Hepatotoxicity of *Teucrium polium* L tea: supporting evidence in mice models

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**Purpose:** The purpose of this study was to assess the possible hepatotoxic effect of *Teucrium polium* (T. polium) lyophilisate in mice.

**Materials and methods:** Inbred BALB/c adult male mice (20-25 g) were used in this study. T. polium lyophilisate was administered orally to male mice. Serum alanine aminotransferase (ALT), gamma glutamyle transferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (AP), cholesterol, triglycerides and bilirubin were determined to assess hepatotoxicity. Mice liver tissues were obtained and processed histopathologically in order to evaluate possible liver lesions.

**Results:** Acute lyophilisate preparations exhibited a significant increase in serum enzymes: ALT, LDH, ALP and GGT. Meanwhile the chronic administration of doses of 0.1 and 0.2 g/kg of lyophilisate given orally for 30 consecutive days resulted in a significant increase in serum: LDH and ALP. Meanwhile, mice receiving different doses of lyophilisate exhibited lobular hepatitis, infiltration of lymphocytes, coagulation necrosis and fatty changes in the liver.

**Key words:** *Teucrium polium*, hepatitis, lyophilisate, liver function enzymes

**Introduction**  
*Teucrium polium* L (Ja’adeh in Arabic) is a dwarf shrub plant which grows wild in Mediterranean countries (Oran 1998). In folk medicine the tea preparation of the aerial parts of the plant is used for the treatment of abdominal colic, headache, diabetes and as an astringent (Al-Khalil 1995). In experimental animal models the aqueous extract of the plant exhibited antispasmodic, anorexic, antidiabetic and hypolipidemic effects (Garaibeh 1989, Rasekh 2001). Most of these effects have been related to the volatile oil, the flavonoids and the terpenoids coponents of *T. polium* (Malkove 1988, Rizk 1986).

The French Department of Health in 1992 banned the sale of all medicinal preparations containing germander (*T. chamaedrys*) following an outbreak of hepatotoxicity in 27 cases with one death from acute, non viral hepatitis (Castot 1992, Zhou 2004). In 1996 the Italian Health Authority, based on the French decisions, prohibited the sale of herbal preparations containing *T. chamaedrys* alone or in association with other medicinal preparations (Bosisio et al., 2004). Still several cases of germander hepatitis were reported in Canada (De Smet 1997) and Spain (Al Varez 2001). Several reports linked the consumption of *T. polium* with hepatitis in man (Mattie 1995, Mazokopakis 2004, Starakis 2006). *T. polium* is consumed by many Jordanians and other people in Mediterranean countries for the treatment of several ailments, and since there is no detailed information on the liver status after the consumption of the plant tea, the objectives of this work were to assess the acute and chronic effects of *T. polium* tea in the liver of adult male mice as model.

**Materials and methods**

**Animals**

Inbred BALB/c adult male mice (20-25 g) were obtained from the animal house of Yarmook University, Irbid, Jordan. Mice were kept for one week for acclimatisation before being used in the experiments. The mice were provided with food water and ad libitum and maintained under laboratory enviromental conditions (light, temperature and humidity). Mice were housed in transparent plastic cages with stainless steel cover lids. Animals were treated in this work in accordance with internationally accepted principles for laboratory animal use and care as found in the European Comunity guidelines.

**Plant**

The flowering aerial parts of *T. polium* were collected from the hills of Al-Ardah (25 km north west of Amman) during the first week of May 2007. The plant was authenticated by Professor Barakat Abu-Imaileh, of the Faculty of Agriculture, University of Jordan. The plant material was rinsed from dust by tap water and dried under shade at room temperature for one week. The dried plant material was ground into fine powder using electric grinders and was used as such in the subsequent experiments.
Preparation of *T. polium* tea

In this method *T. polium* tea was prepared to mimic the human way of preparation. Lyophilisation of tea was carried out according to Loeper et al (1994). Fifty grams of the powdered aerial part of the plant was placed in one litre of distilled water. The mixture sonicated for 30 mins, then boiled for 10 mins and left to infuse. The tea was filtered, the filtrate was frozen and then connected to a lyophiliser machine until dry. The yield of lyophilisate was 10% of the starting plant powder. The lyophilisation steps were repeated several times to obtain enough material to be used in all experiments. The lyophilised powder was kept in a refrigerator (– 20˚c) until used.

