Hepatoprotective activity of *Thymus vulgaris* extract against *Toxoplasma gondii* infection

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**ABSTRACT**

**Objective:** To evaluate the hepatoprotective activity of *Thymus vulgaris* (*T. vulgaris*) extract against *Toxoplasma gondii* (*T. gondii*) infection in experimentally infected mice.

**Methods:** Sixty mice were divided into six groups (Group I–Group VI). Group I was normal control (non-infected, non-treated); Group II was non-infected and treated with *T. vulgaris* extract (500 mg/kg); Group III was *T. gondii* infected-non-immunosuppressed control; Group IV consisted of infected immunosuppressed mice; Group V was infected and treated with *T. vulgaris* extract; Group VI consisted of infected immunosuppressed mice treated with *T. vulgaris* extract. Hepatoprotective effect of *T. vulgaris* extract was evaluated by histopathological examination of tissue sections stained with hematoxylin and eosin, determination of liver function parameters (alanine aminotransaminase, aspartate aminotransaminase, alkaline phosphates, total bilirubin, total protein concentrations) and assessment of hepatocytes genotoxicity by comet assay. Antigenotoxic effect of *T. vulgaris* was assessed by several comet assay parameters that were provided by the image analysis software, including % tailed cells, % of DNA in the tail, tail length, and tail moment.

**Results:** Treatment with *T. vulgaris* in both Groups V and VI improved *T. gondii* induced pathological lesions in the infected liver that regressed to near the normal picture especially in Group V. Also, it restored the altered values of liver function parameters near to the normal levels significantly (*P* < 0.05) compared with Groups III and IV respectively. Regarding comet assay parameters, all of them were significantly increased (*P* < 0.05) after *T. gondii* infection (Group III) and reached the greatest values in infected immunosuppressed group (Group IV) compared to the normal controls (Group I). With treatment by *T. vulgaris* in Groups V and VI, there was a significant decrease (*P* < 0.05) in all values compared to Groups III and V respectively. The results indicated that *T. vulgaris* reduced DNA damage induced by *T. gondii* infection in liver cells.

**Conclusions:** *T. vulgaris* ethanol extract exhibited notable hepatoprotective activity against *T. gondii* infection.

1. Introduction

Liver is frequently affected by parasitic infections. The parasites may either inhabit this organ or pass through it during their normal development. Mechanism of liver tissue damage is either due to the direct effect of the parasite on the tissues or related to the excessive immunological response to the parasite(1). *Toxoplasma gondii* (*T. gondii*) is an obligate intracellular protozoan parasite, and is capable of infecting almost all the internal organs and tissues of the mammalian host. In the host cells, *T. gondii* causes DNA damage and rapid cell death with rupture and release of the organisms and soluble antigens that cause many pathological changes ranging from mild congestion to severe degeneration within the affected organs(2,3). Within the liver, it causes pathological changes that progress to hepatomegaly, granuloma, hepatitis, and necrosis(4,5). The association between *T. gondii* infection and chronic liver diseases and abnormal liver function has been confirmed by several researchers(6-8).

Many medicinal plants exhibit anti-*Toxoplasma* activity, including *Zingiber officinale*, *Nigella sativa*, *Piper nigrum*, myrrh, *Azadirachta indica*, *Curcuma longa*, and *Melia azedarach*. These plants have a beneficial effect in prophylaxis as well as treatment of both acute and chronic toxoplasmosis through being safer, acceptable and available at low cost(9). Also, some scientific reports stated that certain medicinal plants have protective effect on the liver as they contain a variety of chemical constituents like flavonoids, phenols, triterpenoids, coumarins, lignans, essential
oil, monoterpens, carotenoids, glycosides, organic acids, lipids, alkaloids, xanthenes and steroids[10,11]. The mechanism of hepatoprotection by these compounds is by exerting antioxidant, immunomodulatory and anti-inflammatory effects[10].

