduced glutathione (GSH) levels exhibited a significant decrease after three weeks and a significant increase after six weeks. On other hand, GSH of the hypothyroid rats displayed no significant change after three and six weeks. The present study also showed a histopathological damage in liver of either hyper- or hypothyroid rats. These results suggested that both hyper- and hypothyroidism induced adverse effects on liver physiology and histology in female rats.

INTRODUCTION

Thyroid gland is a unique large endocrine gland being largest, superficially located in the neck region and being amenable to physical examination and biopsy (Zaidi *et al.*, 2004). The human's thyroid gland is a brownish-red organ consists of two lobes connected by an isthmus and weighs between 20 - 25 grams (Choksi *et al.*, 2003; Fox, 2008). It has a very rich blood supply and it requires iodine to function properly (Petersen, 2007). The gross structure of thyroid gland of laboratory animals is similar to that described for human (U.S. EPA, 1998). The thyroid gland secretes two iodine containing amine hormones; 3, 5, 3′, 5′ tetraiodo-l-thyronine (T4 or thyroxine) and 3, 5, 3′ triiodo-l-thyronine (T3) (Diekman *et al.*, 2000).

Thyroid hormones (THs) influence the functions of all body organs and cells (Soliman, 2013). No other hormone affects such wide range of cells and tissues such as THs (Capuco, 2001). Animal and human studies indicated that THs play a role in the cardiovascular, nervous, immune, and reproductive system development and function (Krassas, 2000; Choksi *et al.*, 2003). In addition, they mediate several physiological processes, including embryonic development, cellular differentiation, metabolism, and the regulation of cell proliferation (Wu *et al.*, 2013). Also, they are responsible for regulation of oxygen consumption, thermogenesis and lipogenesis (Capuco, 2001).

Thyroid dysfunctions are considered as some of the most important endocrinopathies both in human and in veterinary medicine (Shi *et al.*, 2002; Rijnberk *et al.*, 2003). When T4 and T3 levels are too low or too high, the hypothalamus and pituitary glands start to regulate their production (Hadley, 1996). Hypothyroidism is resulted usually from damage, removal, or inhibition of the function of the thyroid gland (Braverman and Utiger, 2005; Daniels & Dayan, 2006; Mitrou *et al.*, 2011). On the other hand, hyperthyroidism is defined as the clinical syndrome of hyper-metabolism resulting from increased freeT4 and/or free T3 serum levels (Braverman and Utiger, 2005; Saraji *et al.*, 2012). Therefore, the purpose of this study was to compare the effects of both hyper- and hypothyroidism on body weight gain, liver weight, food consumption level, glutathione reduced (GSH) level, liver physiology and histology of female rats.

MATERIALS AND METHODS

Animals

36 healthy female albino rats weighing between 120-140g were obtained from a colony at the animal house of Abu-Rawash, Giza, Egypt. The female rats were maintained under constant light, humidity, and temperature in the animal house of the faculty of pharmacy - Sinai University, Al-Arish, North-Sinai, Egypt. Rats were allowed free access of food and water *ad libitum*, and were acclimatized a week before the starting of the experiment. The rats were divided into three groups (12 rats/group), then each group was further divided into two sub-groups (6 rats/group) based on the period of treatment, three and six weeks as follows:

Group 1 (Euthyroid): served as a control group for the other two groups and received distilled water orally.

Group 2 (Hyperthyroid): received thyroxine + distilled water orally.

Group 3 (Hypothyroid): received carbimazole + distilled water orally.

Chemicals and Reagents

All reagents used for the present study were of the analytical grade. Thyroxine in the form of commercial tablets (Eltroxin) was obtained from GlaxoSmithKline GmbH, Germany. Carbimazole in the form of commercial tablets (Carbimazole) was obtained from Chemical Industries Development (CID). T3 and T4 ELISA kits were purchased from Biocheck, Inc Company, Foster City, U.S.A. Glutathione reduced kit was purchased from Biodiagnostics Company, Dokki, Egypt. Kits for quantitative determination of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), albumin and total protein (T.Potein) were purchased from Spinreact Company, Ctra.Santa Coloma, Spain.

Experimental Protocol

In the present study, 600 µg/kg of thyroxine and 1.35 mg/kg of carbimazole were orally administrated to female rats for induction of hyper- and hypothyroidism, respectively for three and six weeks. These doses were selected

after Chakrabarti *et al.* (2007), Paget and Barnes (1964). The body weight of each animal as well as food consumption rate of each group was recorded at the start and on alternate days until the end of experiments. At the end, the female rats were weighed, fasted for 12-14 hrs, anaesthetized with di-ethyl ether and dissected. Then, blood was drawn by a ventricular cardiac puncture. For the estimation of GSH, 1ml of the fresh blood samples was transferred into test tubes that contained an anti-coagulant agent (EDTA). The rest of the blood was transferred into test tubes without any additives where it was further centrifuged at a speed of 3,000 rpm for 10 min and the clear serum was collected in sterilized, properly labeled plastic tubes and frozen at -20ºC till use. Serum was used for determination of THs and liver functions. Thyroid hormones were assayed by the quantitative method using ELISA kit. ALT, AST, Albumin and T.protein were assayed by the methods of Reitman and Frankel (1957), Gendler (1984) and Koller (1984), respectively.

