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HISTOLOGICAL AND PHYSIOLOGICAL STUDY OF THYROID DYSFUNCTIONS ON OVARY IN FEMALE RATS *(RATTUS RATTUS)*

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ABSTRACT

The present study was approved to investigate the effects of thyroid dysfunctions were induced in female albino rats on thyroid gland and ovary, in the case of hyperthyroidism we used Levothyroxine (LVX), and in case of hypothyroidism we used Carbimazole (CBZ), Then measure hormonal levels of thyroid and examine the histological changes of thyroid gland and ovary. Uses for this study forty albino female rats aged twenty weeks old and weighed between 200-300g were separated randomly into four groups: Group I was administration orally 100 µg/kg b.w. of LVX for three months. Group II was administration orally 5mg/kg/ b.w. of CBZ for three months while Group III was orally administration 1ml of normal saline only (0.09%) as a control group for three months. Group IV has orally administration a mix of 50 µg/kilogram for the bodyweight of LVX for six weeks+2.5 mg/kilogram for the bodyweight of CBZ for six weeks. Finally, all the animals were scarified and then blood collection, thyroid, and ovary samples were taken. Physiological study contain thyroid hormones levels measured by using the Enzyme-Linked Immunosorbent Assay (ELISA) kit, we showed significantly ($p < 0.01$) increase levels of Triiodothyronine (T3), Tetraiodothyronine (T4), in contrast decrease levels of thyroid-stimulating hormone (TSH) levels in Group I treated with LVX while group observed significantly ($p < 0.01$) decrease in levels of (T4), (T3), while TSH level increase. The fourth group, showed a slight increase in the levels of thyroid hormones. The results of histological study revealed that, Group I an increase in the colloid resorption with vacuolation of the nuclei of epithelial cells that lining the follicles of the thyroid; formation of corpus luteum in ovarian tissue with the presence of one secondary follicle in Group II. Group III showed a decrease in the colloid resorption with formation of multiple ovarian cysts and hemorrhagic cysts while the histological changes in Group IV showed active thyroid follicles with the presence of resorption vacuoles and congestion blood vessels and formation of many primordial follicles and primary antral follicles of ovarian tissue.

Keywords **:** TSH, Levothyroxine, Carbamazole, Ovary.

Introduction

The thyroid gland is the very important organs of the endocrine glands and its weight ranged (15-20) gram. It has red in clour and soft in consistency, and lie between the C5-T1 vertebrae of columna vertebral is, below the larynx and front of the trachea. It is consisting of two lobes (lobus sinister and lobusdexter) and there are between it. The thyroid has external and internal folium is folded by a fibrosis capsule (Abalovich *et al.*, 2007). The thyroid gland produces Triiodothyronine (T3) and Thyroxin (T4) that have great importance in body functioning. Thyroid hormones have many target tissues in the body with a wide physiological role in the control of growth, development, and metabolic activities of organs. Certain plasma levels of these hormones are required for normal gonadal functions. Inadequate thyroid hormone supply causes abnormal folliculogenesis and anovulation in female rats and mice (Sato and Jiang, 2001)

Hypothyroidism is a mean decrease of the thyroxin hormone concentration, wherever it causes a decrease in the heart rate and weakens the heartbeat the heart becomes less efficient leading to breathing short during the exercise due to the narrowing of the arteries results in of the blood pressure. If the hypothyroidism is not treated, is results in peripheral neuropathy. The peripheral neuropathy included several changes in the nerves that transfer the stimulus between the central nervous system and the body.

In some cases, the thyroid can produce too much of its hormones, which is called hyperthyroidism or overactive thyroid. Clinical signs are include feeling irritable and nervous, low attention, the problem in concentrating, body warm, increased heartbeat, sleep disorder, diarrhea, and losing weight. Sometimes, there is thyroid gland swelling called a nodule. Sometimes, these nodules haven't an impact on the gland function but sometimes results in the secretion of the thyroid hormones at a large amount or sometimes. These nodules may be benign or cancer (Keleş and Keleş, 2008).

Thyroid hormones are included Thyroxine and Triiodothyronine that have great importance in the growth, the differentiation, and metabolism of all organs such as the function of the endometrium and ovaries ((Krassas, Poppe and Glinoer, 2010). The function of the thyroid is essential to general health and reproduction (Muderris *et al.*, 2011), so, the reproduction of the females is decreased in hypothyroidism and hyperthyroidism ((Kang *et al.*, 2013)).

the hypothyroidism causes disorder of female fertility and ovulatory dysfunction (Krassas, 2000).