*Thymus vulgaris* (*T. vulgaris*), a well-known medicinal plant, possesses diverse activities including anti-inflammatory and antioxidant properties[12,13]. *T. vulgaris* and its major ingredients (thymol and carvacrol) were expected to exhibit a DNA-protective effect on DNA lesions induced by a strong oxidant (hydrogen peroxide) on mammalian cells cultured *in vitro*[14]. Additionally, it was found that *T. vulgaris* exhibited antiprotazooal activity against *Trypanosoma cruzi*, *Entamoeba histolytica*, and *Blastocystis hominis*[15-17]. Therefore, this study aimed to evaluate the hepatoprotective activity of *T. vulgaris* extract against *T. gondii* infection in experimentally infected mice.

2. Materials and methods

2.1. *T. vulgaris* extract preparation

*T. vulgaris* leaves were brought from the International Company (Cairo, Egypt), identified and recorded as a reference in Medicinal and Aromatic Plants Department, Horticulture Research Institute, Egypt. The leaves were dried and ground into fine powder. A total of 100 g of this powder was added to half liter of ethanol (95%) and left in a conical flask at 25 °C for three days with repeated shaking. The mixture was filtered through a filter paper (Whatman No. 1), and then the extract was concentrated by using a rotary evaporator (Sigma-Aldrich, USA). The residues were dissolved in Tween-20 (10%) to obtain a concentration of 100 mg/mL[17].

Preliminary experiment was carried out with successive doses (ranged from 100 to 500 mg/kg) for testing the acute toxicity according to the Organization for Economic Cooperation and Development (OECD) guideline 423[18]. A dose of 500 mg/kg daily for 10 days was selected for the oral administration of this extract as it showed neither death, nor other behavioral or toxicological changes in all tested mice.

2.2. Experimental animals

Sixty laboratory-bred male Swiss albino mice, 12 weeks old and weighing 35–40 g, were selected. They were fed with a balanced standard diet, and maintained under controlled environment with an average temperature of (25 ± 2) °C and standard cycle of light and dark through the experiment. The experiment was carried out in the animal house of the Research Institute of Ophthalmology, Giza, Egypt.

2.3. Infectious agent

Me49 non-virulent strain of *T. gondii* was used to infect mice in this study. It was obtained from the brains of the previously infected mice eight weeks prior. The mice brains were ground and diluted, and brain cysts suspension was obtained. Using the haemocytometer, the number of Toxoplasma cysts was adjusted to be 1 × 10⁶ cysts/mL in this brain suspension[3]. For infection, 0.1 mL of the brain cysts suspension was injected intraperitoneally to each mouse. All infected mice were tested positive for *T. gondii* IgG antibodies on Day 21 post-infection using commercial mouse anti-toxoplasmosis antibody (IgG) ELISA kit (MyBioSource, Inc. California, USA) according to the instructions of the manufacturer.

2.4. Experimental design

Mice were divided into six groups (Groups I–VI) of 10 mice/group. Group I was non-infected, non-treated (normal control) and received 0.1 mL of sterile distilled water; Group II was non-infected and received *T. vulgaris* extract daily for 10 days; Group III was *T. gondii* infected-non-immunosuppressed control; Group IV consisted of *T. gondii* infected-immunosuppressed mice injected subcutaneously with methylprednisolone acetate (Depomedrol®, Pfizer Inc.) 40 mg/day/mouse for five successive days one month after infection[3]. Six weeks after infection, both Group V (infected and non-immunosuppressed) and Group VI (infected-immunosuppressed) were treated by *T. vulgaris* extract daily for 10 days.

Eight weeks after infection, the blood was obtained from the mouse’s orbital venous plexus under ether anesthesia. The sera were separated by centrifugation at 3 000 x g for 10–15 min and stored at –20 °C for the determination of liver function parameters. Then, all mice were sacrificed and their livers were obtained. Each liver was divided into two parts; one part was used for assessment of hepatocytes DNA damage by single-cell gel electrophoresis (comet assay), and the other part was used for the histopathological evaluation.