Histological Techniques

For histological study, rat livers were removed, washed in saline, weighed, and small pieces were fixed in aqueous Bouin's solution for 24 hours (Usenko *et al.*, 1998). After fixation, they were washed several times in 70% ethanol then dehydrated in ascending grade of ethyl alcohol concentrations and cleared using xylene. Then, liver sections were stained with hematoxylin and counter stained with eosin for routine histological study using the light microscope at (X 160 & X 640).

Statistical Analysis

The statistical software package SPSS for windows version 19.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Data of the present study were subjected to descriptive statistics and the results presented as Means ± Standard Error of Mean. Data were analyzed using one-way analysis of variance (ANOVA), followed by post hoc multiple comparisons using Sheffe's multiple comparison, with $P < 0.05$ accepted as statistically significant (Mano *et al.*, 1994; Okon *et al.*, 2013).

RESULTS

The Body Weight Gain and liver weight

The rate of body weight gain of hyperthyroid rats exhibited a significant ($P < 0.001$, $P < 0.004$ vs. control) decrease after three and six weeks, respectively. Also, the rate of body weight gain of hypothyroid rats exhibited a significant ($P < 0.000$ vs. control) decrease after three weeks. However, the rate of body weight gain of hypothyroid rats showed no significant change ($P > 0.05$ vs. control) after six weeks (Table.1). The liver to body weight ratio (L/BW) of hyperthyroid rats displayed a significant ($P < 0.000$ vs. control) increase after three and six weeks of study. On the other hand, (L/BW) of hypothyroid rats manifested no significant ($P > 0.05$ vs. control) change after three weeks. However, the liver to body weight ratio of hypothyroid rats showed a significant ($P < 0.03$ vs. control) decrease after six weeks (Table.1).

The Level of Food Consumption

The rate of food consumption was changed in the three groups and even in the same group but at different times. The rate of food consumption of hyperthyroid rats exhibited a significant ($P < 0.006$, $P < 0.000$, $P < 0.000$, $P < 0.002$, $P < 0.000$, and $P < 0.001$ vs. control) increase after the first, second, third, fourth, fifth, and sixth week of treatments, respectively.

Table 1: Mean values of body weight gain (BWG) and liver to body weight ratio (L/BW) of the control (CON), hyperthyroid (HYR) and hypothyroid (HYO) female rats after three, and six weeks of treatment with distilled water, thyroxine, and carbimazole, respectively.

Parameter	CON-3	HYR-3	HYO-3	CON-6	HYR-6	HYO-6
BWG (g)	30±1.0	24±0.6**	14±1.0***	47±1.8	32±3.2**	42±2.5°
Change%	20%	14%	9%	31%	19%	26%
L/BW (g)	3.3±0.1	4.4±0.2***	3.3±0.1°	3.2±0.1	4.1±0.2***	2.6±0.1*
Change%	----	33%	0%	----	28%	-19%

Values are expressed as Mean ± (SE), (n= 6/group).
Key: °, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001 versus control group using Scheffe's test; one way ANOVA.

On the other hand, the rate of food consumption of hypothyroid rats showed no significant (P > 0.05 vs. control) change in the rate of food consumption after the first week of treatments. However, the rate of food consumption of hypothyroid rats showed a significant (P < 0.02, P < 0.04, P < 0.01, P < 0.05, and P < 0.001 vs. control) decrease after the second, third, fourth, fifth, and sixth week of the treatments, respectively (Table 2).

Table 2: Mean values of food consumption of control (CON), hyperthyroid (HYR) and hypothyroid (HYO) female rats recorded from the first to sixth week of treatment with distilled water, thyroxine, and carbimazole, respectively.

Food (g)	CON	HYR	HYO
1st week	76±1.5	88±3.5**	74±1.4°
Change %	----	16%	-3%
2nd week	80±1.8	105±3.4***	70±1.0*
Change %	----	31%	-14%
3rd week	86±2.3	112±4.9***	73±2.0*
Change %	----	30%	-15%
4th week	95±3.8	117±1.6**	78±4.8**
Change %	----	23%	-18%
5th week	90±3.6	119±3.5***	77±3.3*
Change %	----	32%	-14%
6th week	98±2.6	118±1.7***	77±4.4***
Change %	----	20%	-21%

Values are expressed as Mean ± (SE), (n= 6/group).
Key: °, P > 0.05, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001 versus control group using Scheffe test; one way ANOVA.

The Level of Thyroid Hormones

The level of T3 in hyperthyroid female rats showed a significant (P < 0.02 and P < 0.000 vs. control) increase after three and six weeks, respectively. The level of T4 in hyperthyroid female rats also showed a significant (P < 0.001 and P < 0.000 vs. control) increase after three and six weeks, respectively. However, the level of T3 in hypothyroid female rats manifested a significant (P < 0.007 and P < 0.000 vs. control) decrease after three and six weeks, respectively. The level of T4 in hypothyroid female rats also showed a significant (P < 0.05 and P < 0.000 vs. control) decrease after three and six weeks, respectively.