Materials and Methods

This study was conducted at the period October 2019 to January 2020in Anatomy and Histology department of veterinary medicine of AL-Qassim green university.

Drugs

Levothyroxine (LVX.) 100% Natural was purchased from the local market (pharmacy). Carbemazole (CBZ.) was obtained from the local market.

Experimental rats

Forty albino females rats were used in the present study, obtained from the animal house of college veterinary medicine/Baghdad University, their weights ranging between (200-285g), and their ages were twenty weeks. It is put in the animal House of the Al-Qassim green university, the rats were placed in well ventilated wire-plastic cages and left for 14 days to acclimatize and reared under controlled conditions about 12 hours dark and 12 hour light with temperature was 18-20°C. The animals were maintained at standard housing conditions and fed a standard pellet diet and water.

Experimental design

Forty adult females' albino rats were divided randomly to the four equal groups and gavage for 3 weeks as the following:

Group I: Normal saline only:- Received (1 ml) normal saline only as a control group for 3 months.

Group II: Received (100 µg/kg b.w orally) of Levothyroxin (Zarifkar and Hooshmand, 2003) for 3 months.

Group III: Received (5 mg/kg/b.w orally) of Carbimazole(Hossain,2019) for 3 months.

Group IV: 5 rats received (50 µg/kg b.w orally for 6 weeks) + 5 rats received (2.5 mg /kg b.w orally) for 6weeks respectively.

Serum Preparation

At the end of the experimental period, rats were fasted for (10) hrs, anesthetized with ketamine (75 mg/kg) combined with xylazine (2.5 mg/kg) (Molina *et al*., 2015). Blood samples were collected by heart puncture in non-heparinized tubes, centrifuged at (4000) rpm for (10) minutes (Laessig *et al.*, 1976). After separation the serum from the clot, using a sampler, the samples were used to measurement of T3, T4, and TSH level concentration. The rats were sacrificed by cervical dislocation and take the thyroid gland then the abdominal cavity was immediately opened, ovaries were removed and processed for histopathological studies.

Histopathological techniques

Sections were taken from thyroid and ovary tissues from different animals in each group immediately after sacrificed. Tissues were washed with the normal saline solution to eliminate blood, then put in 10% neutral formalin for period (24) hrs to fixation, dried out in different alcohol concentration, and paraffin embedding for processing. Sections of (5) µm thickness were cut using a rotary microtome. The sections were processed and passed through graded alcohol series stained with Haematoxylin and Eosin, cleared in xylene and inspected microscopically according to (Bancroft and Layton,2012).

Statistical analysis

The statistical analysis was carried out using the Complete Randomized Design (CRD) method according to (AL-Rawi and Kalaf-Allah,2000)). The mean differences between the averages of the studied traits were determined at the probability level of (0.01) using the Duncan test (Duncan, 1995). Statistical data were analyzed using the (SAS,2010).

Results

Effect of Levothyroxine and Carbamazole on thyroid hormones

The data results presented in Table (1) indicate a highly significant increase (P≤0.01) in the levels of hormones T3, T4 and decrease of TSH in advocacy of the second rats' group gavaged with (Levothyroxine) compare with other groups gavaged with (Carbemazole), group of rats gavaged the mixture of (Levothyroxine + Carbemazole) and the control group. Carbemazole showing the opposite result (significant decrease, in the level of hormones T3, T4, increase of TSH). While the group that was treated with the mixture (Levothyroxine + Carbemazole) showed a slight non-significant arithmetic increase in concentrations of hormones secretion T3, T4, TSH when compared to the non-treated control group. T3 secretion concentrations (0.61, 0.81, 0.31, 0.61) and T4 hormone (47.40, 73.72,29.98, 51.34) and TSH (0.048, 0.021,0.072, 0.053) for the four groups, respectively.