2.5. Evaluation of hepatoprotective activity of *T. vulgaris* extract against *T. gondii* infection

2.5.1. Histopathological examination

Liver samples were washed in 0.9% sodium chloride solution and fixed in 10% formalin. Then, fixed liver tissues were dehydrated and embedded in paraffin blocks. Tissue sections of 5 μm thickness were stained with hematoxylin and eosin (H&E) and examined microscopically at magnifications of 100×, 400× and 1000×.

2.5.2. Determination of liver function parameters

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin and total protein were estimated by using Sigma diagnostic kits (Sigma Chemical Co., St. Louis, USA) following the manufacturer’s instructions.

2.5.3. Assessment of hepatocyte cell’s genotoxicity by comet assay

Crushed liver samples (0.5 g) were placed in 1 mL ice-cold PBS (pH 7.9), stirred for 5 min and filtered. Then 100 μL of each cell suspension were mixed with 600 μL of low melting agarose (0.8% in PBS). Then 100 μL from the mixture were pipetted onto the slides, and the slides were flooded by lysis buffer which consisted of 0.045 mol/L Tris-borate ethylenediaminetetraacetic acid (TBE, pH 8.4) and 2.5% sodium dodecyl sulfate (SDS) for 15 min. After that, the slides were transferred into an electrophoresis chamber containing TBE buffer only. The electrophoresis was conducted at 2 V/cm for 2 min and 100 mA. Finally, they were stained with ethidium bromide 20 μg/mL at 4 °C and the presence of comets was examined at 40× magnification using a fluorescence microscope [with excitation filter 420–490 nm (issue 510 nm)]. All chemicals were obtained from Sigma Chemical Co., USA.

The Comet 5 image analysis software (Kinetic Imaging, Ltd. Liverpool, UK) linked to a charge-coupled device camera was used to determine the quantitative and qualitative extent of DNA damage in the liver cells by measuring the length of DNA migration, migrated DNA percentage and tail moment through the observation of fifty to hundred cells per sample[19]. Tailed cells indicated by the ratio of the number of comet tails and the number of non-head shapes to the number of total cells. The percentage of tail DNA was calculated from the fraction of DNA in the tail divided by the amount of DNA in the nucleus multiplied by 100. The tail length was measured from the middle of the nucleus to the end of the tail.
The tail moment was calculated by multiplying the tail length and % of DNA in the tail[20].

2.6. Statistical analysis

The statistical analysis was performed by using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Data were represented as mean ± SD. The significant differences between the experimental groups were estimated by ANOVA followed by the student’s t-test. Probability (P value) less than 0.05 was considered significant.

2.7. Ethical considerations

All experimental procedures involving animals were conducted in accordance to EU Directive on the Protection of Animals Used for Scientific Purposes (2010/63/EU) and approved by Research Committee, Research Institute of Ophthalmology, Giza- Egypt.

3. Results

The study has proven that daily administration of T. vulgaris by the oral route at a dose of 500 mg/kg for 10 days did not cause any mortality or any observable toxic effects in the mice of Group II. Mice were alert with no alteration of their behavioral pattern, any gastrointestinal tract disorder or respiratory distress. From the histopathological observations, there were not any observable changes in the livers at the giving dose compared to the normal controls (Figure 1).

3.1. Histopathological results

Macroscopically, the liver of T. gondii infected group showed a mild degree of enlargement and focally extensive necrosis. However, in T. vulgaris treated mice, the livers appeared healthy. Microscopically, the livers of T. gondii-infected mice (Group III) showed focal areas of necrosis with a mild degree of inflammatory cellular infiltrates; mainly lymphocytes that were very obvious in the portal area and Toxoplasma cysts were observed between the hepatocytes (Figure 2). Conversely, in the infected immunosuppressed group (Group IV), the histopathological features of liver progressed from moderate to severe, where there was a dissociation of hepatic cords pattern with generalized necrosis of the hepatocytes. Also, there was marked dilatation and congestion of the hepatic portal blood vessels. It was observed that some of Toxoplasma cysts ruptured, releasing tachyzoites to the sinusoids and invading the other hepatocytes and Kupffer cells. Also, there was Kupffer cell hyperplasia, and proliferation of epithelial lining bile duct associated with chronic cholangitis (Figure 3).