The Liver Function Tests

After three weeks, ALT and AST levels of hyperthyroid female rats showed a significant (P < 0.005 and P < 0.003 vs. control) increase, respectively. However, albumin and T. protein levels showed no significant (P > 0.05 vs. control) change after the same period. The level of ALT and AST of hypothyroid female rats exhibited a significant (P < 0.03 vs. control) increase after three weeks. However, albumin and T. protein levels showed no significant (P

> 0.05 vs. control) change in albumin and T. protein levels after the same period. After six weeks, ALT and AST levels of hyperthyroid female rats showed a significant (P < 0.000 and P < 0.001 vs. control) increase, respectively. However, albumin and T. protein levels showed a significant (P > 0.02 and P < 0.006 vs. control) decrease after the same period. The level of ALT and AST of hypothyroid female rats exhibited a significant (P < 0.000 and P < 0.004 vs. control) increase after three weeks. The level of albumin and T. protein also showed a significant (P > 0.02 and P < 0.03 vs. control) increase after the same period (Table 3).

Table 3: Mean values of T3, T4, ALT, AST, Albumin, T. protein, and GSH levels of control (CON), hyperthyroid (HYR) and hypothyroid (HYO) female rats after three and six weeks of treatment with distilled water, thyroxine, and carbimazole, respectively.

Parameter	CON-3	HYR-3	HYO-3	CON-6	HYR-6	HYO-6
T3 (ng/ml)	2.4±0.3	3.8±0.5*	0.7±0.1**	2.2±0.3	5.7±0.7***	0.3±0.9*
Change%	----	58%	-70%	----	159%	-88%
T4 (µg/dl)	7.3±0.4	11.9±0.8***	4.5±0.8*	6.8±0.6	18.5±1.1***	2.0±0.2***
Change%	----	63%	-38%	----	172%	-71%
ALT (U/L)	18±1.9	32±2.0**	29±3.6*	18±1.8	42±2.4***	35±2.3***
Change%	----	78%	61%	----	133%	94%
AST (U/L)	25±2.2	39±2.6**	36±2.4*	24±3.0	46±3.5***	42±2.6**
Change%	----	56%	44%	----	92%	75%
Albumin (g/dl)	4.1±0.2	3.8±0.2°	4.6±0.2°	4.4±0.2	3.2±0.2*	5.2±0.3*
Change%	----	-7%	12%	----	-27%	18%
T.Protein (g/dl)	6.2±0.4	5.9±0.2°	6.1±0.3°	6.3±0.2	4.9±0.3**	7.4±0.3*
Change%	----	-5%	-2%	----	-22%	17%
GSH (mg/dl)	22±2.3	12±1.3*	18±2.5	19±2.4	29±2.7*	21±2.4°
Change%	----	-45%	-18%	----	53%	11%

Values are expressed as Mean ± (SE), (n= 6/group).
Key: °, P > 0.05; *, P < 0.05; **, P < 0.01; ***, P < 0.001 versus control group using Scheffe test; one way ANOVA.

The Level of Reduced Glutathione

The level of GSH of hyperthyroid rats exhibited a significant (P < 0.003 vs. control) decrease after three weeks. However, hyperthyroid rats showed a significant (P < 0.05 vs. control) increase after six weeks. On the other hand, the level of GSH of hypothyroid rats displayed non-significant change (P >0.05 vs. control) after neither three nor six weeks (Table 3).

The Histopathology of Liver

The examination of histological sections of liver from control group showed normal central veins, portal triads, and a normal structure and a regular arrangement of hepatocytes with clearly visible nuclei and hepatocytes. On the other hand, the histopathological examination of liver in both hyper- and hypothyroid rats revealed some of pathological alterations. Both levothyroxine and carbimazole treatments caused liver injury and hepatocytes destruction parallel to significant increase in liver function indices. Liver sections of both hyper- and hypothyroid rats showed disorganized hepatocyte cords and the normal lobular architecture disappeared in some areas (Fig.1).

The liver tissue of hyperthyroid rats manifested a fusion of some portal triads with the central vein of the liver lobule with presence of increased inflammatory cell infiltration. Also, there was a lobular inflammatory infiltrate in the vicinity of the enlarged and edematous portal tracts. Moreover, the hyperthyroid rats also showed different degrees of

intracanalicular bile plugs with enlarged hepatocytes mainly centri-lobular intrahepatocytic cholestasis. The intrahepatic cholestasis and mild inflammatory infiltrates were indicators of acute liver injury. The hyperthyroid rats also displayed some degree of fatty infiltration in liver tissue, steatosis. There was a vacuolar degeneration as a result of the mixed pattern of small and large lipid droplets in hepatocytes. In some hepatocytes, the nucleus was squeezed into displaced rim of cytoplasm of the fat vacuole.

On the other hand, the liver tissue of hypothyroid female rats showed cellular discontinuity with loss of hepatocytes radial distribution, hyperplasia and irregular arrangement of hepatocytes. There was also a focal necrosis within liver parenchyma; a cluster of inflammatory cells which marked the site of necrotic hepatocyte. An extensive portal tract fibrosis with a moderately dense infiltrate of mononuclear inflammatory cells was noticed. The spaces between hepatic plates were increased in some areas with progression to fibrosis (Fig. 2).