Table 1 : Histometric parameter ofT_3,T_4,TSH in female rats treated with levothyroxine, Carbimazole, and (Carbimazole + levothyroxine) (± SE)

Traits	Control	Levothyroxine	Carbemazole	(Carbemazole+levothyroxine)
1.No.of rats	10	10	10	10
T3** Mean ± SE	0.61± 0.03B	0.81 ± 0.02A	0.31 ± 0.03C	0.61 ± 0.03B
T4**Mean ± SE	47.40±6.28B	73.72 ± 2.58A	29.98±2.53C	51.34 ± 1.56B
TSH**Mean± SE	0.048±0.01B	0.021 ± 0.01A	0.072±0.01C	0.053 ±0.01B

Ns: Non-significant *(P≤0.05). ** (P≤0.01)

Histological study

Thyroid gland

Histomorphological study of the thyroid gland section of rats in the control group (G1)was normal histological structures (follicles, colloid, and nucleus) figure (1). Histomorphological study of the thyroid gland in the group (G 2) treated with (100 µg/kg/ b.w I/P) of Levothyroxine showed hyperthyroidism characterize by an increase in the colloid reabsorption (note multiple follicles that appear have little or without colloid with vacuolation of the nuclei of epithelial cells that lining the follicles (Figure 2). While group (G3) treated with (5µg/kg b.w orally) of Carbimazole showed a decrease in the colloid resorption (note absences of resorption vacuoles) with tinctorial variation in the colloid (Figure 3) and in mix group (G4) treated with (50 µg/kg/ b.w) of LVX for 6 weeks+ (2.5mg / kg /b.w) of CBZ for 6

weeks was manifested by active thyroid follicles with the presence of congestion blood vessels and resorption vacuoles (Figure 4).

Ovary

Histomorphological study ovarian tissue of the control group showed active ovary with the formation of ovarian follicles in different stages (Figure 5). G2 treated with (100 µg/kg/ b.w I/P) of LVX for 3 months showed the formation of corpus luteum with the presence of one secondary follicle (Figure 6). Also group G3 with (5µg/kg b.w orally) of Carbimazole of ovarian tissue showed the formation of multiple ovarian cysts and hemorrhagic cyst (filled with RBCs) congestion of blood vessels also seen (Figure 7), and groupG4 with (50 µg/kg/ b.w) of LVX + (2.5mg / kg /b.w) of CBZ if ovarian tissue showed formation of many primordial follicles and primary antral follicles (Figure 8)

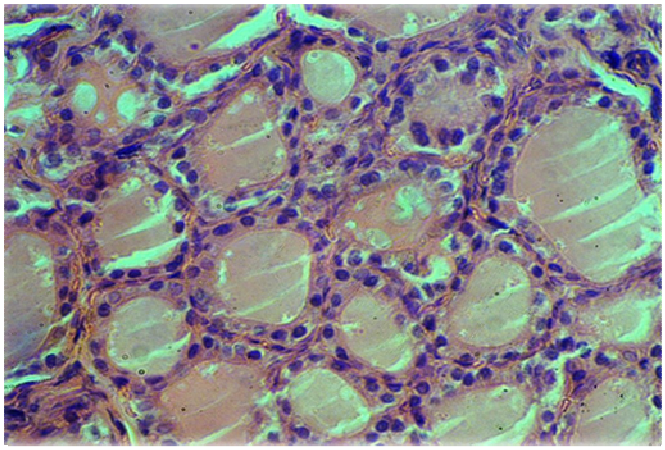

Fig. 1 : (H & E, 400X)

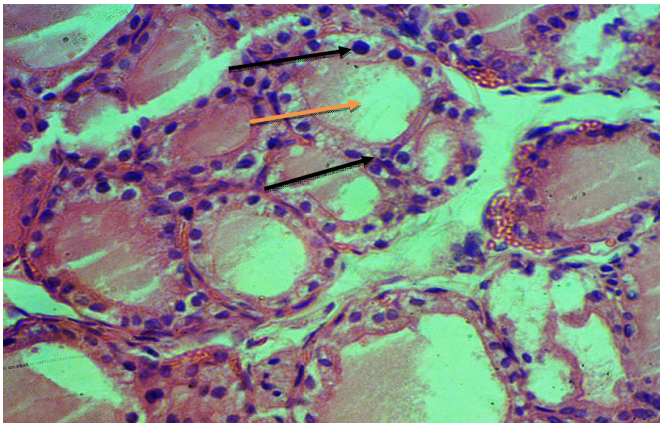

Fig. 2 : (H & E, 400X)

