



ISSN: 0067-2904

A Study Medicinal and Nutritional Effects of Thyme on Hematological Changes in Male Albino Rats

Noori Mohammed Luaihi*¹, Murtadha Ghafil Jasim¹, Amenah Hussein Mousa²

¹Department of Science, College of Basic Education, Sumer University, Baghdad, Iraq.

²Department of Biology, College of Science, Al-Mustansiriyah University, Baghdad, Iraq.

Abstract

The increase in the use of thyme in Iraq and neighboring countries, which may be result in serious side effects necessitate the demand for testing different concentrations of thyme extract (500,750,1000) mg/kg of body weight on rats to be given either by injection or feeding grinded dried thyme leaves added to pellets (50,100,150) g /kg of pellet in of different periods , (10,20,30) days for injection and feeding 2 times weekly. Thyme extracts leaves effects on RBCs, WBCs and Differential WBCs counts were measured. statistical analysis showed significance increase difference ($P \leq 0.05$) in RBCs, WBCs and Lymphocyte, Nutrophile and Monocyte counts and decrease in Eosinphil counts in rats treated with 1000 mg of thyme /kg of body weight and 150 g/kg of pellet in 10 days, in 20 days treatment group was showed highly significance increase difference ($P \leq 0.01$) compared to control group in RBCs, WBCs, Lymphocyte, Nutrophile and Monocyte counts and decrease in Eosinphil counts in rats treated with thyme extract by (750,1000) mg/kg of body weight and (100,150) g/kg of pellet and in 30 days thyme extract cause highly significance increase difference ($P \leq 0.001$) in RBCs, WBCs, Lymphocyte, Nutrophile and Monocyte counts and decrease in Eosinphil counts in rats treated with thyme by (500-750-1000) mg/kg of body weight comparing with the negative and positive controls and (50-100-150) g/kg of pellet comparing with the negative control for feeding .

Keywords: Thyme, Side effects, Hematological .

دراسة التأثيرات الطبية والغذائية للزعر على المتغيرات الدموية في ذكور الجرذان

نوري محمد لعبيبي* ، مرتضى غافل جاسم ، آمنة حسين موسى

قسم علوم الحياة ، كلية العلوم، الجامعة المستنصرية، بغداد، العراق.

قسم العلوم، كلية التربية الاساسيه، جامعة سومر، العراق.

الخلاصة

نظرا لزيادة استخدام نبات الزعر في العراق والبلدان المجاورة والتي من الممكن ان تنتج تاثيرات جانبية لذلك من الضروري اختبار تراكيز مختلفة من مستخلص الزعر (500-750-1000) ملغم /كغم من وزن الجسم المعطاة لذكور الجرذان اما عن طريق الحقن او أوراق الزعر المجففة المطحونة المضافة الى العليقة (50-100-150) غم/كغم من العليقة في فترات زمنية مختلفة (10-20-30) يوم لكل من الحقن والتغذية بمعدل مرتين أسبوعيا. تم قياس تأثير مستخلص الزعر في عدد كريات الدم الحمر والبيض والفحص التقريبي لكريات الدم البيض. أظهرت النتائج الإحصائية وجود زيادة معنوية ($p \leq 0.05$) في عدد كريات الدم الحمر

*Email: noori.alsudani63@gmail.com

والخلايا البيض (العدلة، اللمفاوية والوحيدة) وانخفاض في عدد خلايا الدم البيض (الحمضة) للجرذان المحقونة بمستخلص الزعتر بتركيز 1000 ملغم/كغم من وزن الجسم ولمدة 10 أيام وأيضاً في الجرذان المتغذية على العليقة الحاوية على الزعتر المطحون 150 غم/كغم من العليقة مقارنة مع مجموعة السيطرة، وظهرت مجموعة المعاملة بـ 20 يوماً حصول ارتفاع معنوي عالي ($p \leq 0.01$) في عدد كريات الدم الحمر والخلايا البيض (العدلة، اللمفاوية والوحيدة) وانخفاض في كريات الدم البيض (الحمضة) للجرذان المحقونة بمستخلص الزعتر (750-1000) ملغم/كغم من وزن الجسم وكذلك للجرذان المتغذية على العليقة المحتوية على الزعتر المطحون (100-150) غم/كغم من العليقة أما في 30 يوماً فقد حصل ارتفاع معنوي عالي ($p \leq 0.001$) مقارنة مع مجموعة السيطرة في عدد كريات الدم الحمر والخلايا البيض (العدلة، اللمفاوية والوحيدة) وانخفاض في كريات الدم البيض (الحمضة) في الجرذان المحقونة بمستخلص الزعتر (500-750-1000) ملغم/كغم من وزن الجسم مقارنة مع مجموعة السيطرة الموجبة والسالبة والمتغذية على العليقة الحاوية على الزعتر (50-100-150) غم/كغم من العليقة مقارنة مع مجموعة السيطرة السالبة .

Introduction

Medicinal plants are used in many countries as replacement to synthetic drugs. Scientists are now paying attention towards herbal extracts to do as microbial agent due to increase in bacterial resistance to antibiotics which to an increasing extent led to world health issue. diverse spices and herbal extracts are used for preservation of food, as well some are used as appetizers and many of them are utilized medicinally in old times [1].

Medicinal herbs are high natural source of medicinal products used in traditional medicine and chemical entities for modern drugs. Medicinal plants are broadly used either directly (home remedies) or indirectly (modern medicines) by all sectors of inhabitants [2].

Many pharmacological *in vitro* experiments carried out during the last decades revealed well defined pharmacological activities of both, the thyme essential oil and the plant extracts [3]. The non-medicinal use of thyme is worthy of attention, because thyme is used in the food and aroma industries; it is widely used as culinary ingredient and it serves as a preservative for foods especially because of its antioxidant effect. Thyme essential oil constitutes raw material in perfumery and cosmetics due to a special and characteristic aroma [4].

Thymus vulgaris is an important medicinal plant [5-6] which belongs to the Lamiaceae family, it has been used for centuries as spice, home remedy, drug, perfume and insecticide. In medicine, it is used as antispasmodic, antibacterial, antifungal, secretolytic, expectorant, antiseptic, antelmintic and antitusive as reported by other authors [7].

