IMMUNOMODULATORY EFFECT OF *CURCUMA LONGA* IN MICE

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ABSTRACT

This study was designed to find out the effect of oral inoculation of aqueous extract of *Curcuma Longa* at two doses (1 and 5) mg/ kg body weight daily for 4 weeks on the immune response of Balb/c mice by estimating of serum concentration of interleukin-2 (IL-2) , interleukin-4 (IL-4), interleukin-10 (IL-10) and interferon gamma (INF-γ) using ELISA test. The present results revealed that significant increase ($p<0.05$) in the values of both of IL-2 (133.80 pg/ml and 181.60 pg/ml, respectively) and INF-γ concentration (789.50 pg/ml and 1131.50 pg/ml, respectively) in sera of both mice groups treated with two concentration of *Curcuma longa* 1and 5 mg/kg body weight, respectively in comparison with control group. On the other hand significant elevation of IL-4 (91.00 pg/ml and 64.40 pg/ml, respectively) and IL-10 concentration (50.10 pg/ml and 42.70 pg/ml, respectively) in sera of both mice groups treated with *Curcuma longa* 1 and 5 mg/kg body weight in comparison with control group. IL-2 and INF-γ were used for detection of T_H1 response, while IL-4 and IL-10 used for T_H2 response detection. However, both mice groups treated with *Curcuma longa* (1 and 5 mg/kg) showed increase in the activity of T_H1 in comparison with T_H2. The ratio of IL-2/IL-10 (4.253) for mice group treated with 5 mg/kg body weight *Curcuma longa*, and INF-γ/IL-4 (17.659), and these rates were higher than the ratio of IL T 2/IL-10 (2.671) and INF-γ/IL-4 (8.676) for mice group treated with 1 mg/kg body weight

INTRODUCTION

Recently there has been a renewed interest in improving health and fitness through the use of more natural products .Spices are an important part of the human diet which has been used to enhance the flavor, color and aroma of food (1)

Curcuma longa or commonly known as turmeric is a medicinal plant widely used (2) which belong to the *Zingiberaceae* family distributed throughout tropical and subtropical regions of the world, being widely cultivated in Asiatic countries, mainly in India and China (3). There are several data in the literature indicating a great variety of pharmacological activities of *Curcuma longa* L. *(Zingiberaceae*) (4). The main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions: anti-inflammatory activity - There is a great number of papers in the literature relating the activity of compounds extracted from *C. longa* L.being potent inhibitors of inflammation, antibacterial activity antioxidant effects, hepatoprotective effects, anticarcinogenic effects, and cardiovascular effects (5-7).

$CD4^+$ T helper cells can be functionally differentiated into two subsets called T helper 1 (T_H1) and T helper 2 (T_H2). It has been shown that IL-12, IL-2 and IFN-γ have an essential role in T_H1 pathway but IL-4, IL-5, IL-13 and IL-10 are involved in the T_H2 immune response (8). Thereafter, each subset secretes different series of interleukin, which suppress the biological actions of another series. Thus, T_H1 subset involves in cellular immunity, whereas T_H2 subset drives humeral immunity. T_H1/ T_H2 balance is a clinically available immunologic marker and this balance reflects global immune surveillance, whereas this imbalance underlies various systemic as well as organ specific autoimmune diseases (9). A faulty immune response plays a pathogenic role in a wide spectrum of inflammatory diseases, including hypersensitivity responses to environmental antigens (allergic disorders), false recognition of self-antigen (autoimmune diseases) and immune attack against alloantigens during transplantation. Hence, it becomes crucial to suppress the immune system. This study aimed to evaluate the modulating effect *Curcuma longa* on the immune response

MATERIALS AND METHODS

Laboratory animal model

Thirty BALB/c mice 4-5 weeks old weighting 15-28 gram were obtained from the animals unit, college of medicine, university of Baghdad, Iraq. The animals were divided into three groups, each group consists of 10 mice, and the animals were bred in standard mice cages and fed with a suitable quantity of water and complete diet.

Preparation of aqueous extract

Water extraction was prepared by boiling 100 gram of turmeric in 1000 ml distilled water for 15 minutes. The flask was then plugged and removed from the heat and allowed to cool at room temperature. After cooling the content of the flask was filtered and dried to prepare the required concentrations (11).

