A study effect of thyme on Biochemical and Histological changes in Liver of male rats

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ABSTRACT
The increase in the use of thyme in Iraq and in 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 effects on GOT, GPT and ALP were measured. statistical analysis showed significance significant increase (P≤0.05) in the level of GOT, GPT and decrease in level of ALP in rats treated with 1000 mg of thyme/kg of body weight and 150 g/kg of pellet in 10 days, in 20 days thyme cause highly significance significant increase (P≤0.01) in the level of GOT, GPT and decrease in level of ALP in rats treated with thyme by (750,1000) mg/kg of body weightand (100,150) g/kg of pellet and in 30 days thyme cause significance highly significant increase (P≤0.001) in the level of GOT, GPT and decrease in level of ALP 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:

INTRODUCTION

Medicinal plants are used in many countries as replacement to synthetic drugs, scientists are now paying attention towards herbal extracts to do as antimicrobial 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].

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 [2].

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 [3].

*T. vulgaris* is an important medicinal plant [4,5] 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, seerotolytic, expectorant, antiseptic, antilelmiintic and antitusive as reported by other authors [6,7].
MATERIALS AND METHODS

Laboratory Animals:
All experiments were performed on 120 albino Rats (male), their 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 / Bagdad 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:
The Thyme used in this study were purchased from the Shorja market in the Baghdad, dried thyme leaves have been prepared in two ways, depending on how the dosage:

Injection:
Dry leaves of Thymus vulgaris where put about 50g, 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 [10,11,12,13]. And this extraction examine by I. R. Spectrophotometer Show of screening effective groups in thyme by peaks, and every peak refer to Certain effective group, figure (1).

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 taking 15g of dry extract and dissolved in100ml of Alcohol, therefore the concentration of the stock solution (150 mg / ml), and It was prepared concentrations of (500, 750, 1000) mg /kg of body weight [14].

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 giving to the animals [15].

![Figure 1: Show Effective Groups in Extraction of Thyme](image-url)
Animals Groups:

The experiment was achieved as following:

- **1st experiment:**
  included 75 rats randomly distribution 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, 150 g/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 (experimental and control). The collection of blood were obtained by heart puncture using (3, 5 ml) disposable syringes, the blood put in small plastic tubes container ethylene diaminetetraacitic acid (EDTA), and used for hematological test.

Collection of Organ:

Organ was collected from all rat groups (experimental and control), after an autopsy and the withdrawal of blood was removed by the liver and put it on filter paper, then put in formalin a concentration of 10 % for the histological examinations.

RESULTS AND DISCUSSION

This study included separation blood taken from male rats (120 rat’s) treatment with thyme to see the effect of this substance on the Biochemical tests and histological changes showed results as follows:

Biochemical Tests (GOT, GPT, ALP):

The statistical analysis showed non significance difference (P≤0.05) at the level of GOT, GPT(IU/L) of the rats were treated with thyme in the concentration of (500 or 750) mg/kg of body weight by injection for 10 days as compared with the control groups (con- and con +). Also there was non significance difference (P≤0.05) at (50 and 100) g/kg of pellet by feeding at the same days when compared with the control group (con-). While there was significant increase difference (P≤0.05) in the level of GOT, GPT(IU/L)) in the rats were treated with the concentration of 1000 mg/kg of body weight. Also there was a significance increase difference (P≤0.05) in the rats were treated with thyme by feeding at 150g/kg of pellet as compared with the control group (con-).

The statistical analysis showed non significance difference (P≤0.01) in the level of GOT, GPT(IU/L)) of the rats were treated with thyme in the concentration of 500 mg/kg of body weight by injection for 20 days as compared with the control groups (con- and con +). Also there was non significance difference (P≤0.01) at 50
g/kg of pellet by feeding at the same days when compared with the control group (con-). While there was a highly significant increase difference (P≤0.01) in the level of GOT, GPT(IU/L) in the rats were treated with the concentration of (750 or 1000) mg/kg of body weight as compared with control groups (con- and con+). Also there was a significance increase difference (P≤0.01) in the rats treated with thyme by feeding at (1000or150) g/kg of pellet as compared with the control group (con-).