Aim of the study

Knowledge of side effects of thyme on blood contents and defend cells in body.

Materials and Methods

Laboratory Animals

All experiments were performed on 120 male albino rats, ages ranged between 2-3 months with a body weight ranged between 225-250 g. Rats were obtained from animal house of National center for drug control and researches and housed in the animal house of the College of Medicine / Baghdad University. They were kept in a room supplied with air conditioner to keep the temperature between 18-24 °C, the air of the room was changed continuously by using ventilating fan and light was controlled with range of 12 hours of light and 12 hours of darkness.

The animals were housed in plastic cages (4 rats/cage) with a wire grid covers, supported on ventilated racks [8]. The bedding material used was fine sawdust and wood shaving which was changed every other day to prevent accumulation of urinary pheromones [9]. The cages was washed regularly once a week with hot water, then 70% alcohol as disinfectant, rats were fed with standard balanced pellet that contains special dietary supplement to keep normal activity and growth, before experimentation, all rats were left for at least two weeks for adaptation, during this period, abnormal and sick rats were excluded from the experiment.

The plant

Thymus vulgaris used is wild thyme leaves and has been diagnosed by specialized botanists and used in this study were purchased from the Shorja market in the Baghdad, dried thyme leaves have been prepared in two ways, depending on the way of the administration:

Injection

Dry leaves of *Thymus vulgaris* were put about 50 gm in containers extraction thimbles located in Soxhlet extractor then added 500 ml of ethyl alcohol (70%) to the powder and continued recovery for 24 hours and then took the extraction and put in the electric oven with degree of (40) °C [10,11]. Further were this extract examined by Infrared (I.R.) Spectrophotometer to show of screening effective groups in thyme by peaks, and every peak refers to certain effective group.

Extraction and examination of the extract was conducted in the Ibn Al-Bitar Centre to the board of industrial research and development one of the formations and the Ministry of Industry and Minerals. The stock solution was prepared by dissolving 15g. of dry extract and in 100ml of alcohol ethanol, therefore the concentration of the stock solution (150 mg / ml). It was prepared several concentrations involving (500,750 and 1000) mg/kg of body weight [12].

Feeding

The grinding dried thyme leaves were divided into three different groups according to weights (50, 100, 150) g. every group was mixed with a diet (grinding pellet) (950, 900, 850) g. respectively, kneaded, cut into small pieces, sun-dried and given to the animals [13].

Animals Groups

The experiment was achieved as following:

➤ **1st experiment:** included 75 rats randomly distributed into five groups as follows:

➤ **1st group**

This group included 15 rats were given only water and pellet was considered as negative control animals, this group also considered control to the second experiment.

➤ **2nd group**

This group included 15 rats were injected with alcohol subcutaneously twice a week considered as positive control animals.

➤ **3rd group**

This group included 15 rats were subdivided into 3 subgroups, the 1st was injected thyme dose of (500 mg/kg of body weight twice a week) for 10 days, the 2nd was injected the same dose for 20 days, and the 3rd was injected the same dose for 30 days.

➤ **4th group**

This group included 15 rats were subdivided into 3 subgroups, the 1st was injected thyme dose of (750 mg/kg of body weight twice a week) for 10 days, the 2nd was injected the same dose for 20 days, and the 3rd was injected the same dose for 30 days.

➤ **5th group**

This group included 15 rats were subdivided into 3 subgroups, the 1st was injected thyme dose of (1000 mg/kg of body weight twice a week) for 10 days, the 2nd was injected the same dose for 20 days, and the 3rd was injected the same dose for 30 days.

➤ **2nd experiment:** Contain control as above in first experiment (1st group) and included 45 rats divided into 3 subgroups, the 2nd was administered feeding pellet mixed with thyme (50,100,150g/kg of pellet twice a week) for 10 days, the 3rd was administered the same doses for 20 days, and the 4th was administered the same doses for 30 days.

Collection of blood samples:

Blood was collected from all rat groups. The collection of blood were obtained by heart puncture using 3 and 5 ml disposable syringes, the blood put in small plastic tubes container ethylene diaminetetraacetic acid (EDTA), and used for hematological tests.

Results and Discussion

This study included treated the male albino rats with thyme to see the effect of this substance on the hematological tests (WBCs count, RBCs count and WBCs Differential count) results showed as follows:-

The statistical analysis show non significance difference in the counts of RBCs, WBCs and Differential counts of WBCs (Lymphocyte, Neutrophil, Monocyte and Eosinophil) of the rats that treated with thyme in the concentration of 500 mg/kg of body weight by injection for 10 days, Also there was

non significance recorded at 50 g/kg of pellet by feeding at the same days, while there was significant increase difference ($P \leq 0.05$) in counts of RBCs, WBCs and differential counts of WBCs (Lymphocyte, Nutrophil and Monocyte) and decrease in counts of Eosinphil on the rats that treated with thyme in the concentration of 1000 mg/kg of body weight comparison with that of control groups (-,+), and in the rats treated with thyme by feeding at 150g/kg of pellet comparison with that of control group (-).

The statistical analysis show non significance difference in the counts of RBCs, WBCs and differential counts of WBCs (Lymphocyte, Nutrophil, Monocyte and Eosinphil) on the rats that treated with thyme in the concentration of 500 mg/kg of body weight by injection for 20 days comparison with that of control groups (-,+), Also there was non significance recorded at 50 g/kg of pellet by feeding at the same days comparison with that of control group (-), while there was high significant increase difference ($P \leq 0.01$) in counts of RBCs, WBCs and differential counts of WBCs (Lymphocyte , Nutrophil and Monocyte) and decrease in counts of Eosinphil of the rats that treated with the concentration of (750-1000) mg/kg of body weight comparison with that of control groups (-,+), and in the rats that treated with thyme by feeding at (100-150) g/kg of pellet comparison with that of control group (-).

The statistical analysis show very high significant increase difference ($P \leq 0.001$) in counts of RBCs, WBCs and differential counts of WBCs (Lymphocyte, Nutrophil and Monocyte) and decrease in counts of Eosinphil on the rats that treated with thyme in the concentration of (500-750-1000) mg/kg of body weight by injection for 30 days comparison with that of control groups (-,+), Also there was very high significant increase difference ($P \leq 0.001$) recorded at (50-100-150) g/kg of pellet by feeding at the same days comparison with that of control group (-).

