The Effects of Diethylether Extract of Helichrysum plicatum Dc. Subsp. Plicatum and tanacetum balsamita L. Subsp. Balsamitoides (Sch. Bip.) Grierson (Asteraceae) on the Acute Liver Toxicity in Rats

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Abstract: The aim of this study was to investigate the effects of the diethylether extract of Helichrysum plicatum DC. subsp. plicatum (HP) and Tanacetum balsamita L. subsp. balsamitoides (Sch. Bip.) Grierson (Asteraceae) (TB) in carbontetracloride (CCL4)-induced acute liver toxicity in rats. Acute liver toxicity was induced by injecting CCl4 (0.8 mL kg-1) intraperitoneally for 7 days. The control group received isotonic saline only. The reference group received 50 mg kg-1 silibinin. TB and HP extract was injected in doses of 25, 50 and 100 mg kg-1. Body weights were measured daily during the experiment. On the 8th day of the experiments blood and liver samples were collected. Serum Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were measured in serum samples. Tissue samples were evaluated histopathologically. Some of the rats in TB and HP groups died during the experiments. Serum ALT levels were higher in the TB and HP groups than those in the CCL4 group. Histopathological findings were similar in the CCL4, TB and HP groups. The body weight loss was more in the TB and HP groups compared to that of the CCL4 group. It is concluded that the diethylether extract of Helichrysum plicatum DC. subsp. plicatum and Tanacetum balsamita L. subsp. balsamitoides (Sch. Bip.) Grierson (Asteraceae) did not have a protective effect in carbontetracloride (CCL4)-induced acute liver toxicity in rats and even exacerbated the toxicity.

Keywords: Tanacetum balsamita L. subsp. balsamitoides (Sch. Bip.) Grierson, Helichrysum plicatum DC. subsp. Plicatum, Asteraceae, hepatoprotective effect, rat

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INTRODUCTION

*Tanacetum balsamita* L. subsp. *balsamitoides* (Sch. Bip.) Grierson (Asteraceae) (Eng. costmary), grown widely in the Northern Anatolian region, is a perennial herbal plant that can grow up to 80 cm. It is called subsp. *balsamitoides* if it has tongue shaped white flowers on its capitulum and subsp. *balsamita* if not. Its branches with flowers are widely used as folk remedy in Turkish folk medicine as diuretic, lithagogue, anti bloating, appetizer, aphrodisiac, vermifuge, emmenagogue and for migraine (Baytop, 1999; Cubukcu et al., 2002a). There has been no study on the biological effects of *Tanacetum balsamita* although, there have been some studies with the other species of the genus *Tanacetum*. A parthenolide-depleted extract of *Tanacetum parthenium* was shown to protect skin from ultraviolet light (Martin et al., 2008). The chloroform extract of *Tanacetum vulgare* L. had cytotoxic effects on various human cancer cell lines (Ramirez-Erosa et al., 2007) while, acidic polysaccharides isolated from it had immunotherapeutic effects (Xie et al., 2007). Aqueous extracts of *Tanacetum vulgare* L. also had vasorelaxing and strong diuretic effects (Lahlou et al., 2007, 2008). Volatile oil of *Tanacetum argentum* subsp. *Flabellifolium* had antibacterial activity (Tabanca et al., 2007). The crude extract of *Tanacetum artemisoides* showed anti-inflammatory, analgesic and calcium channel blocking effects (Bukhari et al., 2007). Some flavonoids extracted from *Tanacetum microphyllum* inhibited the expression of inducible nitric oxide synthase and cyclooxygenase-2 (Guerra et al., 2006).