Determination of the lethal toxicity

The experiment for determining the LD50 of the lyophilisate was done on adult male BALB/c mice, weighing 20-25 g. Mice were divided into seven groups of six mice each. Animals were fasted overnight but allowed free access to water prior to treatment. Aqueous lyophilisate of *T. polium* of different doses i.e. 0.4, 0.75, 1.5, 2.0, 3.0 and 4.0 g/kg body weight were administered orally to mice by gavage. The proper dose of lyophilisate was dissolved in 0.5 mL distilled water. The control group received 0.5 mL of distilled water. Mortality was recorded daily and the LD50 value was estimated by computerised techniques. The live mice were kept under observation for 7 days. Blood samples were collected and centrifuged to obtain serum. Liver, kidneys and spleen were obtained, weighed and placed in formalin for histological evaluation. Some mice treated orally with a single dose of 0.75 and 1.5 g/kg, were sacrificed 24 hr post dosing for toxicological evaluation.

Chronic toxicity study

In this experiment mice were divided into four groups of 10 animals each. The aqueous lyophilisate of *T. polium* at different dose levels: 0.1, 0.2 and 0.75 g/kg body weight, were administered daily for 30 consecutive days. Mice were sacrificed by decapitation under anesthesia 24 hr after the last treatment. Blood samples were collected and serum samples were obtained by centrifugation. Liver, kidneys and spleen were obtained, weighed and placed in formalin for histopathological evaluation.

Measurement of enzymes

The collected serum samples obtained from acute and chronic toxicity experiments were assayed for the following enzymes: ALT, GGT, LDH and ALP. Cholesterol and triglycerides were also evaluated using the commercially available Sigma Kit (DiaSys) in Al-Bashir analytical laboratory in Sahab, Amman, Jordan.

Liver histopathology

Livers were fixed in buffered formalin. Paraffin sections were made and stained with hematoxylin and eosin. Microscopic evaluation was carried out in the laboratory of Pathology Department, Faculty of Medicine, University of Jordan.

Statistical analysis

Data was presented as means ± SD. Variability of organ weight (liver, kidneys and spleen) was recorded as percentages to overcome the differences in the study. The results were recorded and analysed statistically. The differences between body weight before and after treatment were determined using paired T- test. The differences between the results of the treated and control groups were analysed using unpaired T-test. The results were considered significant when p-value was equal to ≤ than 0.05.

Results

Lethal toxicity of *T. polium*

The estimation of 24 h LD50 value of *T. polium* tea, using computerised technique was 3.15 g/kg of body weight. Mice who survived treatment were apparently healthy.

Chronic toxicity of *T. polium* lyophilisate

Body and some organ tissues weight

Table 1 shows the effect of chronic treatment of *T. polium* lyophilisate on mice body weight. Generally there was a significant increase in body weight of all mice groups (control, 0.1 and 0.2 g/kg) however the gain in body weight of lyophilisate treated mice was less than the control group. Table 1 shows a significant increase in liver weight of treated mice compared with controls but no such changes in the weight of kidneys and spleen.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>24.2 ± 1.03</td>
<td>30.05 ± 1.82 *</td>
<td>7.70 ± 0.85</td>
<td>1.8 ± 0.16</td>
</tr>
<tr>
<td>Lyophilisate (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>24.2 ± 1.81</td>
<td>26.4 ± 2.79 *</td>
<td>5.54 ± 1.36*</td>
<td>1.69 ± 0.37</td>
</tr>
<tr>
<td>0.2</td>
<td>4.4 ± 1.17</td>
<td>26.65 ± 1.94*</td>
<td>6.11 ± 0.99*</td>
<td>1.69 ± 0.28</td>
</tr>
</tbody>
</table>