The results of the statistical analysis of the present study are showed in Figure- (1 A , B), (2 A , B), (3 A , B), (4 A , B), (5 A , B) and (6 A , B).

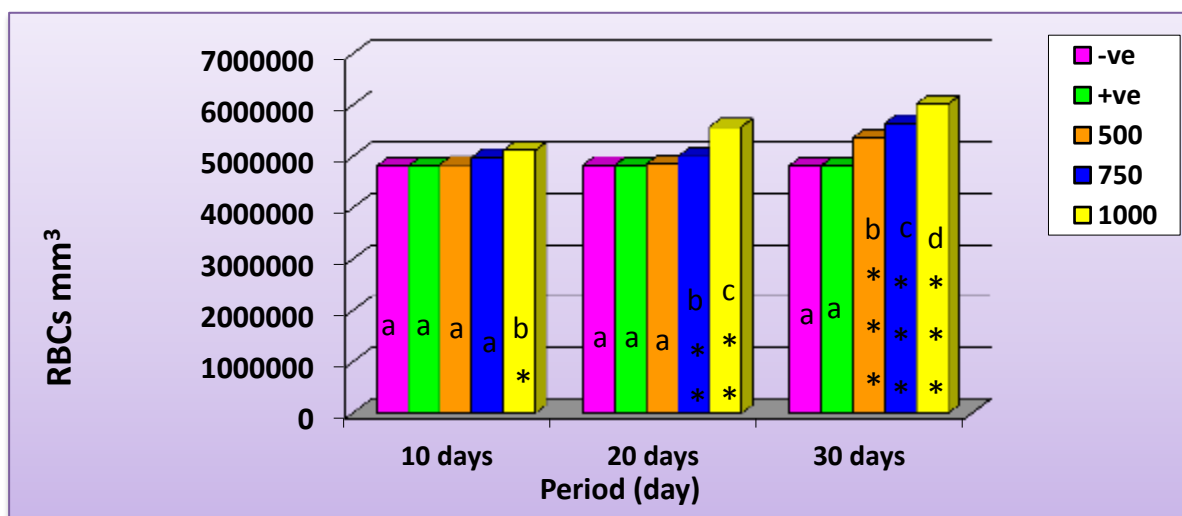


Figure 1A - Effect of Thyme on the count of RBCs by injection with difference period (10, 20, 30) days and various concentration of thyme (500, 750, 1000) mg /kg of body weight comparison with control groups (-, +)

- (*) significant increase ($P \leq 0.05$)
- (**) high significant increase ($P \leq 0.01$)
- (***) high significant increase ($P \leq 0.001$)
- (a, b, c, d) represented the significant different between groups.

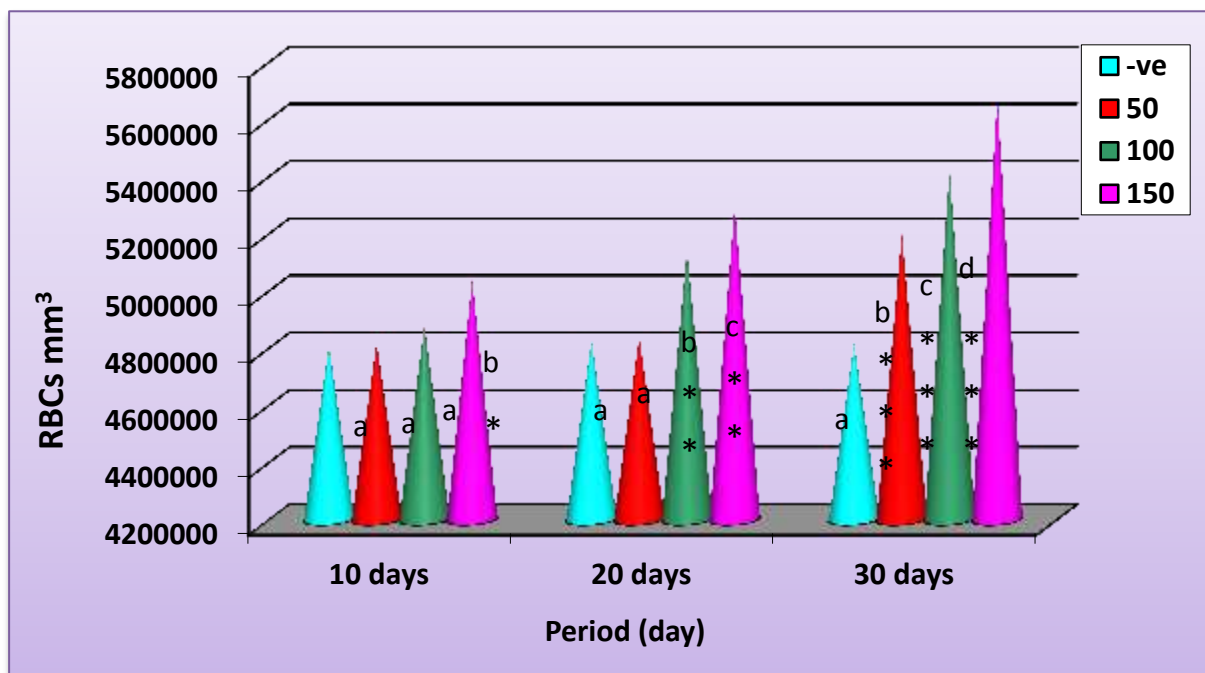


Figure 1 B - Effect of Thyme on the count of RBCs by feeding with difference period (10, 20, 30) days and various weight of thyme (50, 100, 150) g / kg of pellet, comparison with control group (-)
 (*) significant increase ($P \leq 0.05$)
 (**) high significant increase ($P \leq 0.01$)
 (***) high significant increase ($P \leq 0.001$)
 (a, b, c, d) represented the significant different between groups .

