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RESEARCH ARTICLE

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EFFECT OF TRIAMCINOLONE ACETONIDE (TA) DRUG ON SURFACE MARKERES IN LYMPHOCYTES OF MALE MICE FEEDING WITH BETA-CAROTENE

ABSTRACT:

The current study tended to search the impact of beta-carotene and anti-inflammatory Triamcinolone acetonide (TA) drug on the immune system in male mice (Balb / C). It was taken into account for this study to do it through two stages of the life stages of mice; the first stage was before the mice reach adulthood, so the transaction began at the age of four weeks. The second phase was after the arrival of mice the adulthood age where the transaction began at the age of eight weeks. In an attempt to reduce the possible side effects of the TA drug, which is an immunological inhibitor, beta carotene has been used as a natural substance assumed to be immunocompetent. Therefore, the mice were treated with the drug alone and the others with both beta-carotene and the drug concurrently. The study stated and summarized the positive effect of beta-carotene and the drug treatment on surface marker Thy $1.2^{+}$and PNA^{+} in lymphocytes of thymus gland. The negative effect of treated with the drug alone compared with control group. Thus, the present research aimed at trying to help the injecting of the anti-inflammatory Triamcinolone acetonide (TA) drug that contain a derivative of cortisone to alleviate it's side effects through using beta-carotene during the treatment period.

KEY WORDS:

Triamcinolone Acetonide, Lymphocytes, Beta-Carotene.

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INTRODUCTION:

Many of the food products such as fruits and vegetables contains small molecular weight substances which may be vitamins or minerals, also the body contains enzymes, that have the ability to prevent or stop the formation free radicals, which include the reactive oxygen or discourage it's interactions. Such enzymes neutralize the production of the free radicals and protect the body against oxidation properties of free metals, as well as against decomposition processes especially in the elderly, and therefore they protect the body from various diseases and maintain public health (Gaishenets *et al.,* 1989; Ames *et al.,* 1993; Marniemi *et al.,* 1998). (Chaudiere and Ferrari-lliou, 1999) had defined antioxidants within the cell as: materials with very small molecular weights and enzymes analyst, is also a trapper for free radicals including the reactive oxygen, as it removes them or discourage their interactions and thus preventing the attack of vital tissues in the body.

The group of carotenoids is considered to be the most widespread pigments in nature, which is responsible for the yellow, orange and red colors for fruits and many vegetables, flowers, fungi and some animals, it has been identified more than 400 colored material is a under carotenoids. One of the most common carotenoids is some herbs, Fayyolakzantin, beta-carotene, lycopene and Apo-karotinoeidat, (Khachik *et al.,* 1997; Francis, 2000).

Beta carotene have been discovered since ancient times into the world of food, that it gives many fruits and vegetables the yellow color, where it is located in abundance in certain fruits and vegetables such as sweet potatoes, carrots, spinach, yellow squash, turnip green, beet greens, tomatoes, red peppers, apricots, peaches, prunes and oranges, has been found that it is important for public health because it is converted in the body to vitamin A, but in recent years, recent studies showed that it is not only a source of vitamin A but it works as an antioxidant and thus resistant to cancers and many chronic

diseases. Therefore, it can be said that beta carotene works in the body in two ways, one that it is anti-oxidant which strongly affect the reactive oxygen and the as trapper for the free radicals in the blood, and the other as a source of vitamin A in the body provide the body with its needs of vitamin A so it was called Pro vitamin A (Hercberg *et al.,* 1998; Gaby and Singh, 1991).

Studies have shown that beta carotene works as an anti-embryonic mutation in individual smokers and non-smokers, where it works to reduce the incidence of fetal mutations by reducing the destruction incident in the DNA, (Wald *et al.,* 1984; Brock *et al.,* 1988; Van popple *et al.,* 1992).

The beta-carotene as well as other antioxidants might serve to discourage the destruction in the chromosomes, (Dusinská *et al.,* 2003; Riso *et al.,* 2004). In other studies, beta carotene works as an antioxidant and thus reduces the risk of many different types of cancers such as cancer of the lung, cervical cancer, stomach cancer, esophagus cancer, skin cancer, mouth cancer, breast cancer and colon cancer in both human and experimental animals through different feeding by it through different doses (Nesaretnam *et al.,* 2002; Choi *et al.,* 2006; Sampaio *et al.,* 2007).

Nutrition with beta carotene is also linked to the reduction in the rate of death among individuals because of heart disease and atherosclerosis (Toma *et al.,* 2003).

Where a research study demonstrated a low level of beta carotene in the serum in coronary artery disease reflects the activation of the immune system, and that consideration should be given to the inflammation as a key element when analyzing the relationship between beta carotene and coronary artery diseases, (Jonasson *et al.,* 2003). Another study showed that beta carotene positively affects cellular immunity in healthy individuals, by increasing the number of T- helper lymphocytes, which play a key role in cellular immunity, (Richard and Passwater, 1984).

