Effect of *Zataria multiflora bosis* Alcoholic Extract on Some Components of Complement System and Serum IgM in Rat

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Abstract

Common thyme with scientific name Thymus vulgaris is one of the Iran indigenous plants that exist widely in southern and central regions of country. This plant has medical applications in traditional medicine including antibacterial and antifungal activity affecting gastrointestinal and chronic diseases. All Thymuses are rich in terms of volatile compounds and mainly consist of thymol and Carvacrol that are considered as strong disinfectants. With regard to valuable properties of common thyme, we decided to investigate effects of Zataria multiflora bosis alcoholic extract on some components of Complement system (C1 inhibitor, CH50, C3, C4) and serum IgM in rat. In this research two treatment groups were fed with Zataria multiflora bosis alcoholic extract at dose of 200 and 400 mg/kg for 30 days and the third group as whiteness did not received no compound. In this study after measuring components of complement system and IgM, CH50 amount in two treatment groups showed a significant increase than the control group (p<0.05). Amount of C1 inhibitor also showed a significant increase in the group 1 than the control group (p<0.05). Also amount of IgM in two treatment groups showed a relative increase than the control group.

Keywords: Traditional medicine, Zataria multiflora bosis, Complement system, serum, rats, IgM

1. Introduction

Zataria multiflora bosis is an aromatic plant with the scientific name Zataria multiflora bosis and of Lamiaceae family that exists in southern, central and mountain regions and has medical applications (Katooli et al., 2012; Arzani et al., 2013). Common thyme extract has had antibacterial effect on Clostridium perfringens bacterium that is of food spoilage factors. All Thymus are rich in terms of volatile compounds and mainly include thymol and carvacrol that are considered as strong disinfectants (Shati and Elsaid, 2009; Hajimehdipoor et al., 2010; Katooli et al., 2012; Arzani et al., 2013). Aerial organs of this plant are disinfectant mucolytics and is applied for chronic chest pain. Also it is useful to treat diarrhea and indigestion (Arzani et al., 2013; Kensara et al., 2013). Thymol has anticoagulant effect and strongly inhibits platelet accumulation and can be used in inhibiting thrombosis and arteriosclerosis (Al-Khalaf, 2013). Infusion of this plant at dose of 10 grams of leaf powder in 1 liter of water is used to treat lung problems and 25 gram/liter of this plant with some honey is used to treat GI tract (Fachini-Queiroz et al., 2012; Al-Khalaf, 2013; Kensara et al., 2013). Consumption of common thyme extract increases secretion of mucus from bronchus (Duke et al., 2002). Also this plant is used to treat bronchus, colic, diabetes, fever, laryngitis, rheumatism, sciatica, spleen disorders, angina, uterine disorders, verruca, runny nose and pertussis (Composition of Foods, 1977). In
Effect of *Zataria multiflora bosis* alcoholic extract

Each 100 grams of plant powder there is 273 calorie, 7.8 grams water, 9.1 grams protein, 7.4 grams fat, 63.9 grams total carbohydrate, 1890 milli calcium, 201mg phosphorus, 220 mg magnesium, 804 mg potassium, 6.2mg zinc, 3800 international unit of vitamin A, 0.51 mg thiamine, 0.4 mg riboflavin (Boshra and Sunyer, 2006). Using *Thymus vulgaris* essence in bathtub raises body temperature and sever inflammation (Fachini-Queiroz et al., 2012; Kensara et al., 2013). Some medicinal plants stimulate nonspecific immune system components including complementary operating system. Complementary operating system is consisted of about 35 individual proteins and one of its most important activities is ability to kill pathogens by providing pore in their membrane surface (Claire et al., 2002).

Activation of the complement factor is composed of three pathways of classic complement pathway (LCP), Alternative complement pathway (ACP) and Lectin complement pathway (Boshra and Sunyer, 2006).

In this research, we investigated effects of *Zataria multiflora bosis* alcoholic extract on some components of complement system and serum IgM in rat.

2. Materials and methods

This study was conducted in winter 2013 in the Islamic Azad University of Shahrekord Branch laboratory. Some components of the complement system (*C3*- *C4*-CH50- *C1*inhibitor) and IgM were measured in Al-Mahdi Medical Diagnostic Laboratories of Shahrekord, Iran. Sample of *Zataria multiflora bosis* multiflora plant leaves were collected and alcoholic extract of the mentioned plant was prepared in the Islamic Azad University of Shahrekord Branch laboratory. Drying and extracting *Zataria multiflora bosis* was prepared from medicinal plant research center. After Cutting extracting was performed by using Alcoholic distillation method by the rotary device (British Pharmacopoeia, 1988).