* P ≤ 0.05
Table 2
The effect of chronic administration of *T. polium* lyophilisate on the serum level enzymes, cholesterol and triglycerides

Values are mean ± SD of 10 mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT (U/L)</th>
<th>LDH (U/L)</th>
<th>ALP (U/L)</th>
<th>Cholesterol (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute experiment</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>54.9 ± 19.7</td>
<td>1193 ± 193</td>
<td>323 ± 44.3</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Lyophilisate (g/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0.75</td>
<td>64.3 ± 23.9</td>
<td>1123 ± 255</td>
<td>293 ± 70.6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1.5</td>
<td>3474 ± 1313*</td>
<td>1865 ± 728*</td>
<td>552 ± 62*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Chronic experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>51.4 ± 10.65</td>
<td>1236.7 ± 96.69</td>
<td>280.6 ± 41.1</td>
<td>125 ± 16</td>
<td>137 ± 27.5</td>
</tr>
<tr>
<td>Lyophilisate (g/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>45.2 ± 12.1</td>
<td>1901 ± 573*</td>
<td>226 ± 59.5*</td>
<td>104 ± 43.6</td>
<td>111 ± 30.7</td>
</tr>
<tr>
<td>0.2</td>
<td>69.2 ± 0.09</td>
<td>1904 ± 436*</td>
<td>218.6 ± 5702*</td>
<td>105 ± 38</td>
<td>89.8 ± 25.4*</td>
</tr>
</tbody>
</table>

ND = not determined  
* P ≤ 0.05  
U/L = international unit per litre

Serum enzymes
The higher doses of tea of *T. polium* such as 1.5 g/kg, exhibited a significant increase in plasma level of ALT, LDH, ALP and GGT compared with controls. Conversely the chronic administration of 0.1 or 0.2 mg/kg of lyophilisate tea exhibited a significant increase in LDH and ALP values (p<0.05) compared with controls (Table 2), while the same doses resulted in a decrease in cholesterol and triglyceride values (Table 2).

Histopathology of liver tissues
The light microscopic examination of liver tissues of mice treated acutely as control or with 0.75 and 1.5 g/kg of lyophilisate exhibited several pathological changes in the liver such as coagulation necrosis, fatty changes and lobar hepatitis with infiltration of lymphocytes. Such changes were not seen in control mice liver tissue. (Figures 2, and 3). Meanwhile, the microscopic evaluation of mice treated chronically as control (Figure 4) with 0.1, or 0.75 g/kg of lyophilisate exhibited almost same liver lesions as above beside mild lobar hepatitis and infiltration of lymphocytes (Figures 5 and 6) again such hepatic changes were not seen in the control.

Editor’s note: Colour microscopic figures available by emailing ajmh@nhaa.org.au.

Discussion
In this study the LD50 value of lyophilisate in mice was 3.15 g/kg body weight. As far as we know this is the first study to characterise the acute and chronic toxicity of the herb in mice. Taking into consideration the published cases of patients who alleged to have consumed *T. polium* tea for different medical ailments (Starakis 2006, Mazokopakis 2004, Mattei 1995), we decided to conduct this work using mice as a model with two clear objectives in mind. Firstly to evaluate the level of liver enzymes in plasma and secondly to evaluate histopathologically the liver lesions following mice exposure to the herbal tea.

The clinical pictures of most human cases presented with hepatitis reported in the literature exhibited elevation in plasma levels of liver enzymes such as LDH, GGT and ALP. The liver lesions of those cases were similar to our animal model. For example Mattei et al (1995) reported a massive hepatocyte necrosis predominantly in the centrilobular areas of the liver in a patient with acute liver failure after consumption of *T. polium*. In our animal model and in the diabetic rats treated with *T. polium* tea, similar hepatic lesions were obtained (Zal 2001).

In conclusion this study reminds us again that traditional medicines can be hepatotoxic.

Acknowledgment
This research was fully financed by the Deanship of research at the University of Jordan, Amman Jordan.

References
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