Inoculation of experimental animals

Three groups of mice including 10 mice /group were treated with *curcuma longa* by oral inoculation for 4 weeks, the first group A (n=10) daily each mouse was swallowed single dose of 0.1 ml of *Curcuma* extract at the concentration of 1mg/kg B.W. The second group B (n=10) daily each mouse was swallowed orally single dose of 0.1 ml of *Curcuma* extract at the concentration of 5mg/kg B.W. The third group C (n=10) daily negative control mice were swallowed with 0.1 ml of normal saline.

The animals were monitored for apparent signs of toxicity for 30 days. On the 31th day after inoculation and the serum was separated after the blood collection to measure the levels IL-2, IL-4, IL-10 and INF-γ.

Estimation of IL-2, IL-4, IL-10 and INF-γ value in serum

The effect of different doses of oral treatment on the concentration of studied serum interleukins was estimated by IL-2, IL-4, IL-10 and INF-γ. These interleukins were measured in serum by using ELISA according to the instructions of eBioscience company, USA.

Briefly, microtiter plate was coated with 100 µl/well of capture antibody (pre-titrate purified anti- IL-2, IL-4, IL-10 or INF-γ antibody). The plate was sealed and incubated overnight at 4˚C. Cover film was removed and the plate was washed with

250 µl/well washing solution (1xPBS, 0.05 Tween-20) this procedure was repeated five times. Wells were blocked with 200 µl/well of 1x Assay Diluent and incubated at room temperature for 1 hour. Washing step was as mentioned above. 1x Assay Diluent was used to perform 2-fold serial dilutions of standards to make the standard curve. 100 µl/well of 1x Assay Diluent was added to the blank well. 100 µl/well of standards and serum samples were loaded to appropriate wells and the wells were covered and incubated at room temperature for 2 hours. Plate was washed as mentioned above. 100 µl/well of detection antibody (pre-titrated biotin-conjugated antibody) was added to each well. The plate was sealed and incubated at room temperature for 1 hour. Cover film was removed and the plate was washed as described previously. 100 µl/well of Avidin-HRP was added to each well and the plate was sealed and incubated for 30 minutes at room temperature. Plate was washed as in step 2 and repeated for total seven washes. 100 µl/well of substrate solution, tetramethylbenzidine (TMB), to each well and incubated for 15 minutes at room temperature. The reaction was stopped by adding 50 µl of stop solution to each well. The absorbance of each well was read at 450 nm using microplate reader. The sample concentrations were determined depending on a standard curve.

Statistical analysis

Data are expressed as the mean values ± standard deviation (SD) of samples. The statistical significance of the differences between various groups was determined by PostHoc test (LSD alpha 0.05) and one-way analysis of variance (ANOVA) using SPSS version 18.0 software. Differences were considered statistically significant for $p<0.05$.

RESULT

Enzyme linked immune-sorbent assay test were done to estimate immune responses after oral inoculation of *curcuma longa* to determine the titers of IL-2, IL-4, IL-10 and INF-γ in mice sera.

Table 1, 2, 3 and 4 show the mean and standard deviation values of serum concentration of IL-2, IL-4, IL-10, and INF-γ, respectively in mice sera.

Table (1) The ELISA results of IL-2 concentration in serum expressed as pg/ml.

Interleukin 2				
Mice groups according to treatment dose	No.	Mean	S.D.	S.E.
1 mg/Kg of *Curcuma*	10	133.80	7.786	2.462
5 mg/Kg of *Curcuma*	10	181.60	7.820	2.473
Control	10	25.00	6.182	1.955

Table (2) The ELISA results of IL-4 concentration in serum expressed as pg/ml

Interleukin 4				
Mice groups according to treatment dose	No.	Mean	S. D.	S. E.
1 mg/Kg of *Curcuma*	10	91.00	25.625	39.726
5 mg/Kg of *Curcuma*	10	64.40	8.847	2.798
Control	10	26.30	6.601	2.087