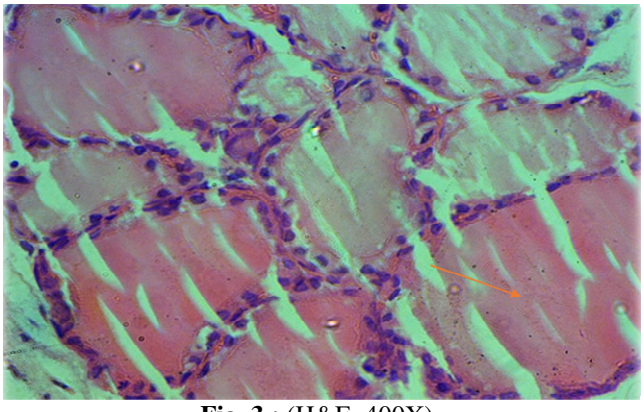

Fig. 3 : (H&E, 400X)

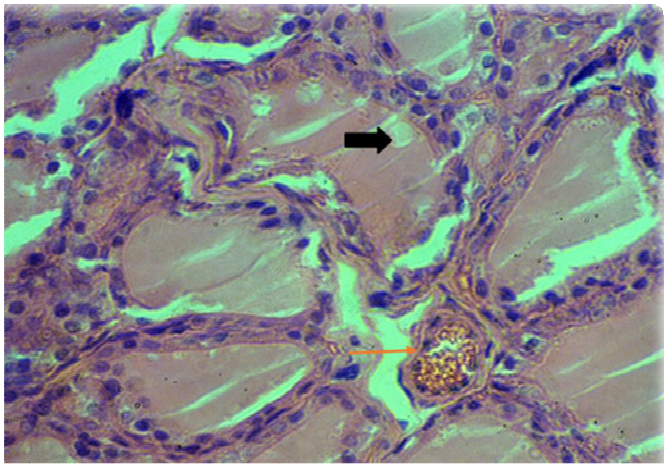

Fig. 4 : (H&E, 400 X)

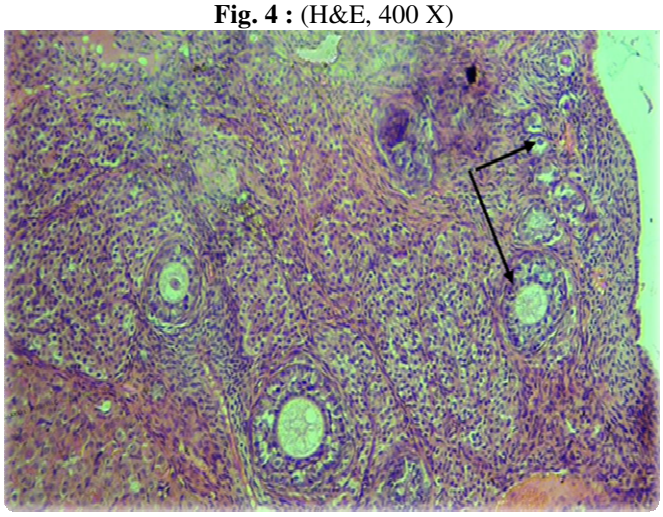

Fig. 5 : (H&E, 200)

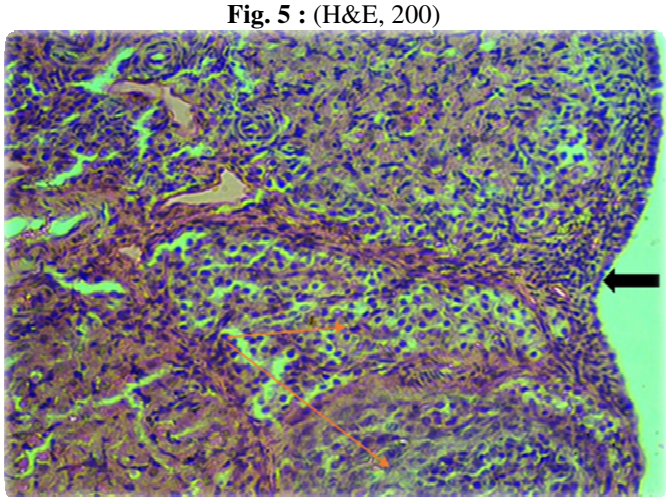

Fig. 6 : (H&E,200)