**Animal**

In this research 30 female white wistar rats prepared from laboratory animals breeding center of Islamic Azad University of Shahrekord Branch laboratory with weight range of 215±15 gr, were maintained in standard cages and had access to food and water. According to ethical code available in university and by considering ethical issues relating animals it was tried to avoid any case including annoyance, unnecessary use of animals or even losses during the testing. Rats were divided into three classes of ten and then extract dose was determined through preliminary experiments in groups 1 and 2 and the control group received no compound (Abdollahi et al., 2003). The group 1 and the group 2 received respectively 200 mg/kg and 400 mg/kg of *Zataria multiflora bosis* alcoholic extract and the control group received no compound. Prescription of extract in groups continued for 30 days and after completion of this period animals were anesthetized intraperitoneally with 100mg/kg of Ketamin hydrochloride and 16 mg/kg of 2% xylazine and blood sample was collected by the Cardiac Puncture Technique (Sumiko et al., 2001). Then its serum was separated by centrifugation.
for 200 rpm and components of complement system and IgM were measured. All experiments were carried out under ethical guidelines of the Islamic Azad University of Shahrekord Branch, for the care and use of laboratory animals.

**Statistical Analysis**
Findings were statistically analyzed by the SPSS software 18.0 (SPSS Inc., Chicago, IL, USA) and significance levels (p<0.05) were compared by means of the Dunnett test.

### 3. Results

Amount of serum C₃, C₄, CH50, C₁ inhibitor and IgM in groups showed in Table 1.

**Table 1.** Below table shows effect of alcoholic extract of *Zataria multiflora bosis* on some components of complement system and serum IgM.

<table>
<thead>
<tr>
<th>Groups</th>
<th><em>Zataria multiflora bosis</em> alcoholic extract was used (SD±Mean)</th>
<th>C₃ (SD±Mean)</th>
<th>C₄ (SD±Mean)</th>
<th>CH50 (SD±Mean)</th>
<th>C₁ inhibitor (SD±Mean)</th>
<th>IgM (SD±Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.61±0.59ᵃ</td>
<td>0.090±0.054ᵃ</td>
<td>5.1±1.3ᵃ</td>
<td>0.054±0.011ᵃ</td>
<td>0.23±0.02ᵃ</td>
</tr>
<tr>
<td>Group 1 200 mg/kg</td>
<td>0.46±0.18ᵃ</td>
<td>0.13±0.076ᵃ</td>
<td>13.9±1.8ᵇ</td>
<td>0.110±0.012ᵇ</td>
<td>0.43±0.13ᵃ</td>
<td></td>
</tr>
<tr>
<td>Group 2 400 mg/kg</td>
<td>0.47±0.20ᵃ</td>
<td>0.084±0.026ᵃ</td>
<td>11.4±3.7ᵇ</td>
<td>0.080±0.012ᵃ</td>
<td>0.43±0.13ᵃ</td>
<td></td>
</tr>
</tbody>
</table>

In each column numbers that have similar letters the difference is not significant (p<0.05).

Amount of serum C₃ in group one was 0.46±0.18 mg/dl that did not show significant differences than control group 0.61±0.59 mg/dl (p>0.05). Amount of serum C3 in group two was 0.47±0.20 mg/dl that shows a relative decreases than control group (p>0.05) (Figure 1).
Effect of *Zataria multiflora bosis* alcoholic extract

Amount of serum C₄ in group one was 0.13± 0.03 mg/dI that did not show significant decrease than control group 0.090± 0.054 mg/dI but shows a relative increase (p>0.05). Amount of serum C₄ in group two was 0.084± 0.026 mg/dI that shows a relative decrease than control group (p>0.05) (Figure 2).

serum CH50 amount in the group 1 was (13.9± 1.8%) that showed a significant increase than the control group (5.1± 1.3%). Amount of serum CH50 in the group 2 was (11.4± 3.7%) that showed a significant increase than the control group (5.1± 1.3) (Figure 3).
Amount of serum C1 inhibitor in the group 2 was (0.080± 0.012 gr/I) that did not show a significant difference than the control group (0.054± 0.011 gr/I) but showed a significant increase than the control group. Amount of C1 inhibitor in the group one was (0.110± 0.012gr/L) that showed a significant increase than the control group (0.054± 0.011gr/L) (Figure 4).