The statistical analysis showed a highly significant increase difference (P≤0.001) in the level of GOT, GPT(IU/L) in the rats were treated with thyme in the concentration of (500or750 and1000) mg/kg of body weight by injection for 30 days as compared with the control groups (con- and con+). Also there was a highly significant increase difference (P≤0.001) at (50 or100 and150) g/kg of pellet by feeding at the same days when compared with the control group (con-).

The statistical analysis showed non significance difference (P≤0.05) at the level of ALP(IU/L) of the rats were treated with thyme in the concentration of (500 or 750) mg/kg of body weight by injection for 10 days as compared with the control groups (con- and con+). Also there was non significance difference (P≤0.05) at (50 and 100) g/kg of pellet by feeding at the same days when compared with the control group (con-). While there was significant decrease difference (P≤0.05) in the level of ALP(IU/L) in the rats were treated with the concentration of 1000 mg/kg of body weight. Also there was a significance decrease difference (P≤0.05) in the rats were treated with thyme by feeding at 150g/kg of pellet as compared with the control group (con-).

The statistical analysis showed non significance difference (P≤0.01) in the level of ALP(IU/L) of the rats were treated with thyme in the concentration of 500 mg/kg of body weight by injection for 20 days as compared with the control groups (con- and con+). Also there was non significance difference (P≤0.01) at 50 g/kg of pellet by feeding at the same days when compared with the control group (con-). While there was a highly significant decrease difference (P≤0.01) in the level of ALP(IU/L) in the rats were treated with the concentration of (750 or 1000) mg/kg of body weight as compared with control groups (con- and con+). Also there was a significance decrease difference (P≤0.01) in the rats treated with thyme by feeding at (1000or150) g/kg of pellet as compared with the control group (con-).

The statistical analysis showed a highly significant decrease difference (P≤0.001) in the level of ALP(IU/L) in the rats were treated with thyme in the concentration of (500or750 and1000) mg/kg of body weight by injection for 30 days as compared with the control groups (con- and con+). Also there was a highly significant decrease difference (P≤0.001) at (50 or100 and150) g/kg of pellet by feeding at the same days when compared with the control group (con-).

The results of the statistical analysis of the effect of thyme on the level of GOT, GPT, ALP(IU/L) was showed in figure (2 A, B), (3 A, B) and (4 A, B).

![Fig. 2A: Effect of thyme on the level of GOT( IU/L) by injection with difference period (10,20,30) days and difference concentration of thyme (500,750,10000) mg / kg of body weight comparison with control groups ( con-, con + )](image-url)

(*) significant increase (P≤0.05)

(**) highly significant increase (P≤0.01)

( *** ) highly significant increase (P≤0.001)

(a,b,c,d) represented the different significant between groups.
Fig. 2B: Effect of thyme on the level of GOT (IU/L) by feeding with different period (10, 20, 30) days and different weight of thyme (50, 100, 150) g/kg of pellet, comparison with control group (con-)

(*) significant increase (P≤0.05)

(**) highly significant increase (P≤0.01)

(***) highly significant increase (P≤0.001)

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

Fig. 3A: Effect of thyme on the level of GPT (IU/L) by injection with different period (10, 20, 30) days and different concentration of thyme (500, 750, 1000) mg/kg of body weight comparison with control groups (con-, con+)

(*) significant decrease (P≤0.05)

(**) highly significant decrease (P≤0.01)

(***) highly significant decrease (P≤0.001)

(a, b, c, d) represented the different significant between groups.
Fig. 3B: Effect of thyme on the level of GPT (IU/L) by feeding with difference period (10,20,30) days and difference weight of thyme (50,100,150) g / kg of pellet, comparison with control group (con – )

(*) significant increase (P≤0.05)

