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RESEARCH ARTICLE

Assessment of Serum IL-2 and Immunohistochemistry Detection of IL-2 in Induced Hypothyroidism of Male Rats

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Abstract

This study was conducted to demonstrate the role of methanolic extract from *Ficuscarica* leaves in the treatment of hypothyroidism disease which induced in male rats by carbimazole drug. Forty male rats were divided into five groups, eight for each. group I selected as a negative control which primed orally with normal saline alone, group II served as a positive control and treated by carbimazole anti-thyroid drug for six weeks ,while group III was treated by plant extract (500 mg /kg, bw) for six weeks ,whereas the fourth group IV was treated by carbimazole drug (5 mg) for six weeks to induce hypothyroidism and then treated by plant extract (500 mg /kg , bw) for six weeks too. Finally the fifth V group was treated by thyroxin drug (100 mg) for six week instead of plant extract.The results showed that a non- significant difference ($P< 0.05$) in the level of IL-2 of the fourth group when compared with the first group , while the results revealed a significant increase when compared with the second group ,whereas a significance decrease when compared with the third and fifth groups. The immunohistochemical sections of rats thyroid gland in the first , second and third groups revealed that weak expression of IL-2 immunopositive cells (brown cytoplasmic deposits) in thyroid sections, while rats thyroid gland in the fourth group and fifth groups revealed that a moderate expression of IL-2 immunopositive cells. In conclusion it is possible to use methanolic extract of *Ficuscarica* leaves in the regulation of immune response in hypothyroidism disease may due to the presence of phytochemical components that enhance the cellular immune response by increase the IL-2 production.

Keywords: *Hypothyroidism, Carbimazole IL-2, Immunohistochemistry, Ficuscarica, phytochemical components.*

Introduction

Thyroid gland is one of the most important endocrine glands in animal and human body. It has an important role in regulating of all metabolism process in the body, central nervous system activity, and the regulation of all the physiological functions of variant tissues such as the liver and the heart [1].

The thyroid gland secretes two important hormones: triiodothyronine (T3) and tetraiodothyronine called (T4) which have essential role in regulating the metabolism process in every cell inside the body. They can promote growth process, development, function and maintenance of all body tissues. They act as regulator of heart rate, body temperature, and cholesterol and body weight [2]. Hypothyroidism means defect in thyroid gland function [3] and can be classified into :firstly Primary hypothyroidism which is due to thyroid gland disorder leading to decreased circulation of thyroid hormones or failure to produce enough thyroid hormone and the second type is called secondary hypothyroidism that caused by a disorder of the pituitary gland or the hypothalamus axis which lead to decreased TSH synthesis and then to decreased synthesis and secretion of thyroid hormones [4].

Hypothyroidism disease is diagnosed by low level of (T4) and (T3) and high level of thyroid-stimulating hormone (TSH) [5]. *Ficuscarica* Linn. (Moraceae) known as the fig plant, is grown and agriculture in tropical and sub-tropical regions [6]. Analyses by using HPTLC to identify phytochemical

components revealed to presence of amino acid tyrosine in the extract from leaf which may be helping or responsible from thyroidal activity when using *Ficuscarica* leaf extract as therapeutic agent. The aim of current study was to determine the effect of methanolic extract for *Ficuscarica* leaves in regulate the immune response and it may be plays ameliorative role when used for pharmaceutical purposes to treatment of induced hypothyroidism.

Materials and Methods

Preparation of Extract

Fresh leaves of *Ficuscarica* were taken from local farms. In order to prepare the methanolic extract, leaves was washed in a tap water and distilled water, and then dried for two weeks in shadow , then crushed into a coarse powder by using a blender , amount of 100 gm of the coarse powder was kept soaked in methanol (500 ml, 99%) and then soxhulat apparatus and rotary evaporator were used in order to get the extract which was stored in refrigerator 4 c until a suspension of this extract was used to treat the animal.

Laboratory Animals

Forty (40) male rats were divided into five groups, eight animals for each. First group served as a negative control primed orally with normal saline alone for six weeks.