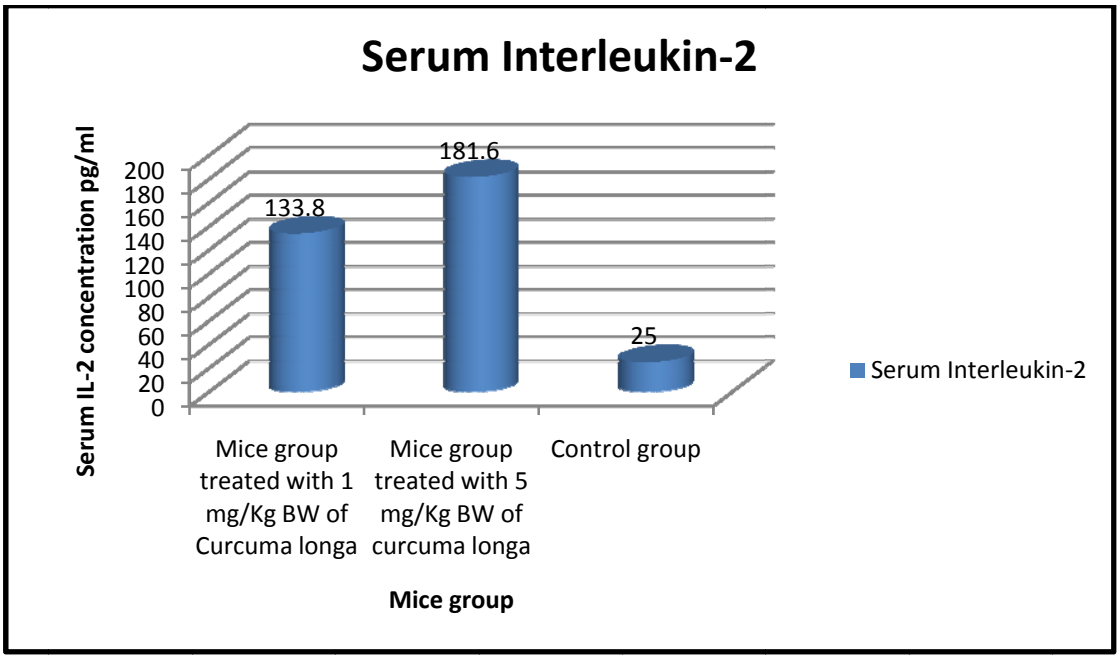
Table (3) The ELISA results of serum IL-10 concentration expressed as pg/ml

Interleukin 10				
Mice groups according to treatment dose	No.	Mean	S. D.	S. E.
1 mg/Kg of *Curcuma*	10	50.10	10.268	3.247
5 mg/Kg of *Curcuma*	10	42.70	7.150	2.261
Control	10	36.10	6.707	2.121

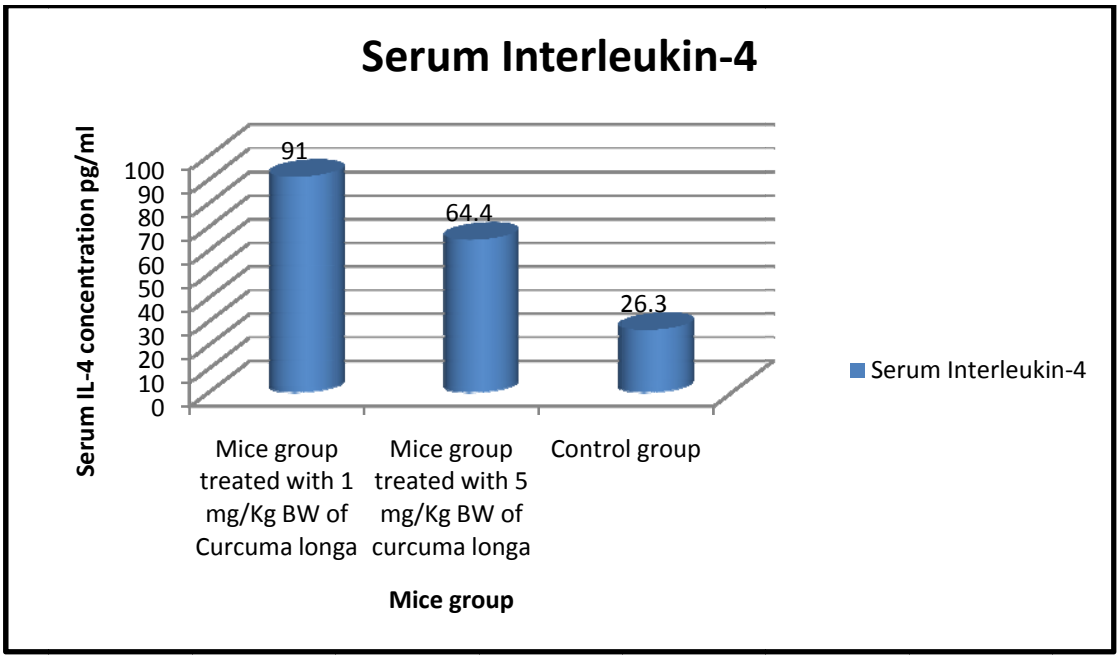
Table (4) The ELISA results of INF-γ concentration in serum expressed as pg/ml

