Effects of *Thymus daenensis* on inflammatory factors and liver toxicity induced by thioacetamide in rats

Banafshe Soosani¹, Hossein Sazegar²*

¹Department of Biology, Faculty of Sciences, Shiraz Branch, Islamic Azad University, Shiraz, Iran
²Department of Biology, Faculty of Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

**Abstract**

**Introduction:** Thioacetamide (TAA) intoxication is underlying acute liver damage, inflammation, and tissue necrosis. The aim of this study was to evaluate the *Thymus daenensis* effect on acute liver disease induced by the thioacetamide and its effects on the tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6) cytokines.

**Methods:** In an experimental study, 36 male Wistar rats were divided into 6 groups of 6 each. The amount of 0.03-g thioacetamide dissolved in 1 mL of distilled water was injected intraperitoneally to all mice groups except the control group for 3 weeks, twice a week. The negative control group received only thioacetamide and the other groups received 8 mg/kg silibinin by gavage, other than to thioacetamide. The experimental groups, after injection of thioacetamide were treated with 5, 10 or 20 mg/kg extract of *T. daenensis* for 2 weeks. Peripheral blood samples were taken from the rats’ hearts after general anesthesia. Then, TNF-α and IL-6 cytokines levels were measured by Elisa kits. Pathology evaluation was also examined on liver.

**Results:** TNF-α and IL-6 levels decreased in the groups treated with 5 mg/mL (respectively, *P* = 0.001, *P* = 0.05), 10 mg/mL (*P* < 0.001, *P* < 0.001, respectively) and 20 mg/mL (*P* < 0.001, *P* < 0.001) extracts compared to thioacetamide group. Histopathological studies indicated that liver lesions were improved in mice treated with *T. daenensis* extract compared with thioacetamide group.

**Conclusion:** *Thymus daenensis* extract has anti-inflammatory and protective effects on liver toxicity induced by thioacetamide. Hence, it might be used for this purpose or for similar toxicities.

**Keywords:**

Thioacetamide, Medicinal plants, *Thyme daenensis*, TNF cytokines, IL-6 cytokine

**Implication for health policy/practice/research/medical education:**

*Thyme daenensis* extracts have antioxidant and anti-inflammatory properties and can improve liver injury in mice by decreasing pro-inflammatory TNF and IL-6 cytokines. So, this extract might be used as anti-inflammatory and hepatoprotective agent.

**Please cite this paper as:** Soosani B, Sazegar H. Effects of *Thymus daenensis* on inflammatory factors and liver toxicity induced by thioacetamide in rats. J Herbmed Pharmacol. 2018;7(1):56-60. doi: 10.15171/jhp.2018.10

**Introduction**

In recent decades, low patient satisfaction from the consumption of synthetic drugs, due to high costs and side effects of these medications caused an increased tendency to traditional treatments (1). Herbal usage to treat a massive spectrum of diseases is developing rapidly. In recent studies special attention has been paid to the protective effects of antioxidants by natural origin compounds against poisoning caused by chemical agents (2). Phenolic compounds with antioxidant activities have been shown to possess protective effects on various organs (3).

*Thymus daenensis* (Thyme) is an aromatic plant from Lamiaeaceae family that is well known as mediator agent in the synthesis of aromatic chemicals and antibacterial agent against especially oral bacteria. Furthermore, its antibacterial effects against Salmonella and Aspergillus have been reported (4). *T. daenensis* is the most important type of thyme in Iran (5). Thymol and carvacrol are 2 original phenolic component of thyme oil, which their concentrations are different between 3% to 6%. In addition, linalool is the main non-phenolic constituent of

*Corresponding author:* Hossein Sazegar,
Email: hoseinsazgar@yahoo.com
the Thyme (6). Thioacetamid (TAA) has been used for several years to induce a model of acute liver injury in rats (7). TAA is used as an antifungal agent and is a powerful oxidizing agent. It could be oxidized to active and toxic metabolites called, thioacetamide oxide, by the liver cell microsomes enzyme cytP450B (8). It attacks to membrane proteins and lipids, causing degradation of them, peroxidation of lipids and oxidative stress (7).

The correlation between inflammation and oxidative stress in the course of liver injury is indisputable (9). The systemic inflammatory response is mediated by activated pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukins (IL-1β and IL-6) and oxygen radicals which may sensitize hepatocytes to the toxicity and damage (10). The TNF-α or IL-6 gene knocked out mice had partial ability to regenerate liver and after liver damage had increased mortality rate (11). Thus, aim of this study was to investigate the effects of Thymus daenensis on serum IL-6 and TNF-α levels as well as hepatotoxicity induced by thioacetamide in rats.