The second group used as positive control was treated with anti-thyroid carbimazole drug (5 mg/kg, bw) for six weeks, the third group was treated with plant extract (500 mg /kg, bw) for six weeks, while the fourth group was treated by carbimazole drug (5 mg/kg, bw) for six week to induce hypothyroidism and then treated by plant extract (500 mg /kg , bw) for six weeks too. The fifth group was treated by carbimazole drug (5 mg/kg, bw) for six week to induce hypothyroidism and then treated by thyroxin drug (100 mg/kg, bw) for six week too instead of plant extract. Hypothyroidism was stimulated by anti-thyroid drug called carbimazole (5mg/kg) which is using in the treatment of human hyperthyroidism disease [7].

Immunohistochemistry Technique

Rats paraffin embedded thyroid gland sections were used for immunohistochemistry technique using the (Abcam, USA). Briefly, samples were deparafnized. Sections, then immune stained with primary antibody to IL-2 (My bio source, USA) and counterstained with hematoxylin.

Evaluation of IHC Results

Immunohistochemistry was given intensity and percentage scores, based on intensity of positive staining and number of cells staining, respectively. [8].

- Score 1: 1-25%.
- Score 2: 26-50%.
- Score 3: >50% [8].

Statistical Analysis

The data were analyzed as mean ± S.E (standard error mean) for eight rats. Statistical analyses were carried out by using SPSS software [version 17]. Differences among control and experimental groups were assessed using one- way Anova and Duncan, least significant differences (L.S.D). Probability less than $P < 0.05$ [9].

Results

Serum Level of Interleukin -2

The results (figure -1) showed that non-significant difference in IL-2 concentration in the fourth group (carbimazole& plant extract) when compared with the first group (normal saline alone) and fifth group (carbimazole& thyroxin drug), while the results revealed a significant increase ($p \leq 0.05$) when compared with the second group (carbimazole alone) and a significance decrease ($p \leq 0.05$) when compared with third group (plant extract alone).

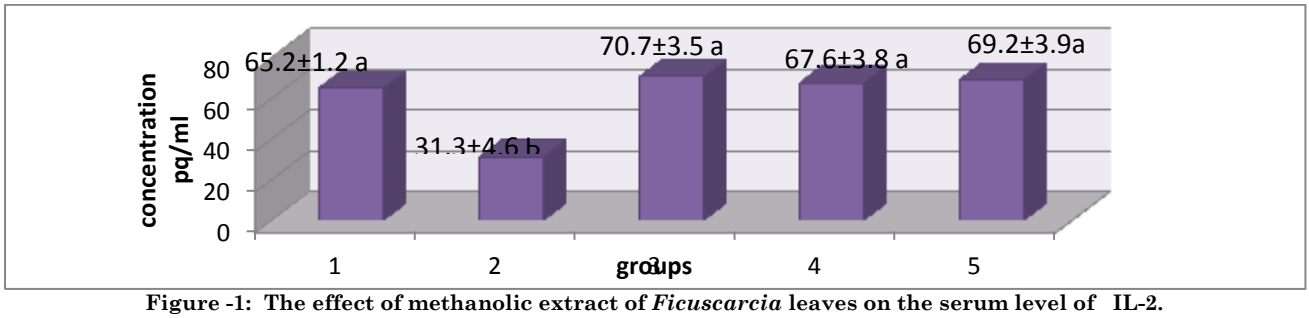

Figure -1: The effect of methanolic extract of *Ficuscarcia* leaves on the serum level of IL-2.

Immunhistochemical Study

Thyroid gland immunohistochemistry

The immunohistochemical study and histological sections of rats thyroid gland in the first, second and third groups (Table-1) revealed that weak expression of IL-2 immunopositive cells (brown cytoplasmic deposits) in thyroid sections (Figure 2 , 3 , 4) , while rats thyroid gland in fourth and fifth groups revealed that a moderate expression of IL-2 immunopositive cells (Figures 5, 6).