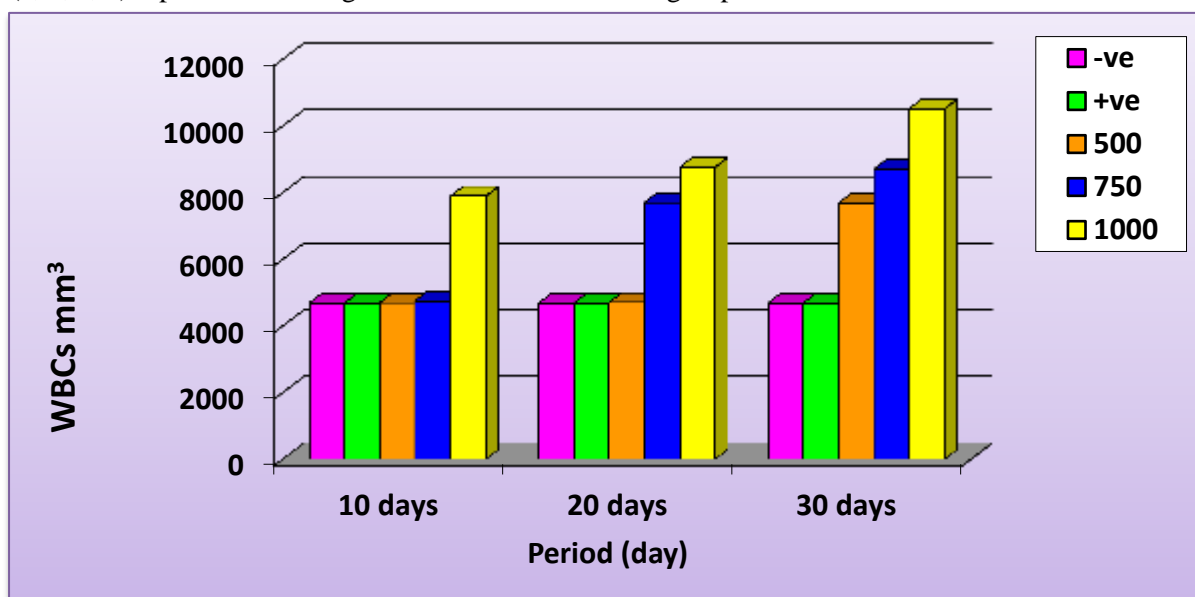


Figure 2 A - Effect of Thyme on the count of WBCs by injection with difference period (10, 20, 30) days and various concentration of thyme (500, 750, 1000) mg / kg of body weight comparison with control groups (- . +) .
 (*) significant increase ($P \leq 0.05$)
 (**) high significant increase ($P \leq 0.01$)
 (***) high significant increase ($P \leq 0.001$)
 (a, b, c, d) represented the significant different between groups .

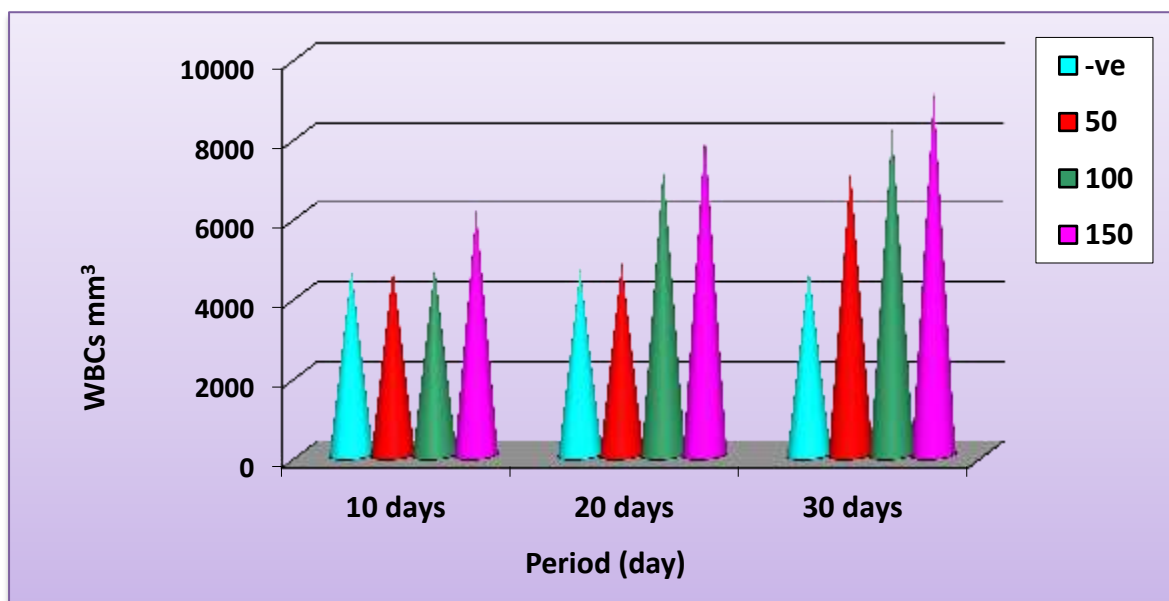


Figure 2 B - Effect of Thyme on the count of WBCs by Feeding with difference period (10, 20, 30) days and various weight of thyme (50, 100, 150) g / kg of pellet, comparison with control group (- .+).
 (*) significant increase ($P \leq 0.05$)
 (**) high significant increase ($P \leq 0.01$)
 (***) high significant increase ($P \leq 0.001$)
 (a, b, c, d) represented the significant different between groups.