Material and Methods

Thymus daenensis leaf extraction

Daenensis aerial parts of thyme plant collected from Chaharmahal and Bakhtiari province mountainous were dried in the room with airflow, away from direct sunlight, in normal temperature and humidity. Then, the plant parts were ground by electric mill to powder. The resulting powder was mixed with ethanol and filtered after 5 hours. The obtained extract was dried using Rotary evaporator. Selected doses of the extract (5, 10 and 20 mg/kg) were used in this study.

Animals

Three-month old male Wistar rats, 300 ± 20 g in weight were obtained from the animal laboratory facilities of Isfahan University of Medical Science. Islamic Azad University of Shiraz Ethics Committee approved the animal protocols. All experimental animals were housed in standard environmental conditions in cages maintained at an ambient temperature of 25 ± 2°C and received 12 hours of light and dark daily.

Experimental design

A total of 36 rats were randomly divided into 6 experimental groups, 6 of rats each. The experimental groups were treated as follows:

1. Rats of group 1 were served as controls and intraperitoneally injected with saline solution (0.9% NaCl), twice weekly for 3 weeks.
2. Rats of group 2 were given 100 mg/kg body weight of TAA (Sigma–Aldrich Corp., St. Louis, MO, USA) intraperitoneal injection, twice weekly for 3 weeks.
3. Rats of group 3 were intraperitoneally injected with TAA at the same dose given to group 2 and were orally supplemented with silibinin in the 8 mg/kg body weight/day for 3 weeks.
4. Rats of group 4 were intraperitoneally injected with TAA at the same dose given to group 2 and were orally supplemented with T. daenensis leaves extract at a dose of 5 mg/kg body weight/day for 3 weeks.
5. Rats of group 5 were intraperitoneally injected with TAA at the same dose given to group 2 and were orally supplemented with T. daenensis leaves extract at a dose of 10 mg/kg body weight/day for 3 weeks.
6. Rats of group 6 were intraperitoneally injected with TAA at the same dose given to group 2 and were orally supplemented with T. daenensis leaves extract at a dose of 20 mg/kg body weight/day for 3 weeks.

Blood serum analyses

After 48 hours of the last gavage, the experimental animals were fasted for 12 hours, water was not restricted, and then anaesthetized with intraperitoneal injection of ketamine and xylazine. Blood samples were taken from the mice hearts. The collected blood was centrifuged at 4000 rpm for 20 minutes and serum was separated. TNF-α and IL-6 cytokines (Eastbiopharm company) were measured by ELISA kits.

Histopathological examinations

After blood sampling, the rats were dissected and the liver tissues were preserved in 10% buffered formalin immediately after removal from the animals, embedded with paraffin. After routine processing, paraffin sections of each tissue were cut into 4 mm thickness and stained with hematoxylin and eosin. All liver sections were examined using a light microscope and photographed. The data were analyzed using the SPSS version 12.0. Each value was expressed as mean± standard deviation (SD) and values were analyzed using two-way analysis of variance (ANOVA) to determine differences between the mean values of the factors in experimental groups. P values less than 0.05 were considered as significant.

Results

The mean of TNF-α and IL-6 cytokines in different groups are shown in Table 1 and Figures 1 and 2. TAA administration to normal rats caused significant elevation of serum TNF-α (23.20 ± 1.02) and IL-6 (84.83 ± 1.47) cytokines compared with control TNF (14.23 ± 1.69) (P < 0.001) and control IL-6 rats (73.33 ± 2.42) (P < 0.001). The level of TNF-α statistically decreased in rats treated with TAA plus T. daenensis leaves extract at a dose of 5 mg/kg (19.20 ± 1.87) (P = 0.001), 10 mg/kg (17.00 ± 1.14) (P < 0.001) and 20 mg/kg (15.41 ± 1.20) (P < 0.001) when compared with TAA group (Figure 1). Moreover, reduction in the level of serum IL-6 was observed in the rats treated with TAA plus T. daenensis leaves extract at a dose of 5
(81.33 ± 1.36) (P - 0.054), 10 (78.16 ± 1.47) (P < 0.001) and 20 (77.00 ± 2.36) mg/kg (P < 0.001) compared with TAA group (Figure 2).

The levels of TNF and IL-6 cytokines in the rats treated with Silibinin + TAA were found respectively (18.15 ± 1.47) and (78.50 ± 2.58) which were significantly less than the ones in the rats treated with thioacetamide (for both of them P < 0.001) (Table 1).