Interferon γ				
Mice groups according to treatment dose	No.	Mean	S. D.	S. E.
1 mg/Kg of *Curcuma*	10	789.50	93158.084	49.990
5 mg/Kg of *Curcuma*	10	1131.50	1483.3	469.090
Control	10	284.30	102.943	32.554

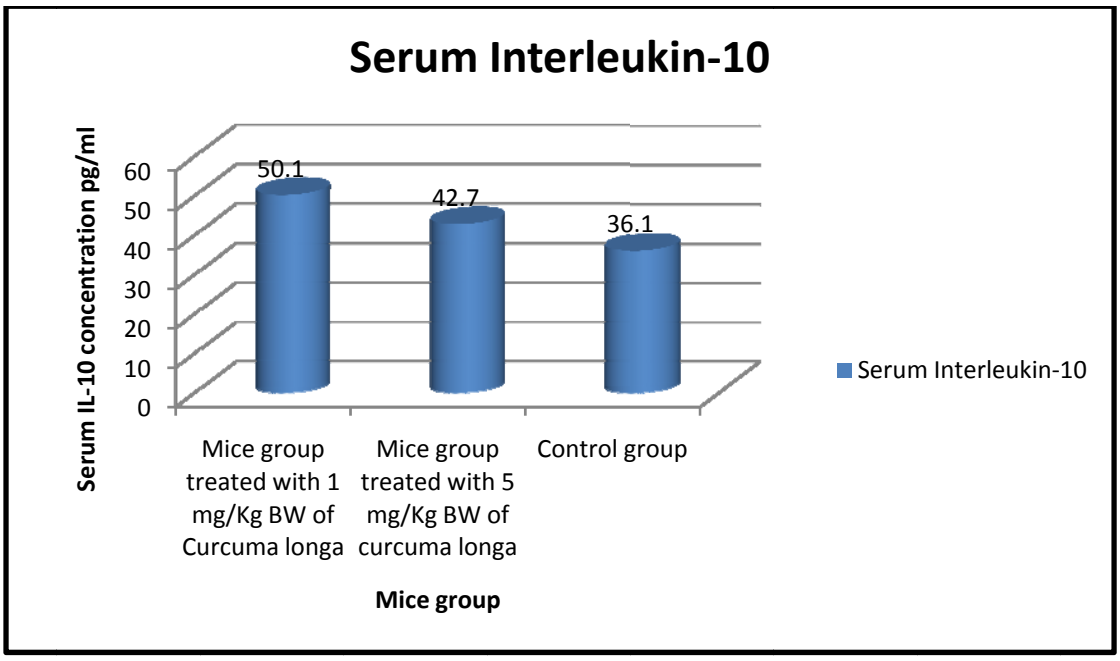
There was significant difference ($p<0.05$) between treated and control groups (Figure 1, 2, 3 and 4) of serum interleukins concentration IL-2, IL-4, IL-10 and INF-γ ,respectively .The highest titer observed in mice group treated with 5 mg/kg of aqueous extract of *Curcuma longa* followed by mice groups treated with 1mg/kg in comparison with control groups. On other hand Figure 1 and 4 show that the concentration of IL-2 and INF-γ 133.8pg/ml ,789.5pg/ml , respectively at dose 1 mg/kg and 181.6 pg/ml, 1131.5pg/ml ,respectively at dose 5 mg/kg observed in mice group treated with two doses were higher than the concentration of IL-4 (91pg/ml, 64.4 pg/ml ,respectively) (Figure.2) and IL-10 (50.1pg/ml, 42.7 pg/ml, respectively) (Figure.3) in comparison with control group.