DISCUSSION

During the experiments, rats from control and treated groups remained alive for their respective periods of treatment. Levothyroxine caused a significant rise in T4, T3 levels when compared with control group, confirming a hyperthyroid state. On the contrast, carbimazole caused a significant fall in T3, T4 levels when compared with control group, confirming a hypothyroid state. The general condition of both treated rats was compared with control rats throughout the study. Control rats appeared healthy, active and showed normal behavior throughout the period of experimental study. On the contrast, the hyperthyroid female rats appeared strange, hyperactive, and nervous especially during administration by gastric tube. They always displayed fast movement in the cage and had increased appetite with decreased body weight gain. At the same time, the hypothyroid female rats appeared ill-looking and suffered from depression as they had a diminished activity and increased sleep periods. They had a sluggish behavior and exhibited decreased response to external stimuli. They also suffered from loss of appetite with reduction in weight gain in the first three weeks of the study.

According to Rajab *et al.* (2015), TH disorders led to emotional and behavioral disturbances and impair patients' everyday life. There is an association between thyroid function and psychiatric disorders particularly mood disorders (Hage and Azar, 2012). According to Marian *et al.*, 2009, hyperthyroidism might be associated with various psychiatric symptoms, such as emotional disturbances, irritability, restlessness and anxiety. However, Brown *et al.* (2005) suggested that, depression is one of the major symptoms associated with hypothyroidism.

The present hyperthyroid rats showed a significant reduction in body weight gain despite the significant increase in food consumption when compared with control rats after three and six weeks. This agrees with Ajayi *et al.* (2013) who suggested that hyperthyroid group displayed a reduction in body weight gain. Thyroid hormones caused a negative energy balance due to the increased metabolic rate & energy expenditure so; there was always a hungry feeling (Costanzo 2010; Ganong, 2010). The present hypothyroid rats displayed a very high significant reduction in body weight gain when compared with control rats after the first three weeks. After the fourth week, there was no significant change in body weight gain of hypothyroid rats when compared with control rats. This agrees with Wang *et al.* (2000) who suggested that, the body weight gain of hypothyroid rats per

week was very low (or even negative) during the whole experimental period and their body growth was practically arrested. This confirms the fact that normal concentrations of THs are required for growth to adulthood and any deficiency in THs can cause arrest in growth (Sharma *et al.*, 2013). Conversely, Perveen *et al.* (2012) displayed that there is a marked weight gain after treatment with carbimazole where weight continued to rise with time.

The thyroid status also affected (L/BW), the present (L/BW) of hyperthyroid rats showed a very high significant increase when compared with control after three and six weeks. This result is in accordance with Ajayi and Akhigbe (2012) who suggested that liver of hyperthyroid group manifested increased weight than control group. The increase in liver weight of hyperthyroid rats could be related to the increase in the rate of absorption of carbohydrates from the gastrointestinal tract (Laycock and Wise, 1983). Malik and Hodgson, (2002) also suggested the usage of T3 as a hepatic growth factor as it induces hepatocyte proliferation and increases liver mass. On the other hand, the present (L/BW) of hypothyroid rats displayed no significant change after three weeks and a significant decrease after six weeks when compared with control. This result disagrees with Ajayi and Akhigbe (2012) who suggested that liver of hypothyroid group manifested increased weight than control group. In fact, T3 mediates several physiological processes, including embryonic development, cellular differentiation, metabolism, and the regulation of cell proliferation (Wu *et al.*, 2013).

The present results revealed significant increase in ALT and AST enzymes activity in the serum of both hyperthyroid and hypothyroid rats when compared with control rats after three and six weeks. The increase in ALT and AST levels was higher in the hyperthyroid rats than hypothyroid rats. The present result agrees with Messarah *et al.* (2011) who suggested an elevation in ALT and AST enzymes in the serum of hyperthyroid group. Also, Christ-Crain *et al.* (2004) suggested the elevation of ALT and AST levels in overt hypothyroidism. The present results disagrees with Khan *et al.* (1999) who found a non-significant increase in AST and no change in ALT in hyperthyroid rats.

The increase in circulating liver enzymes of hyper- and hypothyroid rats might be either due to their increased synthesis and secretion, or to diminished catabolism (Christ-Crain *et al.*, 2004). Malik *et al.* (2000) suggested that increased metabolism in hepatocytes of hyperthyroid rats might lead in turn to increased ALT and AST levels. According to Christ-Crain *et al.* (2004), hypothyroidism is associated with a decreased metabolic rate and a diminished catabolism of AST and ALT. Ajayi and Akhigbe (2012) suggested that the damage which might happen to the hepatocytes of liver of hypothyroid rats leads to leakage of enzymes from the cells.

The present albumin and T.protein levels were significantly reduced in hyperthyroid rats when compared with control rats, only after six weeks. These results agree with the findings of Jalal *et al.* (2010) and Kondaveeti *et al.* (2014). Conversely, the hypothyroid rats showed a significant increase in both albumin and T.protein levels when compared with control rats, only after six weeks. These results agree with Kondaveeti *et al.* (2014). TH is known to promote albumin catabolism (Koga *et al.*, 2009). Larsen and Davies (2002) suggested that TH promotes albumin metabolism so, the mean concentration of albumin and total protein in serum were lower in hyperthyroid patients than the hypothyroid patients. Kondaveeti *et al.* (2014) suggested that, the increased

albumin levels in hypothyroid patients along with non-clinical hyperglycemia is attributed to the deterioration of protein metabolism which further decreases turnover of proteins and increase of half-life of proteins.