Also another study confirmed the impact of beta-carotene as a catalyst for the immune system in individuals as the number of T-helper lymphocytes and killer cells (K) for destroying cancer cells increases (Bendich, 1988). Another study also confirmed that the protection of cancer generated by the beta carotene in the body are the result of beta-carotene encouraging of the immune system in humans, increase the number of NK cells, and The cells (Watson, 1991). Beta-carotene also showed a stimulation effect on the T cell division and the production of IL-2 and the production of antibodies in mice and to promote the cellular response within the body and outside the body (Bendich, 1991; Jyonouchi *et al.,* 1991). Beta carotene was proved to increase the capacity of macrophagies to secrete IL-1 significantly (Potapova *et al.,* 1993). Beta-carotene treatment for either long-term or short term also led to different effects on cellular immunity in healthy elderly individuals, as significantly they have induced increase in the proportions of natural killer cells, which IL-2 appears on its surface receptors, but there was no effect of beta carotene on other immune cells in this study (Santos *et al.,* 1997). As shown the positive impact of beta carotene to increase T-lymphocytes and B-lymphocytes in the older dogs and it improves the respond to sensitivity tests against excessive backloge of red blood cells of sheep (Massimino *et al.,* 2003).

Another study showed the impact of nutrition by beta carotene on lactating mothers, where they increase the number of white blood cells and multiple cell nuclei compared to non-lactating mothers, (Gossage *et al.,* 2000). In the study conducted on mice of strain Balb / C age of 3 weeks old on the impact of nutrition by mouth proved that beta carotene stimulated the production of IFN-γ, IL-12, IL-2 and worked on promotion of T-helper cells to secrete of various cellular secretions, and discourage the production of antibodies of the type of IgE and IgG_1, which effect he appearance of the symptoms of type I allergies (Sato *et al.,* 2004).

The studies identify the TA drug, that it is derived from sugar derivatives cortical hormones, has been used in high doses in the treatment of many diseases, including frozen shoulder, Addison's disease, osteoarthritis, psoriasis, calcium pyrophosphate deposition, rheumatoid arthritis, leukemia, hypersensitivity, where the treated is by injecting either intramuscular, intravenous, intra-articular, intradermal, subcutaneous or intrapreitoneally.

Some of the common side effects of the drug are depression, difficulty in sleeping and increased eye pressure causing glaucoma, osteoporosis, bone fractures, ulcers in the stomach, and inhibition of secretion of the adrenal gland, increased susceptibility to infections, irritation, redness and pain at the injection site and skin paper (Goodman *et al.,* 1985).

It is derived from the derivatives of cortisone and cortisone and its derivatives has been used as widely feedstock drug in the treatment of anti-inflammatory agents and to depress the immune system, and treatment of autoimmune diseases, allergies and organ and tissue transplantation (Hokama and Nakomura, 1982). In a study of Greenspan and others about the impact of cortisone acetate injection under the skin and peritoneal cavity in rats, results showed a significant decrease in body weight accompanied by atrophy of the thymus gland and the cortex of the adrenal gland (Greenspan *et al.,* 1953). Drug also showed a

negative effect on the kidneys, liver and body weight also protein synthesis, functions of the adrenal gland, pituitary and on the heart muscle also several toxic effects had accompanied its usage (Osbaldiston, 1971; Zimmerman *et al.,* 1973; Gilman *et al.,* 1980; Ryrfeldt *et al.,* 1992; Jin *et al.,* 2006).

The study conducted on the impact of acute and chronic treatment by TA drug in fish on glucocorticoid receptor (GR) in white blood cells in the gills and kidney that chronic treatment with TA resulted in a reduced number of GR on the white blood cells in the gills, leading to reduced gravitational capacity in the renal cell (Maule and Schreck, 1991). Another study concluded that treatment with cortisone acetate CA with different doses led to atrophy of the thymus gland and some other organs secreting sex hormones such as adrenal gland and testis in an hamster animal (Hirano *et al.,* 2001). The study by Chen and others proved the inhibitory influence of TA drug on the immune system may be due to its effect on inhibition of appearance of some surface HLA and CD of Ag antigens of dentritic cell (Chen *et al.,* 2001). In a subsequent study, it was shown that the effect of the anti-inflammatory TA drug would be through inhibition of the number of basal cells in the blood (Stelmach *et al.,* 2002).