Table 1: Distribution of IL-2 immunopositive cells in rats' thyroid gland

Studied Groups	Positive IL-2 signaling	Negative IL-2 signaling
First group Normal saline alone	**2/8 (25%)**	6/8 (75%)
Second group carbimazole alone	**1/8 (12.5%)**	7/8 (87.5%)
Third group plant extract Alone	**2/8 (25%)**	6/8 (75%)
Fourth group carbimazole&plant extract	**4/8 (50%)**	4/8 (50%)
Fifth group carbimazole&tthyroxin	**5/8 (62.5%)**	/83 (37.5%)

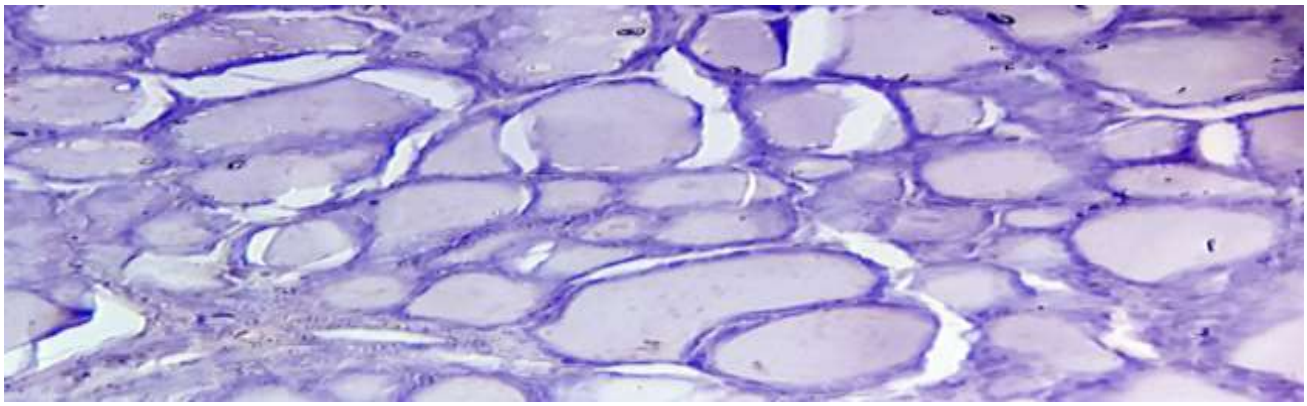

Figure 2: Immunohistochemical study of thyroid gland from rat in the negativecontrol group showed weak IL-2 immunopositive cells (40 X)

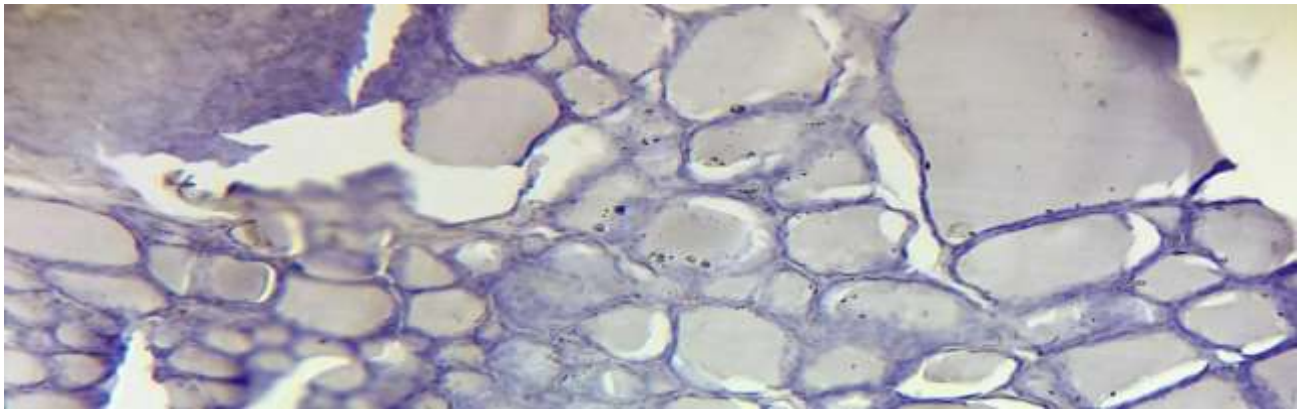

Figure 3: Immunohistochemical study of rat thyroid gland in the second group showed weak immunopositive IL-2 cells (40 X)