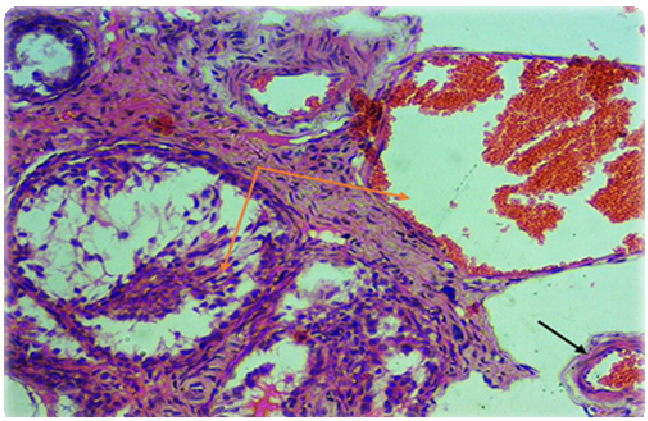

Fig. 7: (H&E,400X)

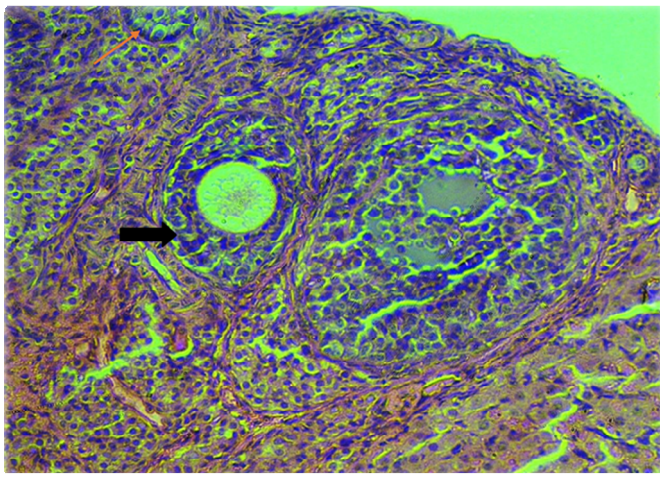

Fig. 8 : (H&E,400X)

Discussion

The result of this study proved that LVX at a dose of (100 µg/kg/BW), produces hyperthyroidism is characterized by a reduction in body weight as that was associated with a significant increase in serum (T4), (T3) levels, and decrease in serum (TSH) level, was showed in LVX rats compared to control, in the current study, the main causes of LVX induced hyperthyroidism due to LVX is a synthetic substance of thyroid hormones, the hypothalamus produces the thyrotropin-releasing hormone, that promotes the anterior lobe of the pituitary for produce thyroid-stimulating hormone, that stimulates the thyroid gland for produce thyroxine (80) % and L-triiodothyronine (20)%. Half of the thyroxine transfer to L-triiodothyronine. The thyroid hormones work by attaching with the thyroid receptor (Sinha and Yen, 2018). The above results of the CBZ group showed a decrease statically in T4, T3 level in the serum of rats attributable to this CBZ is a thionamide. It is converted to methimazole, which inhibits the enzyme Thyroid peroxidase (TPO), thus blocking the conversion of iodide to iodine and inhibiting iodide organification. Methimazole also inhibits the coupling of iodotyrosines, monoiodotyrosine/ diiodotyrosine (MIT/DIT) to DIT. These actions will result in reduced production of thyroid hormones. This study was in agreement with (Herwig et al., 2014) who mentioned that animal studies carried out on pregnant mice, rats, and rabbits showed that treatment with CBZ resulted in hypothyroidism in their offspring.

In this study, CBZ decreased T4 and T3 levels when compared with the control group while it significantly increased TSH levels. In a previous study by (Markou *et al.*, 2001) it was observed that CBZ has an anti-thyroidal property, which inhibits thyroid peroxidase TPO enzyme activities. It might also reduce the T4 level by increasing the level of iodine in circulation, which will inhibit the thyroid gland activity (Wolf Chaicoffs effect) by decreasing iodide organification (inhibition of TPO) and release of T3 and T4. A study by (Vaidya and Pearce, 2008) on the mode of action of CBZ in Graves' disease, observed that CBZ does not work by inhibiting intrathyroidal hormono-genesis, but it affecting thyroid hyperstimulation. It is also possible that CBZ and fresh orange juice (FOJ) might also inhibit the thyroid hormone by inhibiting the hypothalamic-pituitary-thyroid axis.