Light microscopic examination indicated a normal structure of the liver in the control rats. In the rats treated with TAA, there were marked space dilation of sinusoid, vacuolar and degenerative changes of hepatocytes and an increase in the volume of the nucleus of hepatocytes. Nevertheless, in the group treated with Thyme, severe congestion of central venous and presence of mild connective tissue were seen in the rats which received lower concentration of Thyme. By increasing the dosage, it was found evidence of connective tissue in the portal, the vein congestion of portal area, sinusoid mild hyperemia, changes in the structure of liver lobules and disruptive hepatocytes was more acquainted with vesicular nuclei. In the group that received TAA + Silibinin, necrosis, cytoplasmolisis and degenerative changes around central hepatocytes and central venous dilatation were seen (Figure 3).

**Discussion**

The influence of *T. daenensis* preparations on TAA-induced liver injury was determined by assessing histopathologic examinations and serum levels of TNF-α and IL-6. In our study, hepatotoxicity induced by TAA was reflected by a marked elevation of TNF-α and IL-6 activities, but also by notable histopathologic alterations. This is in agreement with the results of many earlier studies using a model of TAA -induced liver damage (7,12,13). Administration of thioacetamide produces thioacetamide S-oxide by cytochrome P 450 enzyme in liver microsomes (14). S-oxide causes oxidative stress and damages to liver cells and eventually caused necrosis and apoptosis of these cells. The free radicals produced by thioacetamide invade the membranes of liver cells and cause lipid peroxidation. This peroxidation makes membranes less fluidity and changes the permeability which facilitates the release of substances such as cytokines from inside the cells into cytoplasm (7).

Administration of thyme preparations independence dose significantly decrease neither proinflammatory cytokines level nor histological markers of hepatic function, when compared to the TAA-treated group. Aziza et al (15) Nafees et al (16) reported the protective effect of thyme species from different regions on liver injury. Treatment with different concentrations of thyme essence significantly reduced proinflammatory cytokine levels as well as histological markers of liver function compared with the TAA-treated group. Protective effects of thyme species and its effector components have been reported in the improvement of liver damage (15,16).

Extract of Thyme leaves has been shown to reduce oxidative stress and liver inflammation induced by aflatoxine in Wistar rats (17). Our result indicated treatment with *T.
Thymus as an anti-inflammatory hepato-protective factor

daenensis extract led to significant decrease in TNF-α, IL-6 serum levels compared with the control group, and TAA group (positive control). Our findings also revealed that the protective effects of Thyme leave extracts were dose dependent as 20 mg/kg had the highest liver protective effect. High concentrations of Thyme have been reported to cause cytotoxicity and liver damage through generation of ROS (18). Difference among the results might be due to Thyme species and Thyme formulated types. They used tincture and syrup types of Thyme on carbon tetrachloride-induced acute liver injury in rats. Moreover, pathological findings confirmed the protective effect of T. daenensis extracts on liver tissue. So that the liver injury significantly decreased in T. daenensis extracts groups. The effect was dose-dependent since the 20 mg/dL extract had the most protective effect. In addition, increased oxidative stress could be due to activated neutrophils, macrophages and monocytes that were reported to release various malicious pro-oxidants that might contribute to cellular damage (19). It should be noted that Thyme daenensis extract includes flavonoids which possess antioxidant activity.

Conclusion
Thyme daenensis can improve liver injury in mice by decreasing proinflammatory TNF and IL-6 cytokines.

Acknowledgements
This article was derived from the thesis of Banafsheh Soosani approved at the Islamic Azad University, Shiraz Branch, Shiraz, Iran. Hereby, we gratefully appreciate the spiritual assistance of the Research Deputy of this university and are also grateful to the participants of this study.

Authors’ contributions
BS was the main researcher and performed the experiments. HS supervised the research, revised and copyedited the manuscript. All read and confirmed the final version of the manuscript for publication.

Conflict of interests
The authors declared no competing interests.

Ethical considerations
Ethical issues in research have been completely observed by the authors.

Funding/Support
This research was financially supported by Islamic Azad University, Shiraz Branch, Iran (grant No. 13330509951001).

References
9. Hamzawy MA, E-Denshary ESM, Hassan NS, Manaa F,

Figure 3. Photomicrographs of liver sections in each group. (A) Control, (B) Silibinin, (C) TAA, (D) TAA + Thyme 5 mg/kg, (E) TAA + Thyme 10 mg/kg, (F) TAA + Thyme 20 mg/kg (×400).