The oxidative stress status was determined by the study of one of the most important antioxidants, GSH. The present GSH level of hyperthyroid rats showed a significant decrease when compared with the control rats after three weeks. The present decrease in GSH content in hyperthyroid rats reflects its consuming through the oxidative stress. This result agrees with Kumar *et al.* (2004) who suggested that GSH level was decreased significantly in the hyperthyroid group when compared with the healthy control group. Glutathione depletion might be due to its exhaustion in the oxidative stress as the increased metabolism in response to hyperthyroidism caused oxidative damage to certain organs, including liver, heart and muscles (Venditti and Di-Meo, 2006). This cellular damage occurred when the balance of oxidants and antioxidants in the body is disturbed, and the antioxidant system of the body is not able to neutralize these oxidants. The increase in oxidative stress caused peroxidation of lipids, damage to proteins and DNA fragmentation (Rapozzi *et al.*, 1999).

After six weeks, the GSH levels in hyperthyroid rats were significantly increased and slightly reached above the normal range when compared with the three weeks hyperthyroid rats and control rats. Blagojević *et al.* (1998) suggested that the antioxidant defense system is an endogenous, dynamic system incorporated in homeostatic regulation lead by internal regulatory signals. So, the body might increase the production of GSH as a response to the increased oxidative stress.

On the other hand, the present hypothyroid rats displayed no significant change in GSH levels when compared with control rats after three and six weeks. This result agrees with Petrulea *et al.* (2012) who reported that GSH levels in the hypothyroid rats showed no significant difference from the control rats and disagrees with Sarandol *et al.* (2005) and Erdamar *et al.* (2008) who suggested an increased production of reactive oxygen species in hypothyroidism. There is a complex relationship between TH levels and oxidative stress, but the general principle is that elevated TH levels (hyperthyroidism) induce oxidative stress, whereas reduced THs levels (hypothyroidism) result in non-detectable to mild oxidative stress (Villanueva *et al.*, 2013). Cano-Europa *et al.* (2011) also suggested that a hypothyroidism-induced hypometabolic state protects against oxidative damage caused by toxins.

The present histological liver sections from control rats showed a normal liver structure including normal central vein, portal triads, and hepatocytes. The histological liver sections from both hyper- and hypothyroid rats showed a hepatic injury and hepatocytes lysis. A lobular inflammatory infiltrate of polymorphonuclear leucocytes was also found in the liver of hyperthyroid rats (fig. 1). This result agrees with Ajayi and Akhigbe (2012) who suggested that, there was a hepatic damage in the hyperthyroid rats and this damage was observed as a rise in ALT and AST levels. Saraji *et al.* (2012) also suggested that moderate parenchymal inflammation and edema were found in hyperthyroid rats. Moreover, Cano-Europa *et al.* (2010) suggested that hypothyroidism caused cell damage in the liver. The mechanism of hepatic injury appears to be relative to hypoxia in the perivenular regions, due to an increase in hepatic oxygen demand without an appropriate increase in hepatic blood flow (Malik and Hodgson, 2002). In addition to hepatic

injury, the present hyperthyroid liver sections showed different degrees of intrahepatocytic and extrahepatocytic cholestasis, mainly centrilobular (fig.2). This result agrees with Shen *et al.* (2010) suggested that, liver biopsy revealed characteristic features of intrahepatic cholestasis with mild inflammatory infiltrates indicative of acute liver injury. Cholestasis might result either from a functional defect in bile formation at the level of the hepatocyte or from an impairment in the secretion of bile and/or obstruction of flow at the bile duct level (Trauner *et al.*, 2007).

The present histological section of hyperthyroid rats also displayed a fatty change of the liver tissue, microvesicular steatosis, in which nucleus is squeezed into the displaced rim of cytoplasm about the fat vacuole (Fig. 2). This result agrees with the finding of Upadhyay *et al.* (2004) who suggested that, in most cases of hyperthyroidism and liver dysfunction without heart failure, liver histology demonstrates some degree of fatty infiltration, cytoplasmic vacuolization, nuclear irregularity, and hyperchromatism in hepatocytes.

The present hypothyroid female rats suffered from liver injury, necrosis, and fibrosis (Figs.1&2). Areas of centrizonal necrosis were observed in the present liver histological sections of hypothyroid rats. The extracellular factors inducing cell death (such as Tumor necrosis factor) increased causing damage to liver cells through the activation of an external pathway of apoptosis (Saraji *et al.*, 2012). Karadeniz *et al.* (2008) suggested that kupffer cells (macrophages) activation and infiltration contributed to tissue injury. Liver fibrosis is a common consequence of chronic liver injury (Albanis and Friedman, 2001) and it is defined as the abnormal accumulation of extracellular matrix in the liver. Cano-Europa *et al.* (2010) suggested that methimazole (anti-thyroid drug) caused cell damage in the liver of hypothyroid objects, whereas hypothyroidism caused by thyroidectomy does not cause hepatic-cell damage. So, it seems that the main cause of the present damage of liver of female rats wasn't the hypothyroidism itself but the method of hypothyroidism induction as long as the anti-thyroid drug-induced hypothyroidism can cause cellular damage.