(**) highly significant increase (P≤0.01)

(***) highly significant increase (P≤0.001)

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

Fig. 4A: Effect of thyme on the level of ALP (IU/L) by injection with difference period (10,20,30) days and difference concentration of thyme (500,750,1000 ) mg / kg of body weight comparison with control groups ( con – , con + )

(*) significant decrease (P≤0.05)

(**) highly significant decrease (P≤0.01)

(***) highly significant decrease (P≤0.001)

(a,b,c,d) represented the different significant between groups.
Fig. 4B: Effect of thyme on the level of ALP (IU/L) by feeding with difference period (10, 20, 30) days and difference weight of thyme (50, 100, 150) g / kg of pellet, comparison with control group (−)

(*) significant decrease (P ≤ 0.05)

(**) highly significant decrease (P ≤ 0.01)

(***) highly significant decrease (P ≤ 0.001)

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

[16] reported that phenolic compounds in the oils thyme that lead to raising the level of (GPT, GOT).

On other hand [17] adding aqueous extract of thyme percentage 10% to drinking water for the mothers of broiler chickens thyme causes decline the level of enzymes (GOT, GPT) is perhaps return back to thyme have compounds enhance the status of antioxidants. Also previous studies agree with the results of the present study [18, 19].

Thyme contain flavonoids that due to decline in level of ALP by obstruction operation in the liver and bones, this results agreed with [20] that fed diet contain 1.5% of the powder mint (mint and thyme belong to same family Lamiaceae [21]) to one hundred sixty chick broiler (Hubbard) day-old divided into four treatment 40 chick each treatment with two replicate 20 bird for 3 and 6 weeks and Results pointed to a decline in level of ALP, but this result disagreed with [22] that said some active compounds found in the leaves of thyme, including the material and thymol, Carvacrol and Simin, which play a role in raising the level of ALP.

This difference in agreed and disagreed results may be cause by different in internal and circumstances of experimental animals used also the material used in the experiment (extract or oil) in addition to the dosage period, used different methods of dosage.

**Histological Changes:**

Histological sections of the liver of rats were treated with 500 mg/kg of body weight for 10 days of thyme does not appear any effect on histological variables and in 20 days have some minor changes including congestion in the blood vessels and some infiltrates of hepatic cells, Figure (6A). In a period of 30 days appeared to clearly show the thyme impact on the liver tissue and have some of histological changes including degeneration of hepatic cells with necrosis mild inflammatory cells near portal area and infiltration with depletion of glycoprotein and granules inside the cells figure (6B), compared with the histological sections of the liver of rats control group, figure (5).
Fig. 5: Sections on Liver of rat from control group, which showing 1. Blood vessels, 2. Portal area, 3. Hepatocyte, 4. Sinusoids, 400 X (H&E).

Fig. 6: Sections on Liver of rat from groups (A) injected with thyme extraction 500 mg/kg of body weight for 20 days, which showing 1. Inflammatory cells, 2. Congestion in the blood vessels, 400 X (H&E) (B) injected with thyme extraction 500 mg/kg of body weight for 30 days, which have 1. Necrosis, 2. Inflammatory cells, 3. Portal area, 4. Depletion of glycoprotein and granules, 5. Blood vessels, 200 X (H&E).

Histological sections of the liver of rats were treated with 750 mg/kg of body weight for 10 days of thyme showing slight in inflammatory cells and congestion in the blood vessels, figure (7A). In 20 days showing degeneration and necrosis near portal area of hepatic cells with inflammatory cells, infiltration and congestion near the portal area, figure (7B). In 30 days it appear increase degeneration of hepatic cells with necrosis, depletion of glycoprotein granules inside the cells and congestion in the blood vessels near portal area figure (7C), compared with the histological sections of the liver of rats control group, figure (5).