Figure 1. Liver sections from mice of Group I (A) and Group II (B) showing normal histological structure of the hepatic lobules (H&E, 400×).

Figure 2. Liver sections from mice of Group III showing focal hepatic necrosis associated with mononuclear inflammatory cells infiltration (A); Toxoplasma cysts between the hepatocytes (B) and portal infiltration with inflammatory cells (C) (H&E, 400×).

Figure 3. Liver sections from mice of Group III stained with H&E showing large focal coagulative necrosis of hepatocytes associated with inflammatory cells infiltration (A) (100×); dissociation of hepatic cords pattern with generalized necrosis of hepatocytes and sinusoidal leucocytosis (B) (400×); necrosis, vacuolation and dissociation of hepatocytes associated with mononuclear inflammatory cells infiltration and T. gondii tachyzoites distributed throughout the liver tissues (C) (400×); marked dilatation and congestion of hepatic portal blood vessel (D) (400×); portal infiltration with massive inflammatory infiltrate (E) (400×); Kupffer cell hyperplasia and T. gondii tachyzoites invading the cells and lying on the endothelial surface of the sinusoidal capillaries (F) (400×); hyperplasia and proliferation of epithelial lining bile duct associated with chronic cholangitis (G) (100×).
These marked histological changes regressed to near the normal picture after treatment with *T. vulgaris* extract; liver tissues of Group V appeared healthy with small foci of inflammatory reaction and Group VI showed hydropic degeneration of hepatocytes and focal hepatic necrosis associated with inflammatory cells infiltration (Figure 4).

**Figure 4.** Liver sections from the mice treated with *T. vulgaris* extract

A: Section from Group V appeared healthy with small foci of inflammatory reaction; B: Section from Group VI showed hydropic degeneration of hepatocytes and focal hepatic necrosis associated with inflammatory cells infiltration (H&E, 400×).

### 3.2. Effect of *T. vulgaris* treatment on liver function parameters

It was found that *T. gondii* infected mice (Group III) and infected immunosuppressed mice (Group IV) showed a significant elevation in ALT, AST, ALP, total bilirubin and reduction in total protein concentration. However, treatment with *T. vulgaris* in Groups V and VI reduced the levels of ALT, AST, ALP, total bilirubin and increased total protein concentration significantly (*P* < 0.05) compared with Groups III and V respectively as shown in Table 1.

### 3.3. Antigenotoxic effect of *T. vulgaris*

*T. gondii* infection induced a statistically significant increase in the tailed nuclei (DNA damage) in mice liver cells (Group III) compared to the normal control group (Group I) which showed some degree of DNA damage. In infected immunosuppressed group (Group IV), the frequency of tailed nuclei in liver cells increased in comparison to both control (Group I) and infected group (Group III). After treatment with *T. vulgaris* in both Groups V and VI, DNA damage in liver cells decreased comparing with Groups III and IV respectively (Figure 5). Also, antigenotoxic effect of *T. vulgaris* was assessed by various comet assay parameters including % tailed cells, % of DNA in the tail, tail length, and tail moment. All these parameters were significantly increased (*P* < 0.05) after *T. gondii* infection (Group III) and reached the greatest values in infected immunosuppressed group (Group IV) compared to the controls (Group I). After treatment with *T. vulgaris* in Groups V and VI, there was significant decrease (*P* < 0.05) in all values compared to Groups III and IV respectively (Table 2). These results indicated that *T. vulgaris* reduced the degree of damage induced by *T. gondii* infection.