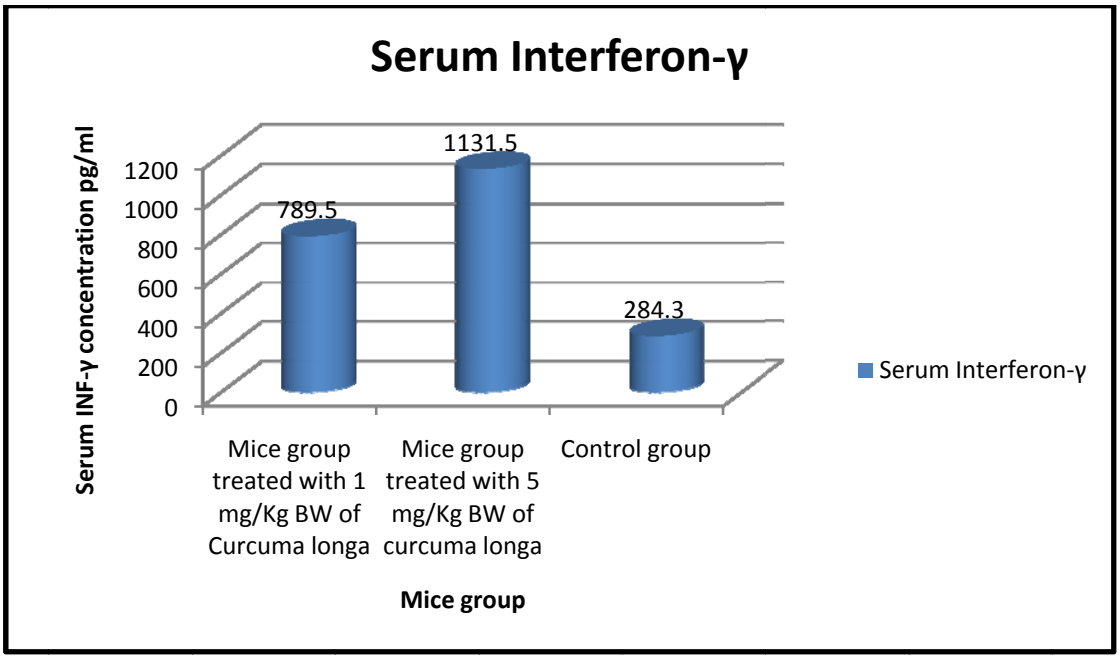
(Figure. 1) Difference between mice groups treated with aqueous extract of *Curcuma longa* according to IL-2 concentration (pg/ml) in serum



(Figure. 2) Difference between mice groups treated with aqueous extract of *Curcuma longa* according to IL-4 concentration (pg/ml) in serum



(Figure. 3) Difference between mice groups treated with aqueous extract of *Curcuma longa* according to IL-10 concentration (pg/ml) serum



(Figure. 4) Difference between mice groups treated with aqueous extract of *curcuma longa* according to INF-γ concentration (pg/ml) in serum.

The ratio of T_H1/T_H2 immune response was estimated by dividing the concentration of IL-2 on the concentration of its antagonist IL-10 and concentration of INF-γ on the concentration its antagonist IL-4 for each treated mice groups. The results are shown in table 5 (14).

Table (5). The ratio of Th1/Th2 immune response

Mice groups according to treatment dose	T_H1/T_H2	
	IL-2/IL-10	INF-γ/IL-4
1 mg/Kg of *Curcuma*	2.671	8.676
5 mg/Kg of *Curcuma*	4.253	17.659

DISCUSSION

Neither signs of toxicity nor death of mice were observed during the 30 days of the experimental period after the mice have been orally given the aqueous extract of *Curcuma longa*. These results are in agreement with many other studies in which they showed that no toxic effects due to feeding turmeric or curcumin but in rat, guinea pig or monkey were recorded (1, 2, and 12).

In this study IL-2 and INF-γ concentration in mice sera were estimated to reflect T_H1 response (Table 1 and 4, respectively). This was significantly different ($p<0.05$) in comparison with control group (Figure 1 and 4) that mean inoculation of *Curcuma longa* played significant role in stimulation of Th1 cell. On the other hand there was a significant difference ($p<0.05$) between IL-4 and IL-10 serum concentration in treated mice groups and control group (Figure 2 and 3) which indicate the stimulation of T_H2 (Table 2 and 3).These finding are in line with many study demonstrated that $CD4^+$ T helper (T_H) cells can be functionally differentiated into T_H1 and T_H2 cells, regarding their interleukin profile production (13).