In the study by Johnson and others on the impact of Triamcinolone acetonide (TA) drug treatment at the level of transforming growth factor-beta (TGF-beta) in fish, the study continued for two weeks, and concluded that drug treatment led to the inhibition of TGF-beta in Neutrophils, lymphocytes and a single nucleus cells, as the drug leds to increase concentration of glucose in the blood (Johnson *et al.,* 2006).

MATERIAL AND METHODS:

Experimental animals:

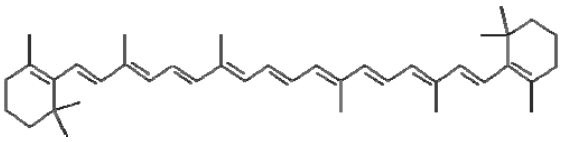
White male mice (Balb/C) had been used in this research, which were obtained from the animal house of the King Fahd Center for Medical Research, King Abdul Aziz University in Jeddah, after making sure absolutely free of any parasites or other diseases, and these Balb/C represents the best example of feeding laboratory animals so as to availability, small size and ease of handling, ease of breeding in the lab, and the possibility of obtaining pure strains of which are valid for research experiments. Experiments were conducted on these mice in laboratories for the Faculty of Education for girls in Jeddah.

Material under test:

*β-carotene Powder with a reddish orange in color, present naturally in some fruits and vegetables, it is non soluble dye in water and soluble in organic solvents, it is also one of the food colorings made from a natural source, (Sigma, St. Louis).

Colour Index Number: (C.I.No.75130) Chemical Formula: $C_{40}H_{56}$

Structural Formula:



Dose used:

Beta-carotene was added to animal feed in the amount of treatment dose (20 mg \ kg \ day), Sato *et al.* (2004).

Drug:

Generic name: Triamcinolone acetonide

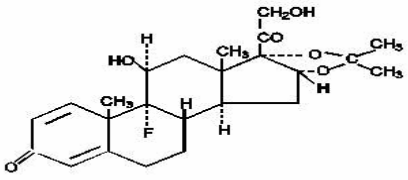
Product name: Kenacort-A

Chemical Composition:

Triamcinolone acetonide 40mg, sodium chloride, alcohol Benzili, sodium Krboxi methyl cellulose, and Bolesorbac.

Chemical Formula: $C_{24}H_{31}Fo_6$

Structural Formula:



Dose used:

The dose was calculated by the following equation: 26 × dose/1000, (Paget and Barnes, 1964).

Calculation of the dose in the mice aged 4 weeks: 26 × dose/1000 = 26 × 10/1000 = 0.26 mg/kg.

Calculation of the dose in the mice aged 8 weeks: 26 × dose/1000 = 26 × 20/1000 = 0.52 mg/kg.

Material used:

Fluorescent clonal antibodies: Monoclonal Fluorescent anti-Thy 1.2 antibody: Is a type of immune globulin united with fluoresceine isothiocyanate (FITC) and has been reduced to 1:100-1:200 PBS solution and then divided into small volumes (0.05ml) in tubes “Epindorff” and saved in a temperature of 20º c and is melted when they are used to avoid re-freezing and thawing. And anti-Thy1.2 is using in marking of all Pan T-cell. (Sigma, St Louis) Fluorescent Peanut Agglutinin (PNA): Is a vegetarian Lectin plant united with FITC has the ability to Union private, non-mature T cells and therefore used to mark these cells in the thymus. It has been diluted to 1: 200 in PBS and was divided into small quantities (0.05 ml) and then saves at a temperature of -20º c until use (Sigma, St. Louis).

Experimental design:

The total number of mice used in the study were165 mice, three groups of mice had been used in this study. Each group was sub divided into two subgroup; (4 weeks old and 8 weeks old mice).

Group I: A control group of male mice aged 4 weeks 35 mice, and 20 mice aged 8 weeks treated with beta-carotene-free diet, and had been injected a known amount of dilute solution of the drug in the peritoneal cavity for a period of seven weeks for the group of mice aged four weeks and four weeks for the group of mice aged eight weeks.

Group II: 35 mice aged 4 weeks, each mouse was treated with the drug with dose of 10mg/kg/wk. 20 mice aged 8 weeks; each mouse was treated with the drug with dose of 20 mg/kg/wk.

Group III: 35 mice aged 4 weeks; each mouse was treated with a dose of beta carotene of 20mg/kg/b.wt/day plus food and drug with dose of 10mg/kg/wk injection in the peritoneal cavity.

20 mice aged 8 weeks; each mouse was treated with a dose of beta carotene of 20mg/kg/b.wt/day plus food and drug with dose of 20mg/kg/wk injected in the peritoneal cavity.