As for the hormone TSH in both the second and third groups, its level differs in the increase and decrease according to thyroid gland activity. TSH test in the blood is a good way of detecting thyroid hormone. TSH was produced in the pituitary lobe and reflecting off the body responding to the thyroxin level. If the thyroid hormone (T4, T3) concentration becomes less, the pituitary gland produces more TSH. If the TSH is low, that should prevent excessive dose of the thyroid hormone dose, and that similar to the results of (Sowers *et al.*, 2003) who found that value above the normal range indicates that the thyroid is underactive, This indicates hypothyroidism

The results of the fourth group (LEVX+CBZ) in this study showed that we have almost normal values of thyroid hormones due to carbimazole may have an important role in the treatment of human autoimmune (AI) thyroid diseases. Also, it was found that methimazole (the active metabolite of carbimazole,) significantly reduced the severity of experimental AI thyroid disease in rats. It hypothesized that carbimazole may improve the outcome for patients with primary hypothyroidism (or at least some categories) when added to LT4 (Elfayoumy *et al*., 2017).

In the experimental group that treated with (100 µg/kg b.w Orally) LVX. of animals, hyperthyroidism and increase in the colloid resorption was noticed in the histological section due to the effect of LVX on the activity of the thyroid gland, as its use led to an increase in the activity of the gland and an increase in its secretion of hormones this result was in agreement with (Petrova *et al.*, 2015)) who reported that cytoplasm is contain colloid resorptive droplets. These cells secrete triiodothyronine and thyroxine. The colloid fill the follicles. The parafollicular cells secreted calcitonin hormone. Hypothyroidism is one of the most common endocrine diseases mainly caused by disorders in the thyroid gland leading to a decrease in production and secretion of the thyroid hormones. In this study, CBZ was chosen to induce hypothyroidism in (group III) revealed a decrease in T3 and T4and increase in TSH versus the control group revealed marked histological alterations compared to control group decrease in the colloid resorption with tinctorial variation in the colloid and that same with results of (Aboul-Fotouh *et al.*, 2018)) who reported that Multiple follicles appeared degenerated with desquamated epithelial cells in their lumen. Some follicles appeared collapsed, some follicles had disrupted walls and appeared coalesced and other follicles were lined with multiple cellular layers in the group of rats treated with the potassium dichromate which led to hypothyroidism. The histological aspect of the study demonstrates histological changes in thyroid gland treated with mix half dose of both LVX and CBZ presence of resorption vacuoles and showed active thyroid follicles this indicates that the LVX dose stimulated the activity of the thyroid gland and served to inhibit the effect of the CBZ dose on the gland tissue this result was confirmed by (Christakis, Müller and Wüstholz, 2015) who reported that the use of combinational therapy of levothyroxine and carbimazole lead control disturbed levels of thyroid hormones.

According to the vitro studies, the growth of preantral follicles in the rats is promoted by thyroid hormones. Administration of FSH, T3 together enhances reducing apoptosis and proliferation in granulosa cells this result is an agreement with (Taha *et al.*, 2018) who revealed induction of hyperthyroidism by daily oral administration of

levothyroxine (100µg/100g b.w) for 10 days lowered significantly serum estradiol and progesterone levels. Also, these results can be explained by the previous reports of (Tamura *et al.*, 1998) who stated that thyroid hormones suppress FSH induced aromatase activity in granulosa cells and therefore inhibit ovulation. Ovulation disorders such as cysts formation dysfunction in primary hypothyroidism could occur for many mechanisms such as changes in estrogens metabolism, changes prolactin metabolism, and hypothalamic-pituitary dysfunction, that same finding of (Krassas and Perros, 2003) who reported that existing evidence indicates that iodine deficiency and the ensuing reduction in thyroid hormones can be manifested as a reduction in fertility, miscarriage, and fatal defects that include defects in the evolution and function of this system. Thyroid disorders can are included hyperthyroidism (excessive production of T4 and T3) or hypothyroidism (reduce production T4 and T3), hyperthyroidism and hypothyroidism have a great effect on the females so the dose of LVX causes hyperthyroidism which led to an increase the ovary activity to formation follicles and starts to ovulation but the dose of CBZ cause hypothyroidism that led to inhibition of ovary activity our result was in agreement with (Treesh and Khair, 2014) who mentioned that female fertility depends on oocyte maturation, maturity degree of the gonads, the granulosa cells differentiation, the growth hormones and regulation of the hormones, all these causes coordinate between follicular development and the cyclic ovary during folliculogenesis. All the paracrine, endocrine, and autocrine affect the follicular cells (atresia or grow). T4 and T3 are factors that affect the ovarian follicles.

Conclusion

Adding CBZ to LVX, increases serum T3, and improves LT4 tolerance and depression in primary hypothyroid female patients.It also worked to improve ovarian activity.

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