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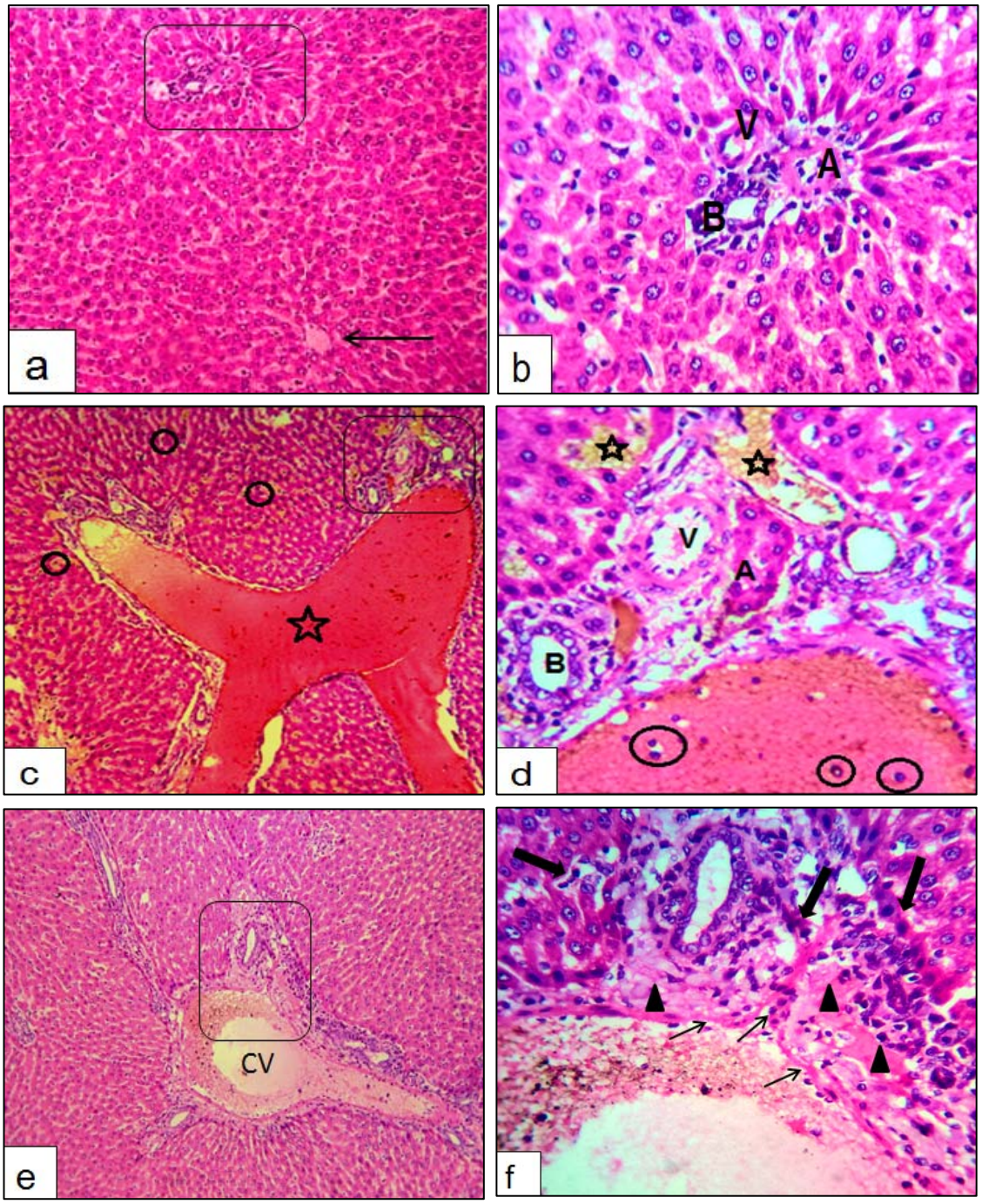

Fig.1: a) Section of liver tissue from a control rat after 6 weeks of treatment with dist. water only, showing a portal triad (square) and a central vein (thin arrow) (X 160).

b) High magnification of the portal triad of Figure 1a to show the portal vein (V), hepatic artery (A) and the bile duct (B) (X 640).

c) Section of liver tissue from a hyperthyroid rat after 6 weeks of treatment with thyroxine, showing the collapse of the liver parenchyma in which a number of portal triads collapsed together, the edematous vein (star), bile accumulation (circle) (X 160).

d) High magnification of one of the collapsed portal triad of Figure 1c to show the damage in in the wall of portal tracts, portal vein (V), portal artery (A), and bile duct (B) (X 640).

e) Section of liver tissue from a hypothyroid rat after 6 weeks of treatment with carbimazole, showing the collapse of some portal triads together, the central vein (CV) (X 160).

f) High magnification of one of the portal triad of Figure 1e magnified to show the damaged wall of central vein (thin arrows) and pyknotic nuclei (thick arrows) of necrotic tissue (triangles) (X 640).