Histological sections of the liver of rats were treated with 1000 mg/kg of body weight for 10 days of thyme showing necrosis of hepatic cells, inflammatory cells infiltration with dilatation of sinusoid figure (8A). In 20 days group showing disruption of adhesion between the hepatocyte cells, shrinkage of hepatocyte cells lead to increase of sinusoid spaces with depletion of glycoprotein granules, figure (8B). In 30 days group showing certain necrotic in hepatocyte cells with increased inflammatory cells infiltration near portal area with disruption of adhesion between the hepatic cells figure (8C), compared with the histological sections of the liver of rats in control group figure (5).
Fig. 7: Sections on Liver of rat from groups (A) injected with thyme extraction 750 mg/kg of body weight for 10 days, which showing 1. Congestion in the blood vessels, 2. Bile ducts, 3. Inflammatory cells, 400 X (H&E), (B) injected with thyme extraction 750 mg/kg of body weight for 20 days, which showing 1. Central vein, 2. Inflammatory cells, 3. Portal area, 4. Congestion in the blood vessels, 5. Necrosis, 200 X (H&E), (C) injected with thyme extraction 750 mg/kg of body weight for 30 days, which showing 1. Depletion of glycoprotein granules, 2. Necrotic in hepatocyte cells, 3. Inflammatory cells, 4. Congestion in the blood vessels, 400 X (H&E).

Fig. 8: Sections on Liver of rat from groups (A) injected with thyme extraction 1000 mg/kg of body weight for 10 days which showing, 1. Necrotic in hepatocyte cells, 2. Inflammatory cells, 3. Dilated sinusoid, 4. Central vein, 200 X (H&E), (B) injected with thyme extraction 1000 mg/kg of body weight for 20 days which showing, 1. Central vein, 2. Shrinkage of hepatocytes, 3. Depletion of glycoprotein granules, 200 X (H&E), (C) injected with thyme extraction 1000 mg/kg of body weight for 30 days which showing, 1. Necrosis, 2. Inflammatory cells, 400 X (H&E).
Histological changes in the liver treatment Thyme concentrations (500,750and1000) mg/kg of body weight at periods of (10,20and30) days, the infiltration of inflammatory cells and congestion that took place in the blood vessels and other changes in the liver tissue due to thyme may act as an antidote to the oxidant therefore stimulates the immune response and thus strengthen the immune system and due to this tissue changes, this result agree with [23] In liver and kidney tissue of rabbits treated with seeds oil (have same active group that found in thyme) orally at a dose of 1000mg/kg of body weight for a period of 6 weeks the results of the histological pointed to a bloody congestion as well as the infiltration of inflammatory cells [24]. Also that another previous studies agreed with results of present study [25,26,27].

The increase in liver enzymes GPT, GOT reflects the histological changes and congestion made in the hepatic vein and sinuses liver, and the decline in the level of enzyme (ALP) and this reflects the decline in the yellow acids and the absence of obstructive icterus, the (ALP) found in bile ducts and thus the lack of its level in the blood indicates the lack of damage in the bile channels[19]. Fed dried leaves of salvia plant (salvia and thyme belong to same family Lamiaceace [21]) had been powdered and added to the diet in different ratios 2.5, 5, 10 g/kg forage. in adult male albino mice, the Salvia causes congestion in the hepatic vein, necrosis of hepatic cells and other histological changes because it contains phenolic compounds, these changes in tissue of liver and enzymes led to imbalance in liver functions and increasing imbalance in the work of the liver by increasing the dose and the time period.

As a result of negative effects of thyme by the results that obtained, suggests that there is smell percentage of thyme that taken during a week as 5 mg / week by varying degrees depending on the geographical areas of humans.

Conclusions:
Intake to leave thyme lead to an increase in the level of liver enzymes (GPT, GOT) and decline in level of ALP, and caused progressive congestion in the blood vessels, with inflammatory cells and degeneration, with necrosis, inflammatory cells near portal area, infiltration with depletion of glycoprotein and granules inside the liver, proportionally with the increasing of the thyme dose and treatment periods.

REFERENCES