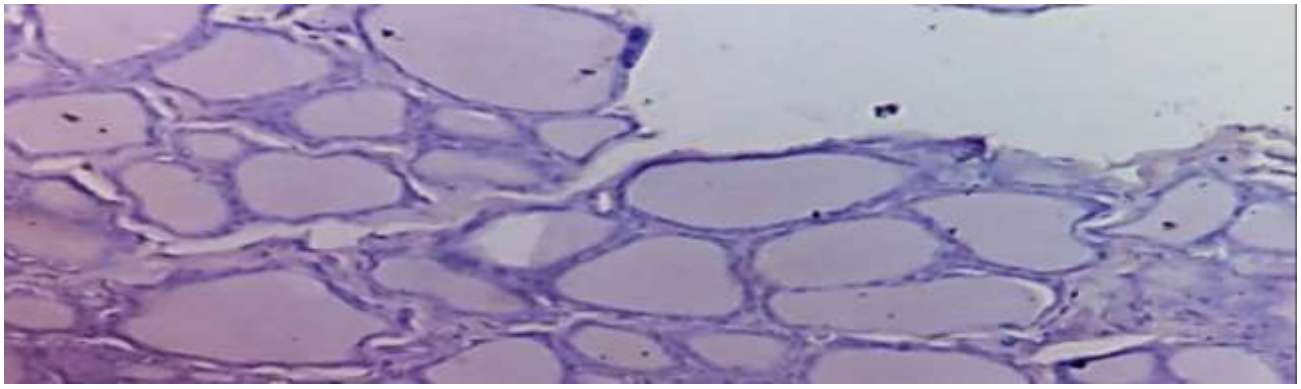

Figure 4: Immunohistochemical study of thyroid gland for rats in the third group showed a moderate immunopositive IL-2 cells (40 X)

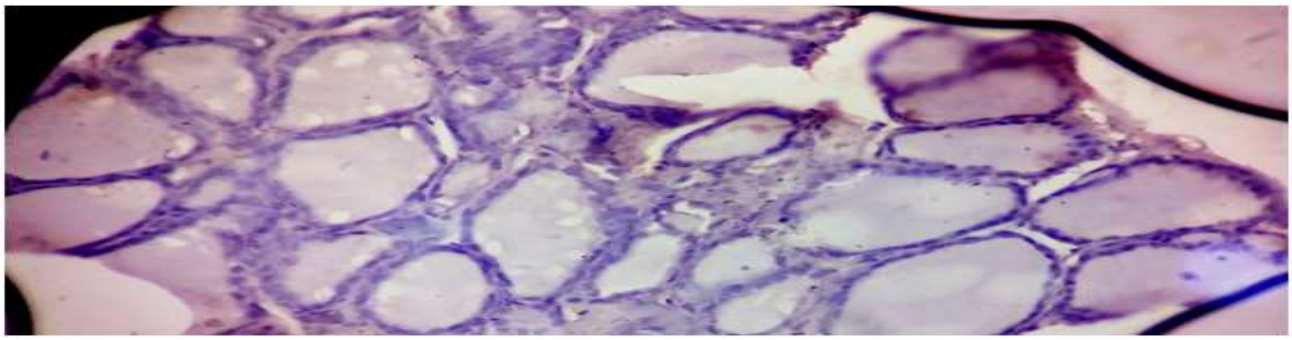

Figure 5: Immunohistochemical study of rats' thyroid gland in the fourth group shown moderate munopositive IL-2cells (40 X)