T_H1 cells secrete mainly IFN-γ and IL-2 and control T_H2 cells proliferation. In contrast, T_H2 cells secrete mainly IL-4 and IL-10, and control T_H1 cells (14). T_H1 activation contributes to cell-mediated immunity whereas T_H2 activation favors the humoral immune response, and T_H1 / T_H2 balance is a prerequisite for the functionality of immune system against infections (15).

On other hand several literatures indicating a great variety of pharmacological activities of *Curcuma longa* L. (*Zingiberaceae*), such as anti-inflammatory, anti-human immunodeficiency virus, anti-bacteria, antioxidant effects and nematocidal activities (16-19).

Our study demonstrated that, *Curcma longa* had modulated immune response via changing theT_H1/ T_H2 ratio (Table 5), which could indicate its usage as anti-inflammatory (16-19), while T_H2 cells synthesize high levels of interleukin IL-4, IL-5, and IL-13, which might lead to the production of IgE and the release of mediators from mast cells (17). T_H1 cells regulate T_H2 activity by secreting IFN-γ. IL-4 induces class switching to IgG1and IgE, whereas IFN-γ is involved in IgG2 subclass switching (20, 21).

These results agree with previous study in which the researchers show that both turmeric and curcumin can act as potent immunomodulatory agents that can modulate the activation of T cells, B cells, macrophages, neutrophils, natural killer cells, and dendritic cells (22, 23).

In conclusion, *C. longa* might have the capacity to modulate the activity of T_H1, and the potential use of *C. longa* crude extract (containing curcuminoids and

polysaccharides) as an adjuvant supplement for cancer patients, whose immune responses were suppressed due to chemotherapies, which supress the cytokine productions (TNF-α, GM-CSF, IL-5, IL-6, IL-8, IL-10, IL-13, etc.) (16, 23).

التأثير المناعي المعدل للكركم(*Curcuma longa*) في الفئران المختبرية

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الخلاصة

صممت هذه الدراسة للكشف عن تأثير التجريع الفموي للمستخلص المائي للكركم بتركيزي ١ و ٥ ملغ/ كغم من وزن الجسم لمدة اربع اسابيع يوميا على الاستجابة المناعية للفئران المختبرية نوع Balb/c بواسطة قياس تراكيز انترليوكين-٢ و انترليوكين-٤ و انترليوكين-١٠ و الانترفيرون-جاما في المصل باستخدام فحص الاليزا.

كشفت النتائج الحالية عن وجود زيادة معنوية ($P<0.05$) في قيم كلا من انترليوكين-٢ (133.80 pg/ml , 181.60 pg/ml على التوالي) والانترفيرون-جاما (789.50 pg/ml, 1131.50 pg/ml على التوالي) في مصول مجموعتي الفئران التي عولجت بتركيزين من الكركم (*Curcuma longa*) وبمقدار (١ و٥ مل/كغم) وعلى التوالي وبالمقارنة مع مجموعة السيطرة .

ومن جانب اخر وجد ارتفاع معنوي ($P< 0.05$) في تركيز كلا من الانترلوكين-٤ (91 pg/ml 64.40) pg/ml , على التوالي والانترلوكين-١٠ (50.10 pg/ml و٤٢.٧٠ pg/ml) على التوالي في مصول كلا المجموعتين من الفئران التي عولجت بجرعتين الكركم بتركيزي (١ و ٥ ملغ/ كغم وعلى التوالي) بالمقارنة مع مجموعة .

استخدام انترليوكين-٢ و الانترفيرون-جاما للكشف عن استجابة استجابة T_H1 بينما استخدم انترليوكين-٤ و انترليوكين-١٠ للكشف عن استجابة T_H2.مع ذلك من مجموعتي الفئران المعالجة بجرعتي الكركم ١ و ٥ ملغ/ كغم اظهرت زيادة في فعالية T_H1 بالمقارنة مع T_H2 .كانت نسبة IL-2/IL-10 (٢٥٣٤.) في مجموعة الفئران المعالجة بجرعة ٥ ملغ/ كغم من الكركم وكانت نسبة INF-γ/IL-٤ (١٧.٥٦٩) وان هذه النسب اعلى من نسبة IL-2/IL-10 (٢.٦٧١) ونسبة INF-γ/IL-٤ (٨.٦٧٦) في مجموعة الفئران المعالجة بجرعة ١ ملغ/ كغم .

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