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

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Received 22 May 2016; Accepted 18 July 2016; Available online 8 August 2016

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 Uric acid , Urea and Creatinine were measured. statistical analysis showed significance significant increase (P≤0.05) in the level of Uric acid , Urea and Creatinine 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 Uric acid , Urea and Creatinine in rats treated with thyme by (750,1000) mg/kg of body weight and (100,150) g/kg of pellet and in 30 days thyme cause significance highly significant increase (P≤0.001) in the level of Uric acid , Urea and Creatinine 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, secrrotolytic, expectorant, antiseptic, antilelmintic 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].

![Fig. 1: Show Effective Groups in Extraction of Thyme](image)

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].

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, 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 (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 kidneys and put them 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 (Uric Acid, Urea, Creatinine):**
The statistical analysis showed no significance difference (P≤0.05) at the level of Uric Acid, Urea, Creatinine (mg/dL) 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 nonsignificance 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 Uric Acid, Urea, Creatinine (mg/dL) in the rats were treated with the concentration of 1000 mg/kg of body weight. Also there was nonsignificance 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 no significance difference (P≤0.01) in the level of Uric Acid, Urea, Creatinine (mg/dL) 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 not 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 Uric Acid, Urea, Creatinine (mg/dL) 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 nonsignificance increase difference (P≤0.01) in the rats treated with thyme by feeding at (100or150) g/kg of pellets compared with the control group (con-).

The statistical analysis showed a highly significant increase difference (P≤0.001) in the level of Uric Acid, Urea, Creatinine (mg/dL) in the rats were treated with thyme in the concentration of (500 or 750 and 1000) 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 or 100 and 150) g/kg of pellet by feeding groups at the same days when compared with the control group (con-).
The results of the statistical analysis of the effect of thyme on the serum level of Uric Acid, Urea, Creatinine (mg/dL) were showed in figure (2A, B), (3A, B) and (4A, B).

**Fig. 2A:** Effect of thyme on the level of uric acid (mg/dL) 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 increase (P≤0.05)

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

(***+) very 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 uric acid (mg/dL) 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)
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Advances in Environmental Biology, 10(7) July 2016, Pages: 301-310

(***) 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 urea (mg/dL) 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 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. 3B: Effect of thyme on the level of urea (mg/dL) 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)
(***) very 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 creatinine (mg/dL) by injection with difference concentration of thyme (500, 750, 1000) mg/kg of body weight comparison with control groups (con-, 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. 4B:** Effect of thyme on the level of creatinine (mg/dL) by feeding with 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.

The thyme causes an increase in the level of Uric acid, Urea and Creatinine because it contains resin material that acts to increase the metabolism process of nucleic acids thereby increase the uric acid level in the blood, causing an increase the protein broken rate and thus presence of urea in the blood increased, also caused increase in the creatine phosphate broken in the muscles therefore increase the level of creatinine.[16] Fed pellet contain different levels of thyme leaves powder ration at levels of 0.2, 0.4, 0.6 % for treatments T1, T2, T3 respectively to broiler for 47 day, the result showed significant increased in urea, uric acid and creatinine. The another previous studies agreed with results of the present study [17,18,19].

Histological Changes:
Histological sections of the kidneys of rats were treated with 500 mg/kg of body weight for 10, 20 days of thyme showed only some histological slight effects, in 30 days showing apoptosis of renal tubules epithelial cells figure (6A), compared with histological sections of the kidneys of rats control group, figure (5).

Fig. 5: Sections on Kidney of rat from control group, which showing 1. Glomerulus, 2. Distal tubules, 3. Proximal tubules 400 X (H&E).

Histological sections of the kidneys of rats were treated with 750 mg/kg of body weight for 10 days apoptosis of renal tubules epithelial cells and congestion in the renal blood vessels, figure (7A). Also in 20 days showing degeneration and apoptosis of renal epithelial cells, figure (7B1). In 20 days in other section showing severe congestion in the renal blood vessels, figure (7B2). In 30 days showing hyaline cast inside the renal.
tubules with degeneration and apoptosis of epithelial cells, figure (7C), compared with the histological sections of the kidneys of rats control group figure (5).

Fig. 7: Sections on Kidney of rat from groups (A) injected with thyme extraction 750 mg/kg of body weight for 10 days which showing, 1. Apoptosis of renal tubules epithelial cells, 2. Congestion in renal blood vessels, 400 X (H&E), (B1) injected with thyme extraction 750 mg/kg of body weight for 20 days which showing, 1. Degeneration of renal epithelial cells, 2. Apoptosis of the renal epithelial cells, 400 X (H&E), (B2) injected with 750 mg/kg of body weight for 20 days of thyme extraction which showing 1. Congestion in renal blood vessels, 200 X (H&E), (C) injected with thyme extraction 750 mg/kg of body weight for 30 days which showing, 1. Hyaline cast, 2. Degeneration of renal epithelial cells, 3. Apoptosis of the renal epithelial cells, 200 X (H&E).

Histological sections of the kidneys of rats were treated with 1000 mg/kg of body weight for 10 days appear showing necrosis of renal epithelial cells with inflammatory cells infiltration, figure (8A). In 20 days appear showing necrosis of renal epithelial cells with inflammatory cells infiltration and congestion in the renal blood vessels, figure (8B). In 30 days appear sever congestion in the renal blood vessels, certain necrosis and increase inflammatory cells infiltration figure (8C), compared with the histological sections of the kidneys of rats in control group figure (5).

Fig. 8: Sections on Kidney of rat from groups (A) injected with thyme extraction 1000 mg/kg of body weight for 10 days which showing, 1. Necrosis of renal epithelial cells, 2. Inflammatory cells, 400 X (H&E), (B) injected with thyme extraction 1000 mg/kg of body weight for 20 days which showing, 1.
Congestion in renal blood vessels, 2. Necrosis of renal epithelial cells, 3. Inflammatory cells. 400 X (H&E), (C) injected with thyme extraction 1000 mg/kg of body weight for 30 days which showing, 1. Necrosis of renal epithelial cells, 2. Inflammatory cells, 3. Congestion in renal blood vessels, 200 X (H&E).

Histological changes in both liver and kidney treatment Thyme concentrations (500,750 and 1000) mg/kg of body weight at periods of (10,20 and 30) days, the infiltration of inflammatory cells and congestion that took place in the blood vessels and other changes in both the liver and kidney 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 [20] In liver and kidney tissue of rabbits treated with seeds oil (have same active group that found in thyme) orally at a dose of 1000 mg/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 [21]. Also that another previous studies agreed with results of present study [22,23,24,25].

The changes that have occurred in the high level uric acid, urea, creatinine may be due to thyme contain the resin, which caused the necrosis and degeneration of kidney cells which prevents a infiltration of glomerular process properly and who was accompanied by the expansion of glades urinary with severe congestion in a bloody and severe bleeding in the tissues of nephron veins, with increasing the dose and duration of exposure thyme which cause an imbalance in the kidney function in rats and thus the reason for the high level of Uric acid, Urea, Creatinine in serum instead of secreted out of the body [18,19].

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 increase in the level of Uric Acid, Urea and Creatinine and caused progressive congestion in the blood vessels, with inflammatory cells and degeneration, with necrosis in kidney tissue intake to leave thyme caused hyaline cast inside the renal tubules, degeneration and apoptosis of renal tubules epithelial cells with inflammatory cells infiltration and congestion in the renal blood vessels, proportionally with the increasing of the thyme dose and treatment periods.

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