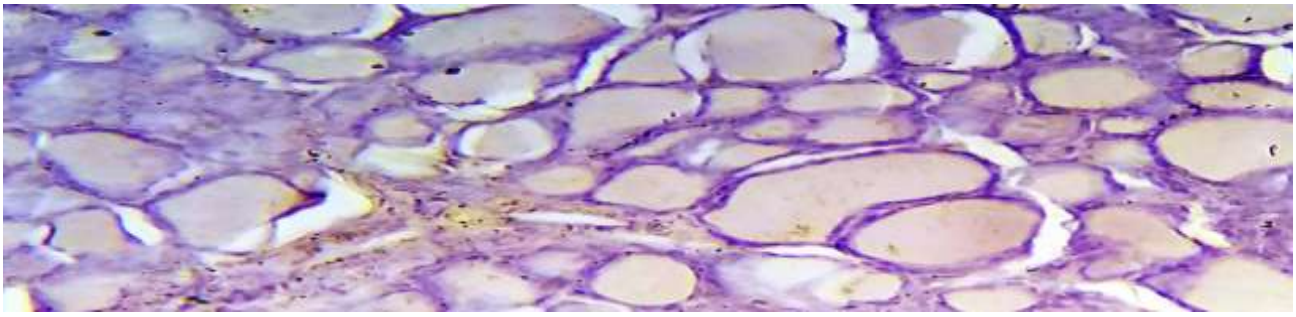

Figure 6: Immunohistochemical study of rats thyroid gland in the fifth group shown a moderate immunopositive IL-2 cells (40X)

Liver Immunohistochemistry

The current study in the (Table-2) and histological sections demonstrated that the IL-2 immunolabeled positive cells were weak expression in liver sections of first control group (normal saline alone) and second group (carbimazole alone) (figure 7,8) , while liver section of animals in the third group treated by plant extract alone showed an increase number of IL-2 immunolabeled hepatocytes around central veins, suggesting that moderate in IL-2 expression (9). Also , the immunohistochemical study of a liver section in fourth group treated by (carbimazole and plant extract) and fifth group treated by (carbimazole and thyroxine) revealed an increase and high expression in IL-2 -immunolabeled positive cells when compared with control group (Figure 10,11).

Table 5-4: Distribution of IL-2 -immunolabeled positive cells in rat livers

Studied Groups	Positive IL-2 signaling	Negative IL-2 signaling
First group Normal saline alone	2/8 (25%)	6/8 (75%)
Second group carbimazole alone	1/8 (12.5%)	7/8 (87.5%)
Third group plant extract alone	4/8 (50%)	4/8 (50%)
Fourth group carbimazole&plant extract	6/8 (75%)	2/8 (25%)
Fifth group carbimazole& thyroxin	7/8 (87.5%)	1/8 (12.5%)

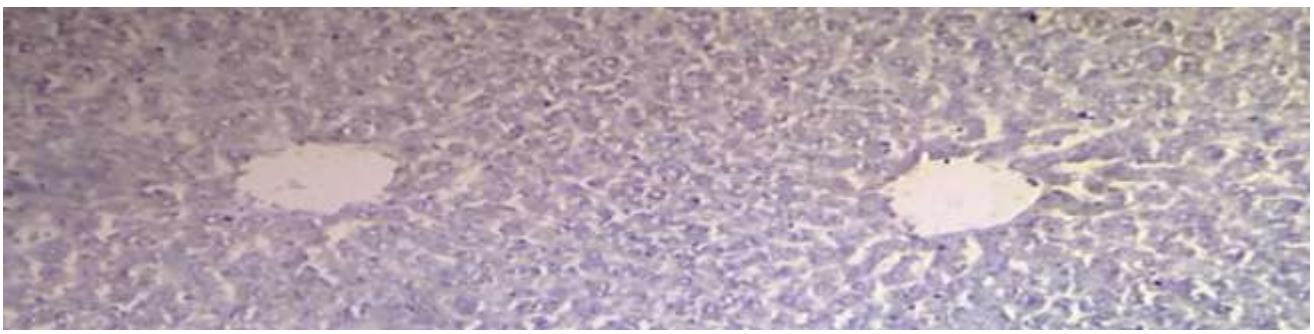

Figure 7: immunohistochemistry study of liver in control group (normal saline alone) showed that IL-2 immunolabeled cells were weak expression (20 X)