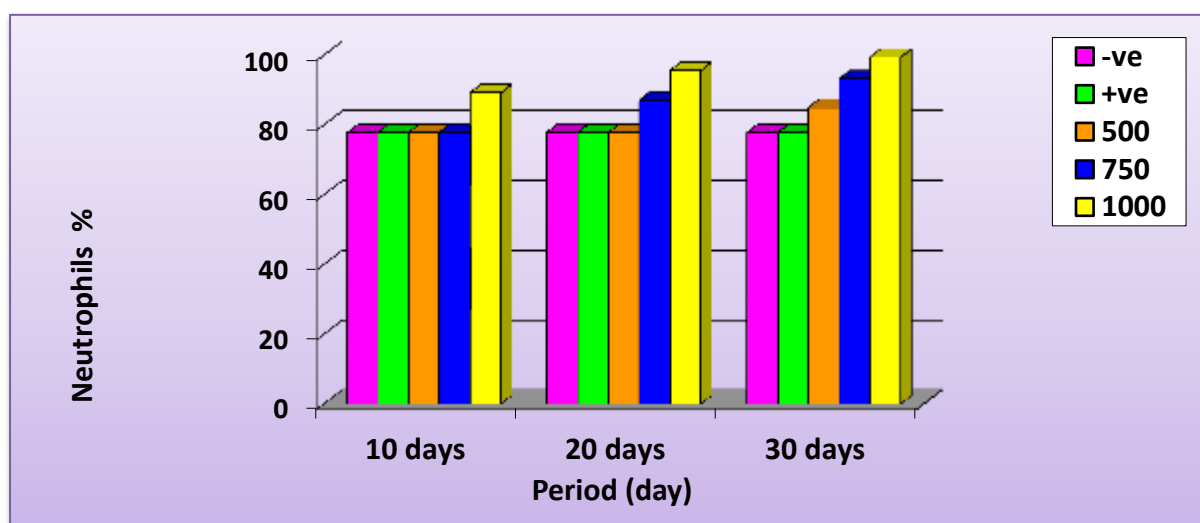


Figure 3 A-Effect of Thyme on the count of Neutrophils by injection with difference period (10, 20, 30) days and various concentration of thyme (500, 750, 1000) mg / kg of body weight comparison with control groups (- . +)
 (*) significant increase ($P \leq 0.05$)
 (**) high significant increase ($P \leq 0.01$)
 (***) high significant increase ($P \leq 0.001$)
 (a, b, c, d) represented the significant different between groups .

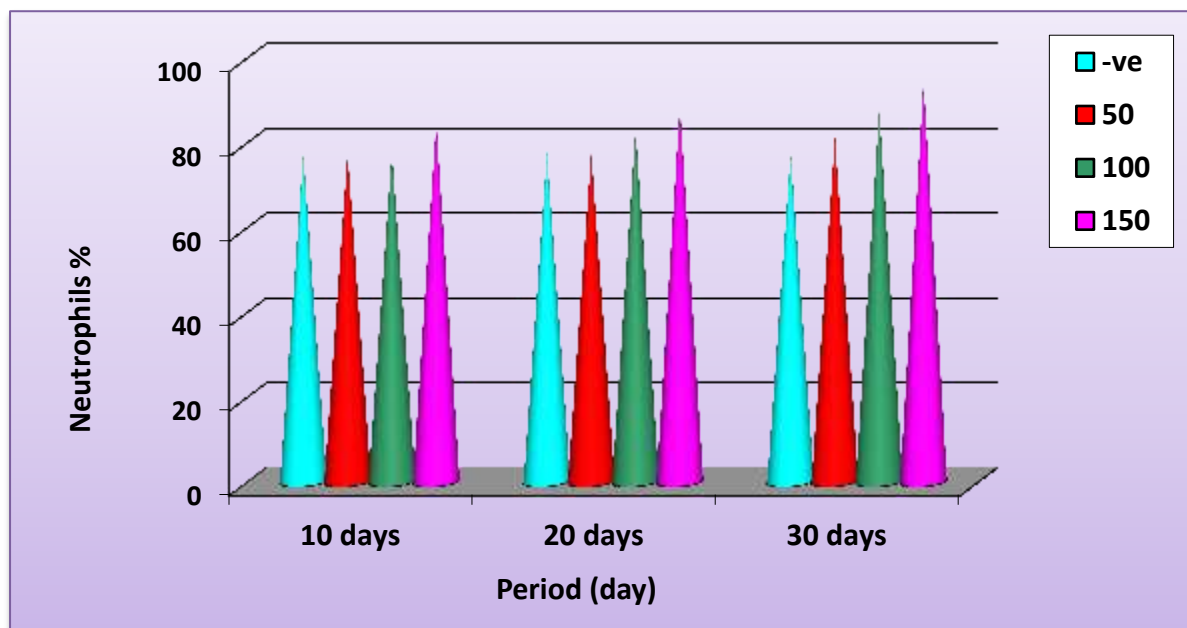


Figure 3 B - Effect of Thyme on the count of Neutrophils by feeding with difference period (10, 20, 30) days and various weight of thyme (50, 100, 150) g / kg of pellet , comparison with control group (- . +) .

(*) significant increase ($P \leq 0.05$)

(**) high significant increase ($P \leq 0.01$)

(***) high significant increase ($P \leq 0.001$)

(a, b, c, d) represented the significant different between groups .

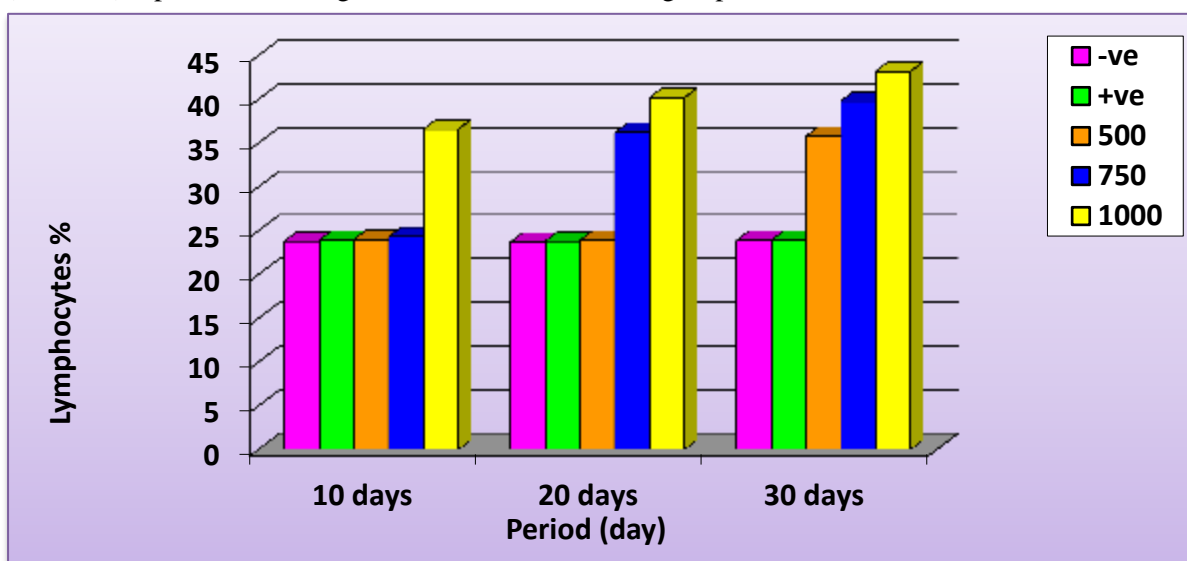


Figure 4 A - Effect of Thyme on the level of Lymphocytes by injection with difference period (10, 20 , 30) days and various concentration of thyme (500, 750,1000) mg / kg of body weight comparison with control groups (- . +) .