*Helichrysum plicatum* DC. subsp. *plicatum* (Asteraceae) (Eng. everlasting flower) is a perennial herbal plant that can grow 10-40 cm and commonly found in Anatolia. Their leaves are flat and pubescent on both sides. The bracts around capitulum are yellow or yellowish white in color. It is used in Turkish folk medicine mainly as diuretic and lithagogue (Gürkan et al., 2003; Cubukcu et al., 2002b; Baytop, 1999). Aqueous and ethanol extracts of *Helichrysum plicatum* sp. were shown to decrease blood sugar in streptozotocin induced diabetes in rats and had antioxidant activity (Aslan et al., 2007) as well as antibacterial effects (Smirnov et al., 1982). Kulevanova et al. (2000) identified flavone aglycones in *Helichrysum plicatum* DC. subsp. *plicatum* using HPLC.

Oztürk et al. (1991) reported hepatoprotective use of both *Helichrysum plicatum* L. and *Tanacetum balsamita* L. in Turkish folk medicine. This study aimed to investigate hepatoprotective effects of diethyl ether extract of *Tanacetum balsamita* L. and *Helichrysum plicatum* DC collected around Van and Mus districts in Turkey in an acute liver toxicity model induced by carbon tetra chloride (CCL4) in rats.

MATERIALS AND METHODS

Plant Material

*Helichrysum plicatum* DC. subsp. *plicatum* was collected around Yukarıköy Village (1975 m) (Gevas, Turkey) in July 2007. *Tanacetum balsamita* L. subsp. *balsamitoides* (Sch. Bip.) Grierson was collected from Laladaş Mountain (Malazgirt, Turkey) in August 2007. The plant was collected and identified (Wagenitz and Davis, 1975) by Dr. F. Özgöke and deposited at the herbarium of the Biology Department, Yüzüncü Yıl University (F: 13192, F: 13194).

Extraction of Plant Material

The above-ground parts of plants were grounded in an electric grinder and macerated in diethyl ether for 2 h using a soxhlet apparatus (Ildam®, Turkey). The extract was separated
from the solvent by evaporation under vacuum using a rotary evaporator (IKA-WERKE, Germany). The yield for *Helichrysum plicatum* DC. subsp. *plicatum* was 3.39% (w/w) and for *Tanacetum balsamita* L. subsp. *balsamitoides* (Sch. Bip.) Grierson was 7.50% (w/w).

**Animals**

Male and female Sprague-Dawley rats weighing 110-220 g, purchased from the Animal House of the Medical School, Yüzüncü Yıl University (Van, Turkey) were used in the present study. The animals were housed at room temperature (20±2°C) in standard cages with standard pellet food and water ad libitum. The approval of Medical School Ethics Committee was obtained (2006/04-06).

**Chemicals**

Silibinin was obtained from Sigma (Germany), CCl₄ and dimethyl sulfoxide (DMSO) from Merck (Germany) and olive oil from Fluka (Germany). Silibinin was dissolved in ethyl alcohol (w/v), CCl₄ in olive oil (1:1, v/v), diethyl ether extracts of *Helichrysum plicatum* DC. (HP) and *Tanacetum balsamita* L. (TB) in DMSO (w/v).

**Induction of Acute Liver Toxicity**

Acute liver toxicity was induced according to Handa and Sharma (1990) and Shenoy et al. (2001) by injecting CCl₄ (0.8 mL kg⁻¹) intraperitoneally once a day for 7 days. The rats were divided into eleven groups of 6 animals each. Group 1 received only Isotonic Saline Solution (ISS) (0.1 mL). Group 2 received CCl₄ (0.8 mL kg⁻¹). Group 3 received CCl₄ and silibinin (50 mg kg⁻¹). Groups 4, 5 and 6 received CCl₄ and 25, 50 or 100 mg kg⁻¹ TB, respectively (TB-25, TB-50 and TB-100). Groups 7, 8 and 9 received CCl₄ and 25, 50 or 100 mg kg⁻¹ HP, respectively (HP-25, HP-50 and HP-100). All injections were made intraperitoneally and once a day for 7 days. The dose of silibinin was chosen according to Horvath et al. (2001). The doses of TB and HP were chosen according to Aslan et al. (2007) and Bukhari et al. (2007). CCl₄ was injected into the right abdomen while, the drugs were injected into the left abdomen. All the animals were observed daily and any dead animals were subjected to post-mortem examination to find the cause of death. Twenty four hours after the last injections (at the 8th day) the animals were sacrificed by cervical dislocation and blood samples were collected by direct cardiac puncture. The livers were taken out from the sacrificed animals and fixed in formaldehyde for histopathological examination.