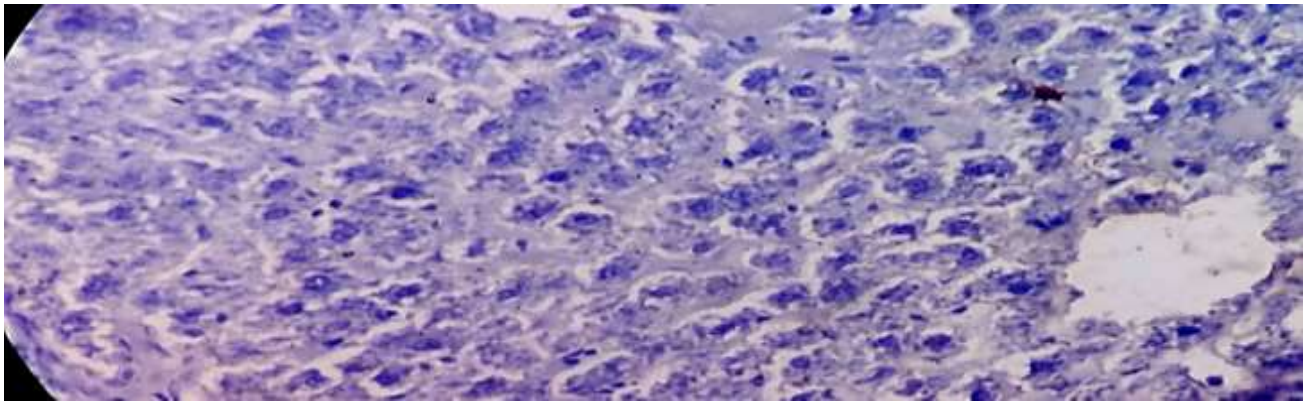

Figure 8: immunohistochemistry study of liver from second group (carbimazole alone) revealed weak expression of IL-2 immunolabeled positive cells (40 X)

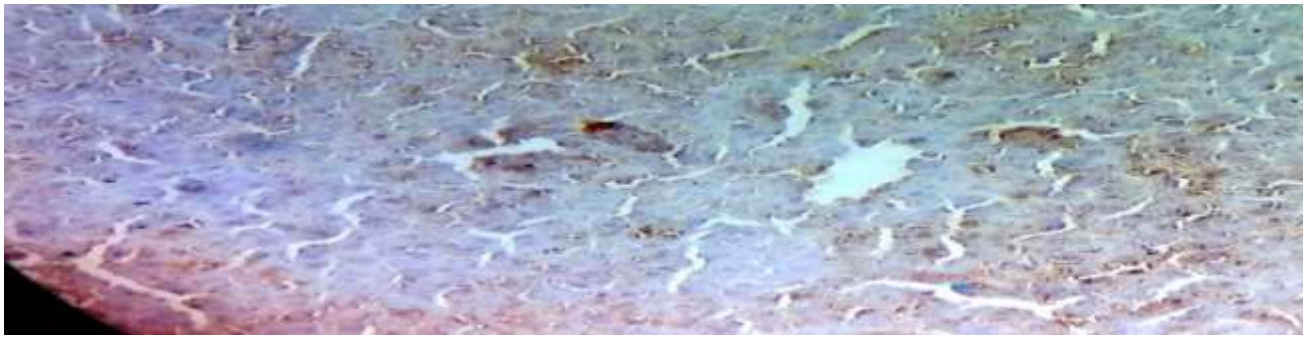

Figure 9: immunohistochemistry study of liver from third group (plant extract alone) showed moderate expression of IL-2 immunolabeled positive cells (20 X)

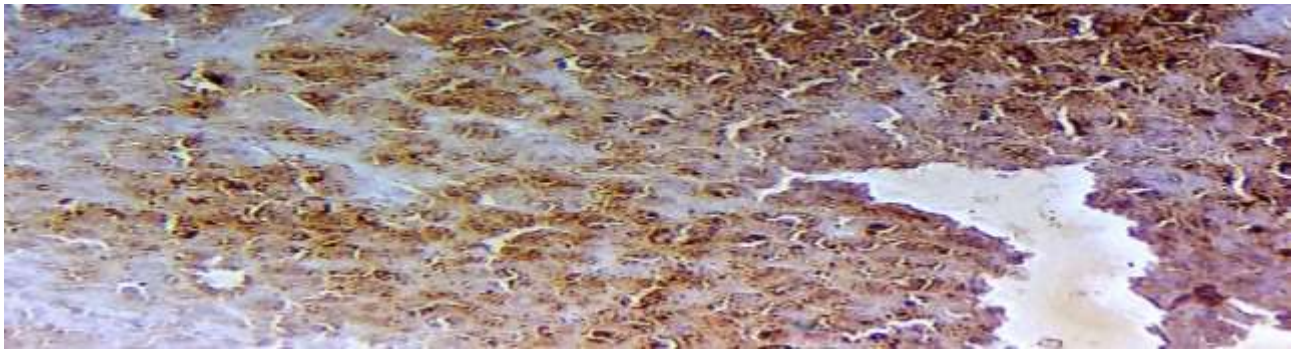

Figure 10: immunohistochemical study of liver from fourth group (carbimazole and plant extract) , observed high expression of IL-2 immunolabeled positive cells (40 X)