(*) significant increase ($P \leq 0.05$)

(**) high significant increase ($P \leq 0.01$)

(***) high significant increase ($P \leq 0.001$)

(a, b, c, d) represented the significant different between groups.

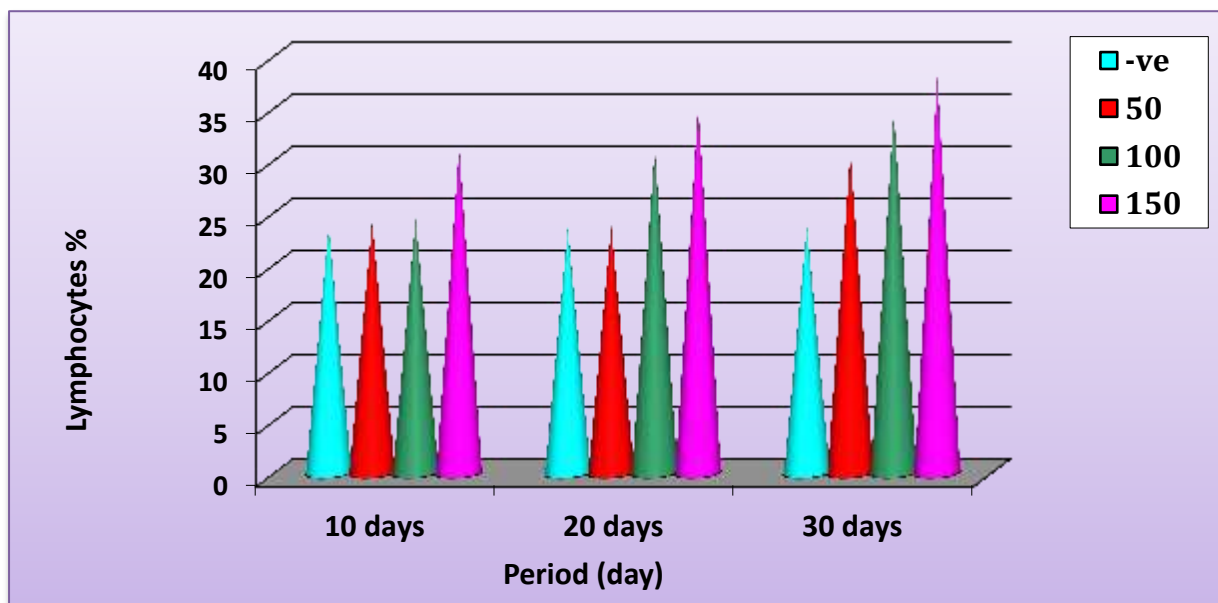


Figure 4 B - Effect of Thyme on the level of Lymphocytes by Feeding with difference period (10, 20, 30) days and various weight of thyme (50, 100, 150) g / kg of pellet, comparison with control group (-) .

(*) significant increase ($P \leq 0.05$)

(**) high significant increase ($P \leq 0.01$)

(***) high significant increase ($P \leq 0.001$)

(a, b, c, d) represented the significant different between groups .

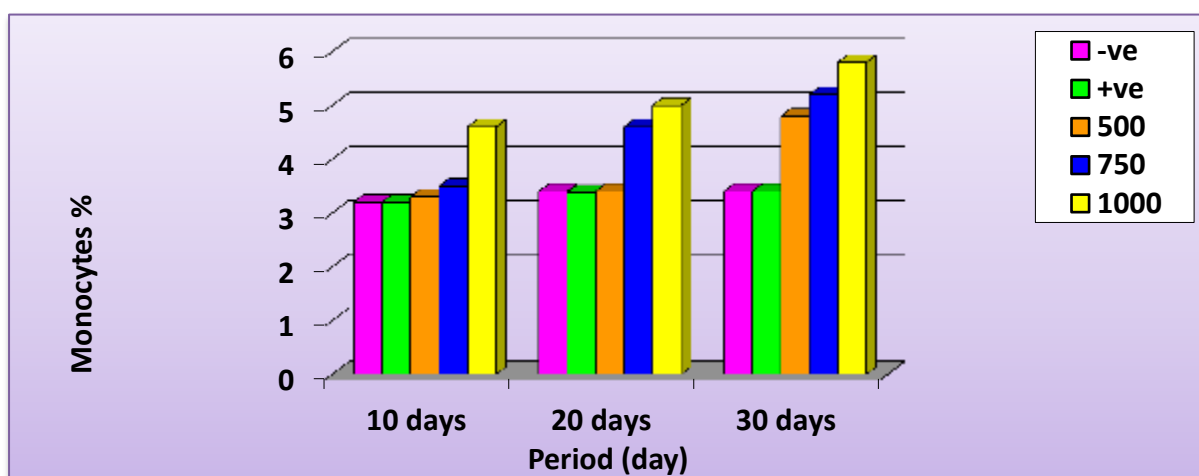


Figure 5 A - Effect of Thyme on the level of Monocytes by injection with difference period (10, 20, 30) days and various concentration of thyme (500, 750, 1000) mg / kg of body weight comparison with control groups (- . +) .

(*) significant increase ($P \leq 0.05$)

(**) high significant increase ($P \leq 0.01$)

(***) high significant increase ($P \leq 0.001$)

(a, b, c, d) represented the significant different between groups.