Measurements:

Assay of Lymphocyte Subpopulations by direct immunoflourescence test:

To determine the percentage of total pan T cells used FITC monoclonal anti Thy 1.2 and to account the number of immature T cells, peanut agglutinin anti PNA well used at the dilution (1: 200) according to the method, described by (Raff, 1971; Barclay *et al.,* 1976; Scollay *et al.,* 1984) as follows:

Incubate 2×10^6 lymph (derived from spleen or thymus) in 0.1 ml of PBS and 0.1 ml of Thy 1.2 or PNA (as antibody) at 4° C for 12-24 hours. Centrifuge the mixture at speed of 2000 r.p.m. wash for 2-3 times with ice-cold PBS.

Cells attached in 0.1 ml of PBS and placed on a glass slide and exanimate by zeiss axiophot at the power 400 x 10. Positive cells appear as result of forming a complete bright circle around the cell, where the link between antigen and receptors are equal on the surface of lymphocytes to form immune complex and forming patches leading to capping followed by coverage of these phenomenon to form immune complexes (Thaler *et al .,*1977). Count about 200 positive and negative cells. The positive cells calculated as follows = the number of cells positive / the total number of cells × 100.

RESULTS:

Effect on the number of lymphocytes Thy 1.2^+ isolated from the thymus, which represents the total number of T-cells:

***Mice aged four weeks:**

I: Treated by TA drug:

A significant decrease in the proportion of lymphocytes in the treat med group appeared as compared with the control group in the first, fourth and fifth weeks of the treatment by - 9.92%, - 22.44%, - 5.34%, respectively, and the second and third week of treat med showed a non significant increase by 2.98%, %4.54, respectively in the treatment group compared to controls.

II: Treatment with TA drug and beta carotene together:

There was the significant increase ($P \leq 0.01$) in the treat med group compared with the control group in the first week, second, third, and fourth week at rates of 8.01%, 100%, 18.97%, and 16.99%, respectively, and did not show any significant differences in the two weeks the fifth and seventh, while showing the sixth week a significant decrease ($P \leq 0.01$) by 13.97% in the group-treatment compared to the control group as shown in (Table 1 and Fig. 1a&b).

Table 1. Percentage of Thy 1.2^+ lymphocyte sub-population in thymus of Control, Triamcinolone acetonide drug, β-carotene and Triamcinolone acetonide drug treated four weeks old male Balb/c mice

Experimental period	Percentage of Thy 1.2^+ lymphocytes			
	Triamcinolone acetonide drug		β_carotene and Triamcinolone acetonide drug	
	Control	Treated	Control	Treated
	$\bar{x}$ ± S.E	$\bar{x}$ ± S.E	$\bar{x}$ ± S.E	$\bar{x}$ ± S.E
1	40.60 ± 1.01	36.57 ± 0.30 **	36.20 ± 0.6	39.10 ± 0.62 *
2	43.50 ± 0.21	44.80 ± 0.40	25.60 ± 0.6	51.20 ± 0.40 **
3	35.20 ± 0.50	36.80 ± 0.22	36.90 ± 0.8	43.90 ± 0.50 **
4	40.10 ± 0.72	31.10 ± 0.20 **	35.30 ± 0.4	41.30 ± 0.40 **
5	43.00 ± 0.71	40.70 ± 0.51 *	38.40 ± 0.1	38.90 ± 0.40
6	35.50 ± 0.50	D	46.50 ± 0.8	40.00 ± 0.32 **
7	42.20 ± 0.80	D	43.80 ± 0.9	44.00 ± 0.10

Four weeks old male mice were treated with β-carotene (20 mg/kg/day) and Triamcinolone acetonide drug (10 mg/kg/week).

$\bar{x}$ Mean data of 5 male mice per group.

*: Significant difference ($P \leq 0.05$).

S.E: Standard Error.

**: Highly Significant difference ($P \leq 0.01$).

Thy 1.2^+, Pan T-cells,

D: Dead.