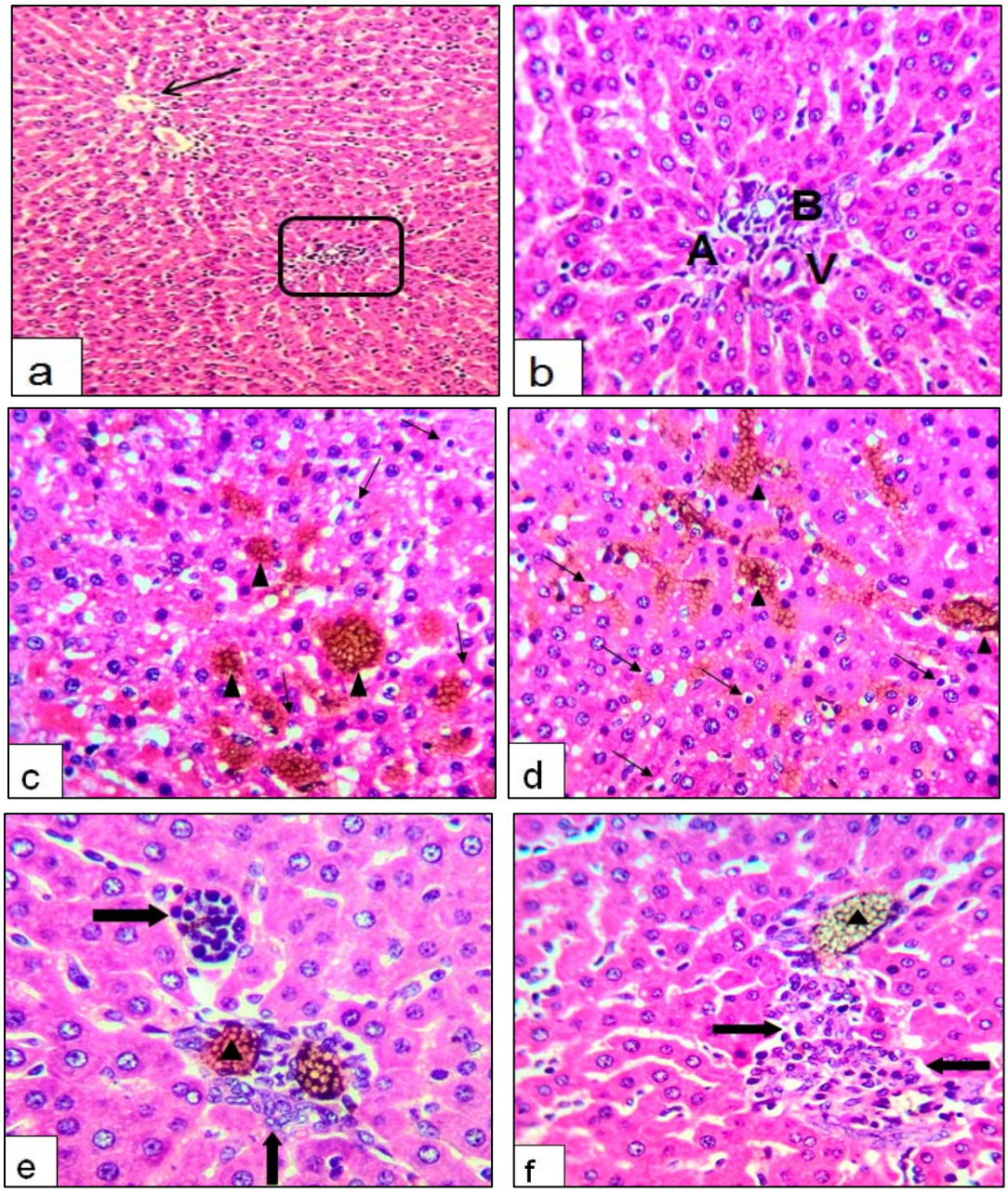

Fig. 2: a) Section of liver tissue from a control rat after 6 weeks of treatment with dist. water only, showing a portal triad (square) and a central vein (arrow) (X 160).

b) High magnification of the portal triad of Figure 2a to show the portal vein, hepatic artery and the bile duct. There is a normal appearance of .the hepatic cells are normal (X 640).

c) Section of liver tissue from a hyperthyroid rat after 6 weeks of treatment with thyroxine, showing the fatty change of the liver tissue (microvesicular steatosis) (thin arrows). In some of cells, the nucleus is squeezed into the displaced rim of cytoplasm about the fat vacuole. There are also liver congestion and bile accumulation (cholestasis) (triangles) (X 640).

d) Another section of liver tissue from a hyperthyroid rat after 6 weeks of treatment with thyroxine, showing showing the fatty change of the liver tissue (microvesicular steatosis) (thin arrows). In some of cells, the nucleus is squeezed into the displaced rim of cytoplasm about the fat vacuole. There are also liver congestion and bile accumulation (triangles) (X 640).

e) Section of liver tissue from a hypothyroid rat after 6 weeks of treatment with carbimazole, showing a centizonal necrosis with an aggregation of the pycnotic nuclei of lysed hepatocytes (thick arrow) and bile accumulation (CV) (X 640).

f) Another section of liver tissue from a hypothyroid rat after 6 weeks of treatment with carbimazole, showing the bile accumulation (triangles) and the pyknotic nuclei of the necrotic cells (thin arrows) (X 640).