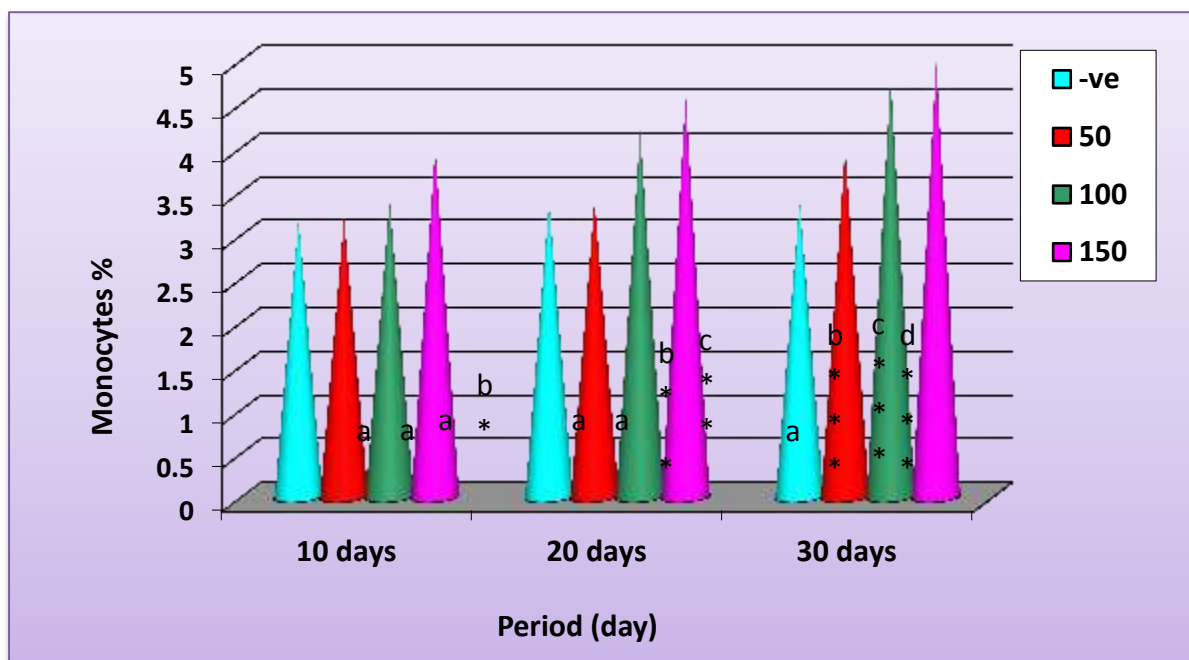


Figure 5 B - Effect of Thyme on the level of Monocytes by Feeding with difference period (10, 20, 30) days and various weight of thyme (50, 100, 150) g / kg of pellet, comparison with control group (-).

(*) significant increase ($P \leq 0.05$)

(**) high significant increase ($P \leq 0.01$)

(***) high significant increase ($P \leq 0.001$)

(a, b, c, d) represented the significant different between groups.

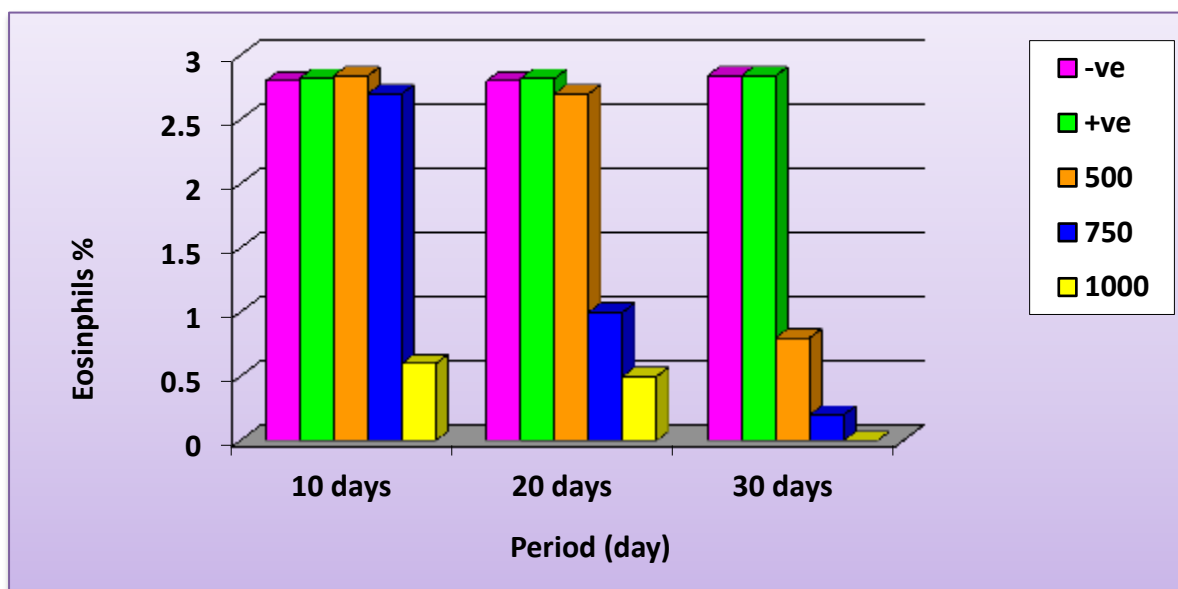


Figure 6 A - Effect of Thyme on the level of Eosinphils by injection with difference period (10, 20, 30) days and various concentration of thyme (500, 750, 1000) mg / kg of body weight comparison with control group (- . +)

(*) significant increase ($P \leq 0.05$)

(**) high significant increase ($P \leq 0.01$)

(***) very high significant increase ($P \leq 0.001$)

(a, b, c, d) represented the significant different between groups .