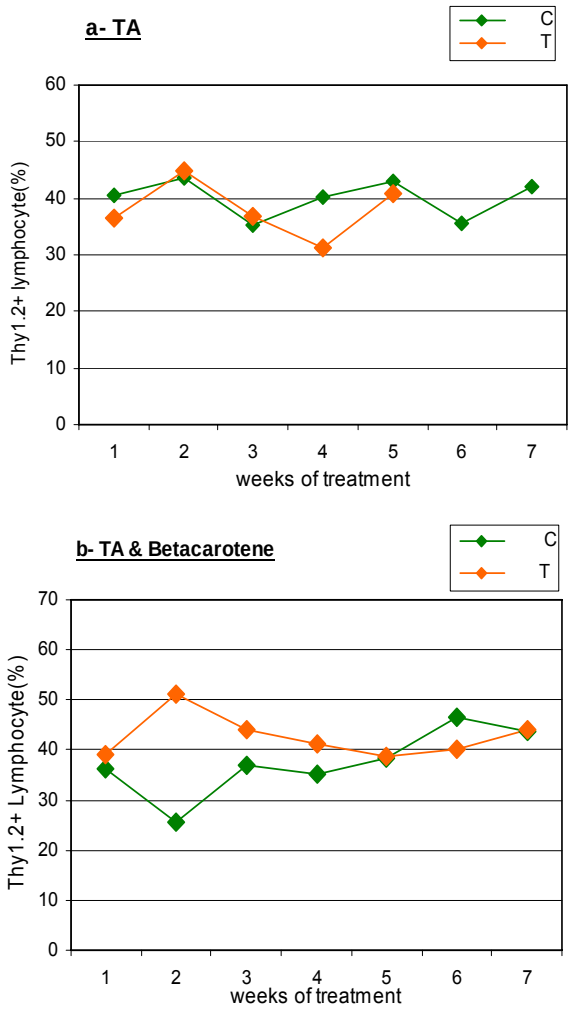


Fig. 1. Percentage of Thy 1.2$^+$ lymphocyte isolated from thymus of four weeks old male Balb/c mice of Control, Triamcinolone acetonide drug, β-carotene and Triamcinolone acetonide drug treated groups.

***Mice aged eight weeks:**

I: Treated by TA drug:

Significant decrease in the percentage of lymph cells in the treatment group appeared compared with the control group in the first week of treat med by-20.8%, while no significant different for both group in other weeks.

II: Treatment with TA drug and beta carotene together:

Significant rise in the proportion of lymphocytes Thy 1.2$^+$ taken from thymus gland in the first and second week of treatment by 26.82% and 12.04%, respectively, while no significant different in the third week of treat med, while the fourth week showed a significant decrease (P ≤ 0.05) by -5.39% in the treat med group compared to controls (Table 2 & Fig. 2a&b).

Table 2. Percentage of Thy 1.2$^+$ lymphocyte sub-population in thymus of Control, Triamcinolone acetonide drug, β_carotene and Triamcinolone acetonide drug treated eight weeks old male Balb/c mice

Experimental period	Percentage of Thy 1.2$^+$ lymphocytes			
	Triamcinolone acetonide drug		β_carotene and Triamcinolone acetonide drug	
	Control	Treated	Control	Treated
	$\bar{x}$ ± S.E	$\bar{x}$ ± S.E	$\bar{x}$ ± S.E	$\bar{x}$ ± S.E
1	43.20 ± 0.30	34.20 ± 0.32 **	36.90 ± 0.6	46.80 ± 0.3 **
2	42.70 ± 0.40	43.90 ± 0.11	33.20 ± 0.3	37.20 ± 0.2 **
3	45.80 ± 0.21	44.90 ± 0.11	37.90 ± 0.5	37.70 ± 0.1
4	31.10 ± 0.11	30.60 ± 0.22	35.20 ± 0.2	33.30 ± 0.6 *

Eight weeks old male mice were treated with β_carotene (20 mg/kg/day) and Triamcinolone acetonide drug (20 mg/kg/week). $\bar{x}$: Mean data of 5 male mice per group.
*: Significant difference (P ≤ 0.05). S.E: Standard Error.
**: Highly Significant difference (P ≤ 0.01). Thy 1.2 $^+$, Pan T-cells

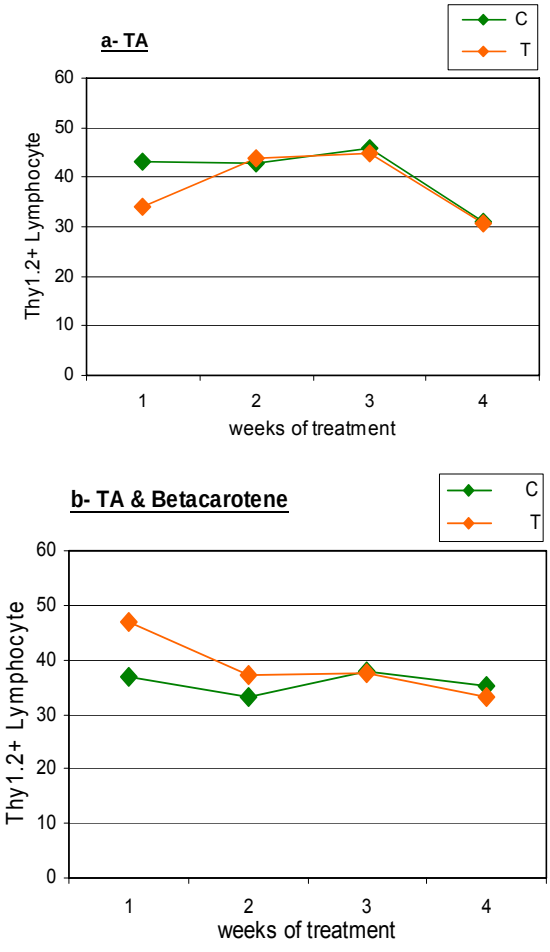


Fig. 2. Percentage of Thy 1.2+ lymphocyte isolated from thymus of eight weeks old male Balb/c mice of Control, Triamcinolone acetonide drug, β-carotene and Triamcinolone acetonide drug treated groups.