Figure 11: immunohistochemistry study of rats' liver in the fifth group carbimazole and thyroxin showed high expression of IL-2 immunolabeled positive cells (20 X)

Discussion

Cytokines are synthesis bynumerous cells of immune system and provide several functions, composed of mediation in the immune responses according on a several factors, such as local, amount, receptor expression style and the integration of different signaling pathways in response to immune cells [10]. Interleukins are small protein molecules that signal specific cells to regulate the immune system, primarily synthesized by T cells, monocytes, macrophages and endothelial cells. There functions interleukins include the facilitation

of communication among immune system proliferation antibody secretion and cellular regulation [11]. It has been showed that thyroid follicular cells can produce and release cytokines, which activate T and B lymphocytes and then play a key role in coordinating the immune response in the pathogenesis of Graves' disease (Hyperthyroidism) [12]. Serum concentration of these cytokinereveal a good relationship with endocrine system, indicating that they could even function as biomarkers of thyroid status in hyperthyroidism [13].

Hashimoto's thyroiditis regarded as a local autoimmune disease that is distinguished by the synthesis of auto- antibodies against thyrocytes auto-antigens and infiltration of auto reactive cytotoxic T- cells in the thyroid gland that lead to increase in damage degree of follicles [14]. It is originally described as stromalymphomatosa that is distinguished by the formation of lymphoid follicles, clear variety in the thyroid epithelial cells, heavy formation of new connective tissue and diffuse infiltration of round cells [15].

In current study, the IL-2 serum level was found to be significantly decreases in animals with induced hypothyroidism than in controls. The immunohisto chemical study may appear some aspect to the pathogen degree of this disorder, in which the histological changes demonstrated that in thyroid gland were characterized by five sorts of follicular epithelial and stromal alterations: hyperplastic change in follicles, depletion in colloid components inside follicular lumen, clear and vesicular cytoplasmic change in follicular epithelium, clear differences in follicular size, and vascular dilatation of the stroma.

The most essential and specific of the five sorts of histological alterations were the lack of colloid materials in the follicular lumen in addition, clear and vesicular cytoplasmic change in the follicular cells. The lack of colloid materials may result from interference with TG synthesis, because excess iodine inhibits the organification of iodine in the follicular cells, also the clear and vesicular cytoplasmic change seemed to be related to the interference with hormone synthesis in this disease. The increase in the serum level of IL-2 is associated with increase serum level of IL-12 which released by APCs activates TH1-type CD4+ T cells, lead to balance in the immune between effector and regulatory cells to breakdown. TH1 cells can release IL-2, which can be induce specific pre-cytotoxic T cells (Pre CTL) to become cytotoxic (CTL), andIFN-g, that enable to stimulate macrophages to become cytotoxic.

These cytotoxic macrophages release cytokines including IL-1b, TNF-a, and IFN-g, and free radicals. TH1 cells can also to secrete cytokines that are directly cytotoxic cells. These CTLs release granzyme and perforin (cytolysin), which are toxic to b cells. In addition, Fasand TNFR-mediated apoptosis are involved in b cell destruction. In this way, macrophages, Tcells, and cytokines synergistically act to destroy b cells, resulting in the development of autoimmune disease [16].

The histochemical study showed moderateimmunopositiveIL-2 cells (Figure 5, 6) and negative AB reaction inthe colloid, which indicates the presence of a glycoprotein rich in neutral glycosaminogly can. The expression level of IL-2 in animal with hypothyroidism disease (second group) was significantly lower than that in animals with plant extract treatment (p<0.05) (fourth & fifth) groups. IL-2 isan inflammatory cytokine secreted by Th cells and it is known that elevated levels of IL-2 can often prompt some autoimmune diseases, such as rheumatoid arthritis. Current IL-2 cans be used as biomarkers for the identification of hypothyroidism [17, 18].

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