ARABIC SUMMARY

التأثير المعاكس للثيروكسين والكاربيمازول على الكبد فى إناث الجرذان

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إستهدفت الدراسة الحالية التحقق من بعض التأثيرات الفسيولوجية والهيستولوجية لإثنين من المواد الكيميائية وهما (الثيروكسين – الكاربيمازول) على وظائف الجسم المختلفة وتركيب الأنسجة فى إناث الجرذان. فى نهاية الدراسة أظهرت إناث الجرذان التى تعانى من فرط نشاط الغدة الدرقية إنخفاض ملحوظ فى معدل زيادة وزن الجسم عند مقارنتها بالمجموعة الضابطة بعد ثلاثة وستة أسابيع من الدراسة. أظهرت أيضا إناث الجرذان التى تعانى من قصور فى الغدة الدرقية إنخفاض ملحوظ فى معدل زيادة وزن الجسم بعد ثلاثة أسابيع ولم يوجد فرق ملحوظ فى معدل زيادة وزن الجسم بعد ستة أسابيع من الدراسة عند مقارنتها بالمجموعة الضابطة. كما أظهرت إناث الجرذان التى تعانى من كلا من فرط نشاط الغدة الدرقية وقصور فى الغدة الدرقية زيادة فى الوزن النسبى للغده الدرقية عند مقارنتها بالمجموعة الضابطة بعد ثلاثة وستة أسابيع من الدراسة. وأتضح أيضا أن الوزن النسبى للكبد فى إناث الجرذان التى تعانى من فرط نشاط الغدة الدرقية قد زاد بطريقة ملحوظة عند مقارنته مع المجموعة الضابطة بعد ثلاثة وستة أسابيع من الدراسة وفى الناحية الأخرى لم يتغير الوزن النسبى للكبد فى إناث الجرذان التى تعانى من فرط نشاط الغدة الدرقية عند مقارنتها بالمجموعة الضابطة بعد ثلاثة وستة أسابيع من الدراسة. كما وجد أيضا إختلاف فى درجة حرارة الجسم عند قياسها من خلال فتحة الشرج فأظهرت إناث الجرذان التى تعانى من فرط نشاط الغدة الدرقية إرتفاع ملحوظ فى قيم درجة حرارة الجسم عند مقارنتها بالمجموعة الضابطة بعد ثلاثة وستة أسابيع من الدراسة وعلى العكس فقد أظهرت إناث الجرذان التى تعانى من قصور فى الغدة الدرقية إنخفاض ملحوظ فى قيم درجة حرارة الجسم عند مقارنتها بالمجموعة الضابطة بعد ثلاثة وستة أسابيع من الدراسة. كما تبين أن معدل إستهلاك الطعام كان مختلفا بين المجموعات الثلاثة وأحيانا كان مختلفا فى نفس المجموعة ولكن فى أوقات مختلفة.

أظهرت أيضا إناث الجرذان التى تعانى من فرط نشاط الغدة الدرقية إرتفاع ملحوظ فى مستوى هرمونى T3 و T4 فى الدم عند مقارنتها بالمجموعة الضابطة بعد ثلاثة وستة أسابيع من الدراسة. وعلى العكس فإناث الجرذان التى تعانى من قصور فى الغدة الدرقية عانت من إنخفاض ملحوظ فى مستوى هرمونى T3 و T4 فى الدم عند مقارنتها بالمجموعة الضابطة بعد ثلاثة وستة أسابيع من الدراسة. أيضا مستويات ALT و AST قد إزدادت بشكل ملحوظ فى إناث الجرذان التى تعانى من كلا من فرط نشاط وقصورالغدة الدرقية عند مقارنتها بالمجموعة الضابطة بعد ثلاثة وستة أسابيع من الدراسة. أما عن مستويات كلا من Albumin و T.Protein فلم تتغير فى كلتا المجموعتين عند مقارنتهم بالمجموعة الضابطة بعد ثلاثة أسابيع من الدراسة. ولكن بعد ستة أسابيع من الدراسة عانت إناث الجرذان المصابة بفرط نشاط الغدة الدرقية من إنخفاض ملحوظ فى مستويات كلا من Albumin و T.Protein وعلى العكس إناث الجرذان المصابة بقصور فى الغدة الدرقية قد عانت من إرتفاع ملحوظ فى مستويات كلا من Albumin و T.Protein عند مقارنتهم بالمجموعة الضابطة. ومن ناحية أخرى فقد أظهرت إناث الجرذان المصابة بفرط نشاط الغدة الدرقية إنخفاض ملحوظ فى مستوي GSH فى الدم بعد ثلاثة أسابيع مع إرتفاع مستواه فى بالدم بعد ستة أسابيع عند مقارنتها بالمجموعة الضابطة. كما لم تظهر إناث الجرذان المصابة بقصور فى الغدة الدرقية أى تغيير ملحوظ فى مستوي GSH فى الدم بعد ثلاثة وستة أسابيع من الدراسة عند مقارنتها بالمجموعة الضابطة. أظهر الفحص الميكروسكوبى لقطاعات الغدة الدرقية والكبد أيضا تضرر أنسجتهما. وتشير كل هذه النتائج السابقة إلى أن كلا من فرط نشاط وقصور الغدة الدرقية قد تسبب في آثار خطيرة على وظائف الجسم فى إناث الفئران.