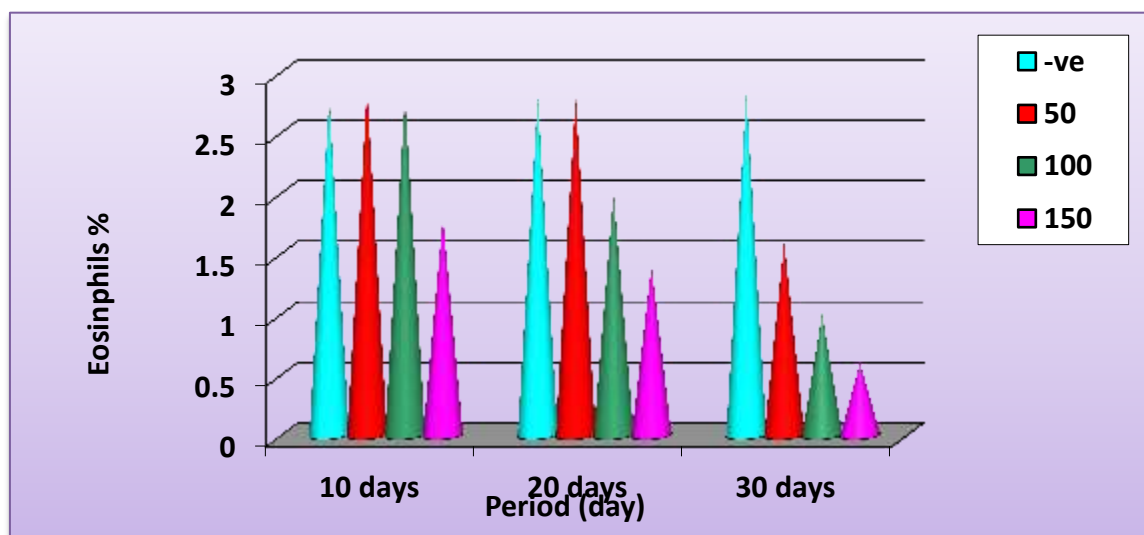


Figure 6 B - Effect of Thyme on the level of Eosinophils by Feeding with difference period (10, 20, 30) days and various weight of thyme (50, 100, 150) g / kg of pellet, comparison with control group (-).

(*) significant increase ($P \leq 0.05$)

(**) high significant increase ($P \leq 0.01$)

(***) very high significant increase ($P \leq 0.001$)

(a, b, c, d) represented the significant different between groups.

This result agree with [12] who used alcoholic extract of *Thymus vulgaris* leaves on white male mice groups that treated with differences concentrations (500 , 600 , 700) mg/kg body weight , result increasing in total number of red blood cell and white blood cell , the reason may be to the existence of material Eriodicytol which is one of the flavonoids compounds found within the installation of thyme and dependent as an anti-oxidant that protects red blood cells from oxidative decomposition this lead to increase in RBCs counts , the ability of thyme to increase the total number of white blood cells as it found that the phenols (carvocrol, thymol) its effectiveness in stimulating the immune system and help to increase the number of white blood cells, this lead to increase in WBCs counts [14].

Study of [13-15] add thyme crushed leaves to feed the chicks broilers 444 of the number of day-old and the hottest distributed randomly on four factors (four replicates per treatment), represented add crushed thyme leaves at level of 500, 750 and 1000 mg / kg feed respectively and for 8 weeks and was results increase in the number of red blood cells and white blood cells, may be that thyme leaves transaction is grinding to contain some phenolic compounds that help protect the blood of the damage that may occur as a result of oxidative stress the fact that the material is a phenolic antioxidant, the phenolic compounds of antioxidants highly effective protection through the red blood cells against oxidative stress various factors through Mechanical get rid of free radicals [16]. The increase in white blood cell may be due to thyme contain phenolic compound which lead important biological roles and a great ability to enhance the function of the immune system in the body [17, 18].

Study of [19] used aqueous extract of thyme in broiler chickens (ROSS), from 3 to 6 weeks old. The birds were distributed to 3 treatments groups with three replicates per treatment (12 males and 12 females per treatment) was mixed at the rate 4 , 6 and 8% with water offered to treatments , result increase in red blood cells and white blood cells , Because of thyme essential oils (thymol , linalool) , which would increase the effectiveness of digestive enzymes and stimulate the digestive system and take advantage of the nutrients in addition to do as antioxidants that's lead to increase in number WBCs and RBCs , supported by [20].

Our results were disagree with [21] who add 2 % thyme leaves to diet of rabbits for 10 weeks , the rabbits (12 weeks old) were divided into 4 groups 6 rabbits each (2 replicates) for males and females also [22] used boiled extract of thyme *Thymus vulgaris* in male rabbits (3-4 months old). Rabbits were divided into 6 rabbits groups each group received orally the boiled extract of thyme at a dose of 100 mg / Kg Body weight daily for 28 days, the results of both studies revealed reduction in total

leukocyte and erythrocyte counts because thyme contain a substance saponin that cause decomposition of the red blood cells and also the thyme which decrease the total number of white blood cells due to effect on the immune system, supported by [22, 23], the ratio of thymol, carvocrol and linalool in thyme are larger than saponin [24] so that we believe the effect of phenolic compound and linalool more than of saponin.

This result agree with [25] who study on twenty mature male rabbits divided randomly into two equal experimed groups of ten animals each, the first group regards as controls and dosage (2ml) normal saline orally daily and the other group were drenched (20 mg/kg) body weight of water extract of *origanum vulgare* for thirty days , the result increase in nutrophils due to treatment, the function of neutrophils are phagocytosis and the *Origanum vulgare* Considered foreign material to the body so the count of neutrophils increase as aresult attack to Origanum , this result supported by [26, 27].

And disagree of the present study with [28] when they found that high dose of *origanum vulgare* oil had adverse effects on metabolism of mice on the contrary to the lower concentration which have appositve effects on antioxidant status.

ct of phenol in olive (200 mg/kg) effect on twenty rats for 30 days, the result increase in count of lymphocytes and monocyte, the exposure to phenol component leading to inflammation , and this reaction characterized by movement of lymphocytes and monocytes from blood to the extravascular tissue this led to increase in count of lymphocytes and monocytes, Therefore, monocytes increased in circulation and phagocytosis increased in tissue, to prevent extensive damage to the body and disagree with [26] The decreases in the lymphocytes by thyme affect the effector cells of the immune system due to inhibit the body's immunity.

Thyme contain Rosmarinic acid which acts as an inhibitor that inhibition syntheis of Eosinphils, this result agree with [28].

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