Amount of serum IgM in group one was 0.32± 0.19 gr/L that did not show significant decrease than control group 0.23± 0.16 gr/L amount of serum IgM in group two was 0.43± 0.13 that didn’t show significant difference to control group but shows a relative increase (p>0.05) (Figure 5).
Common thyme is consisted of various compounds like Alpha-Pinene, thymol, cis sabinene, hydrate, paracymene, myrcene and sabinene. It seems that presence of phenolic compounds like thymol and carvacrol as main compounds of essence can be effective in obtaining antifungal and anti-aspergillus (Fakoor et al., 2007). Nobakht and Mehnannavaz (2010) observed minimum percent of heterophils and maximum percent of lymphocytes in experimental treatment containing equal proportions of thyme, Mentha and Mentha pulegium (Nobakht and Mehnannavaz, 2010). In this research effect of Zataria multiflora bosis alcoholic extract at doses of 200 and 400 mg/kg in two treatment group on the complement system and IgM were discussed that extract of this plant and it was shown that it has not any effect on C3 and C4 amount of complement system in treatment groups than whiteness group. In Lee et al., (2003) research it was suggested that using Zataria multiflora plant cannot change Newcastle, influenza, Gumboro and bronchitis diseases significantly and influences on immune system of broilers (Lee et al., 2003). In Fani Makki et al., (2013) consumption of thyme and seed of Silybum marianum plant as combined in poultry ration provided a significant increase in number of heterophils in broilers serum compared to whiteness treatment but in this regard consumption of treatments containing Silybum marianum and thyme did not provide significant change in number of heterophils in broilers serum (Fani makki et al., 2013). In association with effects of medicinal plants on immune level of broilers, using thyme has raises percent of lymphocytes (Toghyani et al., 2011) that indicates useful effects of this plant on immune level of broilers. In research conducted by Bobadilla et al. (2005) they stated that plant compounds like common thyme, that contain hydroxyl, amine, carbohydrates groups or protein, increase amount of complement system factors (Bobadilla et al.,

**Figure 5.** The effect of Zataria multiflora bosis alcoholic extract level of IgM of rat blood serum.

### 4. Discussion

Common thyme is consisted of various compounds like Alpha-Pinene, thymol, cis sabinene, hydrate, paracymene, myrcene and sabinene. It seems that presence of phenolic compounds like thymol and carvacrol as main compounds of essence can be effective in obtaining antifungal and anti-aspergillus (Fakoor et al., 2007). Nobakht and Mehnannavaz (2010) observed minimum percent of heterophils and maximum percent of lymphocytes in experimental treatment containing equal proportions of thyme, Mentha and Mentha pulegium (Nobakht and Mehnannavaz, 2010). In this research effect of Zataria multiflora bosis alcoholic extract at doses of 200 and 400 mg/kg in two treatment group on the complement system and IgM were discussed that extract of this plant and it was shown that it has not any effect on C3 and C4 amount of complement system in treatment groups than whiteness group. In Lee et al., (2003) research it was suggested that using Zataria multiflora plant cannot change Newcastle, influenza, Gumboro and bronchitis diseases significantly and influences on immune system of broilers (Lee et al., 2003). In Fani Makki et al., (2013) consumption of thyme and seed of Silybum marianum plant as combined in poultry ration provided a significant increase in number of heterophils in broilers serum compared to whiteness treatment but in this regard consumption of treatments containing Silybum marianum and thyme did not provide significant change in number of heterophils in broilers serum (Fani makki et al., 2013). In association with effects of medicinal plants on immune level of broilers, using thyme has raises percent of lymphocytes (Toghyani et al., 2011) that indicates useful effects of this plant on immune level of broilers. In research conducted by Bobadilla et al. (2005) they stated that plant compounds like common thyme, that contain hydroxyl, amine, carbohydrates groups or protein, increase amount of complement system factors (Bobadilla et al.,
In Soltani et al. (2013) research compounds available in Zataria multiflora bosis had affect on amount of fish serum proteins that has increased activity level of alternative complement pathway and of course this increase has occurred in certain period. In fact Zataria multiflora bosis essence has shown positive effect and has increased complement factor (Soltani et al., 2013). In present research also Zataria multiflora bosis extract in both treatment groups provided significant increase in serum CH50 amount compared to the control group and also 200mg/kg of extract created significant increase in C1 inhibitor amount in group 1 than the control group (p< 0.05). Also IgM amount in both treatment groups than the control group sowed a relative increase. Therefore, using Zataria multiflora bosis can stimulates and increases some immune factors including complement factor.

References


Effect of Zataria multiflora bosis alcoholic extract


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