Body weights of the rats were measured once a day during seven days. Daily changes in body weights as percentages were recorded. The percentage of daily changes in body weights was calculated according to the following formula:

\[
\text{Change in body weights as percentage} = 100 \times \frac{(\text{Weight}_n - \text{Weight}_{\text{initial}})}{\text{Weight}_{\text{initial}}}
\]

Where:

- Weight_{initial} = Measurement of first day
- Weight_n = Measurement of 2, 3, ..., 8 days

**Assessment of Liver Damage**

The serum Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) concentrations were determined with commercial slides (Vitros, USA) by using Vitros D60 II autoanalyzer (USA). For histopathological examination, the fixed livers were sectioned at 4 μ thickness and stained using Hematoxylin-Eosin (HE) after routine processing in paraffin-embedded blocks.
Statistical Analysis

Results of the biochemical analyses were reported as Mean±SEM. The total variation was analysed by performing one-way Analysis of Variance (ANOVA). Tukey's Honestly Significant Difference test (Tukey's HSD test) was used for determining significance. Probability levels of less than 0.05 were considered significant.

RESULTS

Results of the biochemical analyses in all groups are presented in Table 1. There were some deaths in the groups where, TB and HP were injected. Three animals in TB-25 and TB-50 groups, one animal in TB-100 group, five animals in HP-25 and HP-100 groups, two animals in HP-50 group died during the experiments. There was no death in ISS, silibinin and CCI groups.

Serum AST and ALT levels were significantly higher in the CCI group compared to those of the ISS group (p<0.05). In the silibinin group, serum AST and ALT levels were significantly lower compared to those in the CCI group (p<0.05). Serum ALT and AST levels in TB and HP groups were higher than those of the CCI group, but these differences were not statistically meaningful except in TB-100 group, probably because of high standard errors due to the deaths in some groups. High serum ALT and AST levels and high rate of deaths in TB and HP groups showed that diethyl ether extracts of Tanacetum balsamita L. subsp. balsamitoides (Sch. Bip.) Grierson and Helichrysum plicatum DC. subsp. plicatum did not have any protective effect in acute liver toxicity.

Percentage changes in weight were 7.98% in the ISS group, -11.12% in the CCI group, -10.24% in the silibinin group, -22.25% in the TB-25, -17.34% in TB-50, -17.63% in TB-100, -13.42% in HP-25, -15.21% in HP-50 and -32.99% in HP-100. The animals in the TB and HP groups showed a larger weight loss compared to those in the ISS and CCI groups.

There were no pathological changes in the livers of the rats in the ISS group while, in the CCI group diffuse ballooning degeneration was observed. Ballooned hepatocytes were of different sizes and much larger than normal hepatocytes, occasionally apoptosis and centrilobular necrosis were observed and an increase in connective tissue was evident (Fig. 1). Ballooning degeneration, apoptosis and centrilobular necrosis were rarely seen in the silibinin group. Histopathological changes in the livers of the TB and HP groups were similar to those of the CCI group. In TB and HP groups widespread ballooning degeneration were observed (Fig. 2a, b).

Table 1: The effects diethyl ether extracts of Tanacetum balsamita L. subsp. balsamitoides (Sch. Bip.) Grierson and Helichrysum plicatum DC. subsp. plicatum on serum ALT and AST levels