Effect on the number of lymphocytes PNA$^+$ isolated from the thymus, which represents the number of immature T cells:

*** Mice aged four weeks:**

I: Treated by TA drug:

The results showed significant increase ($P \leq 0.01$) in the percentage of lymphocytes cells, PNA+ in the treatment group in the second week and third with 18.20% and 41.20%, respectively, no significant different in the first and fourth week of treatment, while a significant decrease in the fifth week by 15.94% followed by the death of the mice in the sixth and seventh weeks of treatment in the treat med group compared to controls.

II: Treatment with TA drug and beta carotene together:

There was significant increase at most weeks of treatment in the group where data showed an increase 13.15%, 49.51%, 40.28%, 36.49%, and 43.73% from the first week and until the fifth week of treatment, and the result no significant different in the sixth and seventh weeks of treatment for both groups (Table 3 and Fig. 3a&b).

Table 3. Percentage of PNA+ lymphocyte sub-population in thymus of Control, Triamcinolone acetonide drug, β-carotene and Triamcinolone acetonide drug treated four weeks old male Balb/c mice

Experimental period	Percentage of PNA$^+$ lymphocytes			
	Triamcinolone acetonide drug		β_carotene and Triamcinolone acetonide drug	
	Control	Treated	Control	Treated
	$\bar{x}$ ± S.E	$\bar{x}$ ± S.E	$\bar{x}$ ± S.E	$\bar{x}$ ± S.E
1	34.5 ± 0.91	34.9 ± 0.3	36.50 ± 0.70	41.30 ± 0.51 **
2	34.6 ± 0.31	40.9 ± 0.31 **	41.60 ± 0.41	62.20 ± 0.52 **
3	33.2 ± 0.71	46.9 ± 0.51 **	35.00 ± 0.80	49.10 ± 0.50 *
4	39.8 ± 0.50	38.3 ± 0.50	33.70 ± 0.10	46.00 ± 0.32 **
5	43.9 ± 0.82	36.9 ± 0.40 **	40.70 ± 0.62	58.50 ± 0.60 **
6	45.3 ± 0.70	D	40.90 ± 0.40	41.10 ± 0.31
7	41.2 ± 0.70	D	41.40 ± 0.50	41.70 ± 0.31

Four weeks old male mice were treated with β-carotene (20 mg/kg/day) and Triamcinolone acetonide drug (10 mg/kg/week).

$\bar{x}$: Mean data of 5 male mice per group.

**: Highly Significant difference ($P \leq 0.01$).

S.E.: Standard Error.

D: Dead.

PNA$^+$: Immature T-cells.

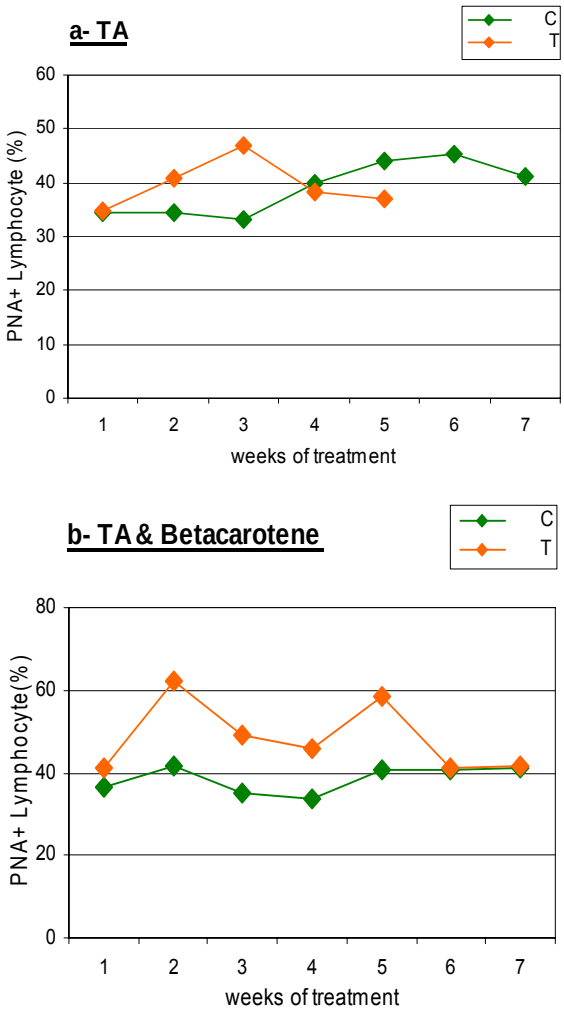


Fig. 3. Percentage of PNA+ lymphocyte isolated from thymus of four weeks old male Balb/c mice of Control, Triamcinolone acetonide drug, β-carotene and Triamcinolone acetonide drug treated groups.