**Table 1**

<table>
<thead>
<tr>
<th>Experimental Groups (n = 10)</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Total bilirubin (mg/dL)</th>
<th>Total protein (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (non-infected, non-treated)</td>
<td>23.30 ± 5.61</td>
<td>37.80 ± 41.15</td>
<td>95.20 ± 5.19</td>
<td>0.80 ± 0.12</td>
<td>6.69 ± 0.77</td>
</tr>
<tr>
<td>Group II (non-infected + <em>T. vulgaris</em>)</td>
<td>21.90 ± 3.16</td>
<td>35.80 ± 10.55</td>
<td>91.40 ± 9.12</td>
<td>0.78 ± 0.52</td>
<td>7.01 ± 0.15</td>
</tr>
<tr>
<td>Group III (infected-non-immunosuppressed)</td>
<td>51.30 ± 4.42</td>
<td>67.40 ± 14.36</td>
<td>188.90 ± 32.12</td>
<td>2.30 ± 0.10</td>
<td>4.00 ± 0.10</td>
</tr>
<tr>
<td>Group IV (infected-immunosuppressed)</td>
<td>66.80 ± 21.26</td>
<td>88.30 ± 17.25</td>
<td>229.60 ± 34.71</td>
<td>4.10 ± 0.16</td>
<td>3.38 ± 0.05</td>
</tr>
<tr>
<td>Group V (infected-non-immunosuppressed + <em>T. vulgaris</em>)</td>
<td>36.90 ± 6.18</td>
<td>41.80 ± 7.22</td>
<td>119.50 ± 71.19</td>
<td>1.96 ± 0.12</td>
<td>5.99 ± 0.68</td>
</tr>
<tr>
<td>Group VI (infected-immunosuppressed + <em>T. vulgaris</em>)</td>
<td>43.10 ± 7.33</td>
<td>44.90 ± 9.32</td>
<td>159.50 ± 71.19</td>
<td>2.68 ± 0.60</td>
<td>4.40 ± 0.50</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SD; *: No significant difference compared with Group I; #: Significant difference compared with Group I; %: Significant difference compared with Group III; ^: Significant difference compared with Group IV.

### 4. Discussion

In this study, the hepatoprotective activity of *T. vulgaris* against

**Table 2**

<table>
<thead>
<tr>
<th>Experimental Groups (n = 10)</th>
<th>% Tailed cells</th>
<th>% Tail DNA</th>
<th>Tail length (µm)</th>
<th>Tail moment (Unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (non-infected, non-treated)</td>
<td>3.6 ± 0.9</td>
<td>1.57 ± 0.30</td>
<td>1.44 ± 0.05</td>
<td>2.26 ± 0.40</td>
</tr>
<tr>
<td>Group II (non-infected + <em>T. vulgaris</em>)</td>
<td>3.1 ± 0.9</td>
<td>1.44 ± 0.50</td>
<td>1.38 ± 0.10</td>
<td>2.17 ± 0.20</td>
</tr>
<tr>
<td>Group III (infected-non-immunosuppressed)</td>
<td>12.3 ± 1.9</td>
<td>4.85 ± 0.30</td>
<td>4.51 ± 0.20</td>
<td>21.87 ± 1.00</td>
</tr>
<tr>
<td>Group IV (infected-immunosuppressed)</td>
<td>22.0 ± 1.6</td>
<td>6.46 ± 0.40</td>
<td>8.13 ± 0.60</td>
<td>52.52 ± 5.00</td>
</tr>
<tr>
<td>Group V (infected-non-immunosuppressed + <em>T. vulgaris</em>)</td>
<td>5.2 ± 1.7</td>
<td>1.82 ± 0.10</td>
<td>1.59 ± 0.30</td>
<td>2.89 ± 0.40</td>
</tr>
<tr>
<td>Group VI (infected-immunosuppressed + <em>T. vulgaris</em>)</td>
<td>10.2 ± 1.3</td>
<td>4.21 ± 0.20</td>
<td>3.79 ± 0.20</td>
<td>15.95 ± 1.00</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SD; *: No significant difference compared with Group I; #: Significant difference compared with Group I; %: Significant difference compared with Group III; ^: Significant difference compared with Group IV.
**Conflict of interest statement**

We declare that we have no conflict of interest.

**References**


[2] Şamdancı-Türkmen E, Taylan-Özkan A, Babür C, Mungan M, Aydin...