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT (U L⁻¹)</th>
<th>AST (U L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISS</td>
<td>41.27±3.53</td>
<td>152.18±12.20</td>
</tr>
<tr>
<td>CCI</td>
<td>94.33±18.39*</td>
<td>1963.33±286.75*</td>
</tr>
<tr>
<td>Silibinin</td>
<td>137.33±18.75*</td>
<td>510.17±10.32*</td>
</tr>
<tr>
<td>TB-25</td>
<td>2458.00±1643.08*</td>
<td>2674.00±1282.24*</td>
</tr>
<tr>
<td>TB-50</td>
<td>2354.00±1677.26*</td>
<td>2494.00±1362.74*</td>
</tr>
<tr>
<td>TB-100</td>
<td>5665.20±1914.72*</td>
<td>5246.40±1432.68*</td>
</tr>
<tr>
<td>HP-25</td>
<td>2742.00</td>
<td>1586.00</td>
</tr>
<tr>
<td>HP-50</td>
<td>2448.00±649.45*</td>
<td>1194.00±250.71*</td>
</tr>
<tr>
<td>HP-100</td>
<td>1164.00</td>
<td>474.00</td>
</tr>
<tr>
<td>Fp Value</td>
<td>5.000/0.001</td>
<td>8.170/0.000</td>
</tr>
</tbody>
</table>

The values represent the Mean±SEM. Post-hoc Tukey’s HSD test: *p<0.05 with respect to the ISS group, †p<0.05 with respect to the CCI group, ‡p<0.05 with respect to the silibinin group.
Fig. 1: Diffuse ballooning degeneration and an increase in connective tissue are seen in the liver of a rat from the CCl4 group. (Hematoxylin-eosin stain, original magnification, x10)

Fig. 2: Numerous ballooned hepatocytes are seen in the livers of a rat from (a) HP-50 group and (b) TB-50 group. (Hematoxylin-eosin stain, original magnification, x10)

**DISCUSSION**

In the model of acute liver damage used in this study, 0.8 mg kg⁻¹ CCl4 induced biochemical and pathological changes related to liver damage; increase in serum AST and
ALT levels and degeneration in hepatocytes. Severe hepatic lesions induced by CCl₄ were remarkably reduced by silybin, an agent known for its hepatoprotective effect, which were in good agreement with the results of the biochemical tests. Serum AST and ALP levels in the silybin group was significantly lower compared to those in the CCl₄ group. However, the ethyl ether extracts of *Helichrysum plicatum* L. subsp. *plicatum* and *Tanacetum balsamita* L. subsp. *balsamitoides* (Sch. Bip.) Grierson did not reduce the liver damage caused by CCl₄ at all doses used in this study, evident in both biochemical and histopathological examinations. The serum AST and ALT levels were even higher, although not statistically significant, in the TB and HP groups compared to those in the CCl₄ group. There were also widespread ballooning degeneration, apoptosis and centrilobular necrosis in the livers of TB and HP groups. TB and HP also caused a larger weight loss compared to those in the ISS and CCl₄ groups. Although, we did not find a beneficial effect of TB and HP on the liver some studies showed hepatoprotective effects of TB. Coprean et al. (1991) showed that *Chrysanthemum balsamita*, a synonym of *Tanacetum balsamita*, extract reduced some of the toxic effects in ethanol intoxicated rats, another model for hepatotoxicity. Rusu et al. (2005) showed that alcoholic extract of *Chrysanthemum balsamita* reversed the hepatotoxic effects of CCl₄ in rats and attributed the effect to its phenylpropanoids and flavones contents. The difference in the administration method of CCl₄ and the plant extract could be the reason why we did not find a hepatoprotective effect of TB in the current study. Rusu et al. (2005) gave both CCl₄ and the plant extract through gavage while we administered them intaperitoneally.

Although, hepatoprotective use of both *Helichrysum plicatum* L. and *Tanacetum balsamita* L. was reported in Turkish folk medicine, this study showed that the ethyl extracts of both plants did not have protective effect on acute liver damage and on the contrary may aggravate the damage (Oztürk et al., 1991). Therefore, one should be cautious when using *Helichrysum plicatum* L. subsp. *plicatum* and *Tanacetum balsamita* L. subsp. *balsamitoides* (Sch. Bip.) Grierson for their other effects, such as for diuretic, lithagogue, anti bloating, aphrodisiac and vermifuge effects.

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**REFERENCES**