*** Mice aged eight weeks:**

I: Treated by TA drug:

There was significant decrease of the average percentage of the lymphoid PNA$^+$ in the treatment group increased by -13.75%, -22.11%, and -27.68%, respectively, in the first week and the third and fourth of treatment as compared to the control group, while not shown in the second week of any significant differences in the values of averages in the group treatment.

II: Treatment with TA drug and beta carotene together:

Rises were in the group significantly with treatment in the first and second weeks of treatment 26% and 12.02%, respectively, while the significant decrease appeared ($P \leq 0.01$) in the third and fourth weeks by -10.07% and -11.76%, respectively, in the treatment group compared to the group control (Table 4 and Fig. 4 a&b).

Table 4. Percentage of PNA^+ lymphocyte sub-population in thymus of Control, Triamcinolone acetonide drug, β-carotene and Triamcinolone acetonide drug treated eight weeks old male Balb/c mice

Experimental period	Percentage of PNA^+ lymphocytes			
	Triamcinolone acetonide drug		β_carotene and Triamcinolone acetonide drug	
	Control	Treated	Control	Treated
	$\bar{x}$ ± S.E	$\bar{x}$ ± S.E	$\bar{x}$ ± S.E	$\bar{x}$ ± S.E
1	37.80 ± 0.20	32.60 ± 0.41 ••	37.3±0.3	47.0±0.5 ••
2	42.00 ± 1.30	41.70 ± 0.51	42.4±0.4	47.5±0.4 ••
3	40.70 ± 0.30	31.70 ± 0.20 ••	40.7±0.4	36.6±0.3 ••
4	41.90 ± 0.40	30.30 ± 0.30 ••	40.8±0.3	36.0±0.5 ••

Eight weeks old male mice were treated with β-carotene (20 mg/kg/day) and Triamcinolone acetonide drug (10 mg/kg/week).

$\bar{x}$:Mean data of 5 male mice per group.
••: Highly Significant difference (P ≤ 0.01).
S.E.: Standard Error.
PNA+: Immature T-cells.

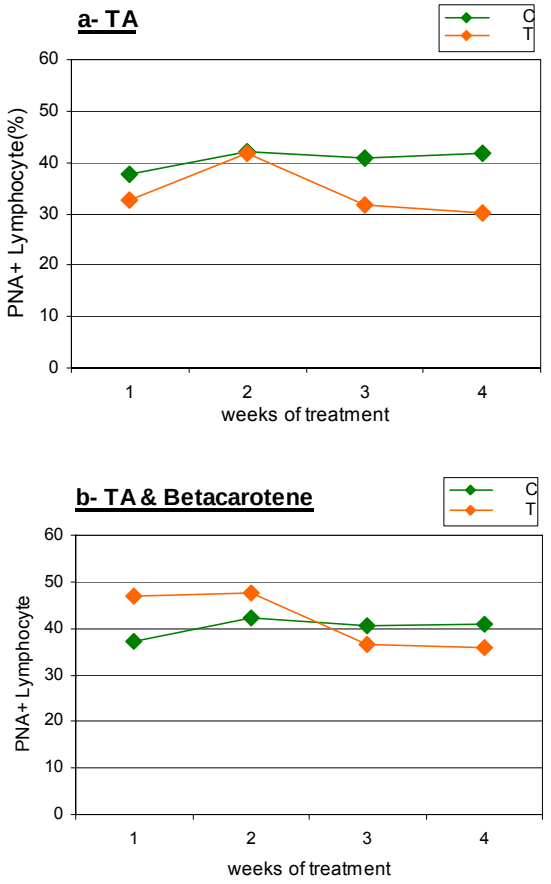


Fig. 4. Percentage of PNA+ lymphocyte isolated from thymus of eight weeks old maleBalb/c mice of Control, Triamcinolone acetonide drug, β-carotene and Triamcinolone acetonide drug treated groups.

DISCUSSION:

In the present study, the effect of two types of treatment on the thymus lymphocytes by direct immunofluorescence test using fluoresecent anti thy 1,2 antibody or fluorescent peanut agglutinin (anti - PNA).

Thy 1, 2 antibody has the ability to mark all members of T cells in the central and peripheral lymphocytes (Barclay *et al.,* 1976; Scollay *et al.,* 1984). On the other hand, the peanut agglutinin (anti-PNA) has the ability to mark immature T cells of the thymus (Scollay *et al.,* 1984). thus we can study any changes in these types of cells in the treated groups .there was a negative impact of TA drug treatment on a Thy 1, 2 cells in the mice group (4weeks age)in the thymus from the first week, but the second and third week showed significant then returned to decline till the fifth week followed by the subsequent death of the mice under experiment , as well in eighth week age mice , a marked significant decrease in Thy 1,2 cells was observed while a positive effect of double treatment was appeared as an increase in the percentage $Thy1,2^+$ of the thymus in 4^{th} and 8^{th} weeks age mice .

Thy 1, 2^+ cells showed sensitivity to the single and double treatment (increase or decrease). Both single and double treatment showed an inhibitory influence of drug on Thy 1, 2 cells. Double treatment of beta carotene reduces the negative impact of the drug on the T cells receptors. There was a significant increase in the percentage of Thy1, 2^+ cells when compared with the control group. These may be due to that drug could inhibits the respiration in the mitochondria which directly lead to failure of protein synthesis inside the cell.

Receptors are protein in nature which was affected by the drug during its synthesis. The drug affecting the lymphocytes by preventing the receiving surface indirectly, as considering these receptors as a protein (Shoji *et al.,* 1976). These agree with that obtained by Murata *et al*. (1994) who found that the ratios of different types of blood lymphocytes were affected as a result of beta carotene treatment, where positive impact on T cells of $CD4^+$ was detected and $CD8^+$ cells were negatively affected, also they observed simple changes in the numbers of memory, inhibitory and cytotoxic T cells. Also, the effect of carotene nutrition was studied by Ekam *et al.* (2006) who found that the number of lymphocytes in the peripheral blood was affected as a result of beta carotene treatment.

While Van popple *et al.* (1993) did not record any effect to beta carotene on the peripheral blood lymphocytes as it increase protein synthesis.

PNA^+ cells in thymus showed less sensitivity than Thy1,2 cells , where there is a significant increase in second and third weeks

and record a significant decrease in fifth week followed by a death of the mice in sixth and seventh weeks, while the impact of double treatment on the percentage of PNA^+ cells clear in mice aged 4 weeks or 8 weeks, where there was an increase in the proportion of cells, PNA^+ immature T starting from the first week and until the fifth there was no significant difference in the other aged weeks mice. In the 8 weeks mice there was a recovery as a result of treatment as compared with drug treated group.

This can be explained by the drop in PNA^+ lymphocytes in the drug treated group due to the process of acquiring the immature cell lymphoma to the $Thy1.2^+$ surface mark and most immature cells remained carrying the sign surface PNA^+ only, this may be due to preventing the formation of mature lymphocytes (klein, 1982; Scollay *et al.,* 1984; Roitt, 1997).

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تأثير عقار التريامسينولون أسيتونيد على العلامات السطحية للخلايا اللمفاوية في ذكور الفئران المغذاه بالبيتاكاروتين

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اتجهت الدراسة الحالية إلى البحث عن تأثير البيتاكاروتين وعقار التريامسينولون أسيتونيد المضاد للالتهاب على الجهاز المناعي في ذكور الفئران. وقد أُخذ في الاعتبار إجراء هذه الدراسة على طورين من أطوار عمر الفئران ، كان الطور الأول قبل أن تصل الفئران مرحلة البلوغ ، لذا فقد بدأت المعاملة في عمر أربعة أسابيع . أما الطور الثاني فقد كان بعد وصول الفئران مرحلة البلوغ حيث بدأت المعاملة في عمر ثمانية أسابيع . وكمحاولة للتقليل من الآثار الجانبية المتوقعة من عقار والتي تعتبر مادة مثبطة مناعياً ، فقد استخدم البيتاكاروتين كمادة طبيعية افترضت في أن تكون مادة منشطة مناعياً . لذلك عوملت الفئران بالعقار بمفرده ثم بالبيتاكاروتين والعقار كمعاملة مزدوجة ، ولخصت الدراسة التأثير الايجابي للمعاملة المزدوجة على العلامات السطحية في الخلايا الليمفاوية في الغدة الثيموسية ، وكذلك التأثير السلبي للمعاملة بالعقار وحده مقارنة بالمجموعة الضابطة . وبالتالي فإن هذا البحث يهدف إلى محاولة مساعدة متناولي عقار التريامسينولون أسيتونيد المضاد للإلتهابات والمحتوي على أحد مشتقات الكورتيزون للتخفيف من حدة الآثار الجانبية له بتناول البيتاكاروتين أثناء فترة العلاج.

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