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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 carbontetrachloride (CCl_4)-induced acute liver toxicity in rats. Acute liver toxicity was induced by injecting CCl_4 (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 CCl_4 group. Histopathological findings were similar in the CCl_4, TB and HP groups. The body weight loss was more in the TB and HP groups compared to that of the CCl_4 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 carbontetrachloride (CCl_4)-induced acute liver toxicity in rats and even exacerbated the toxicity.

Key words: *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 argenteum* subsp. *Flabellifolium* had antibacterial activity (Tabanca *et al*., 2007). The crude extract of *Tanacetum artemisioides* 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 cyclooxigenase-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 hepatoprtective effects of diethylether 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 carbontetrachloride (CCl_4) in rats.

MATERIALS AND METHODS

Plant Material

Helichrysum plicatum DC. subsp. *plicatum* was collected around Yukariköy Village (1975 m) (Gevas, Turkey) in July 2007. *Tanacetum balsamita* L. subsp. *balsamitoides* (Sch. Bip.) Grierson was collected from Laladağ Mountain (Malazgirt, Turkey) in August 2007. The plant was collected and identified (Wagenitz and Davis, 1975) by Dr. F. Özgökçe and deposited at the herbarium of the Biology Department, Yüzüncü Yil 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 Yil 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), CCl_4 and dimethyl sulfoxide (DMSO) from Merck (Germany) and olive oil from Fluka (Germany). Silibinin was dissolved in ethyl alcohol (w/v), CCl_4 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 CCl_4 (0.8 mL kg^{-1}) intraperitoneally once a day for 7 days. The rats were divided into eleven groups of 6 animals each. Group I received only Isotonic Saline Solution (ISS) (0.1 mL). Group 2 received CCl_4 (0.8 mL kg^{-1}). Group 3 received CCl_4 and silibinin (50 mg kg^{-1}). Groups 4, 5 and 6 received CCl_4 and 25, 50 or 100 mg kg^{-1} TB, respectively (TB-25, TB-50 and TB-100). Groups 7, 8 and 9 received CCl_4 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). CCl_4 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:

$\text{Weight}_{\text{initial}}$ = 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 CCl_4 groups.

Serum AST and ALT levels were significantly higher in the CCl_4 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 CCl_4 group ($p<0.05$). Serum ALT and AST levels in TB and HP groups were higher than those of the CCl_4 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 CCl_4 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 CCl_4 groups.

There were no pathological changes in the livers of the rats in the ISS group while, in the CCl_4 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 CCl_4 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

Treatment	ALT (U L^{-1})	AST (U L^{-1})
ISS	41.27±3.53	152.18±12.20
CCl_4	944.33±158.39^a	1903.33±296.75^a
Silibinin	130.33±18.75^b	510.17±49.30^{ab}
TB-25	2458.00±1643.08^a	2674.00±1282.24^a
TB-50	2354.00±1677.26^a	2494.00±1362.74^a
TB-100	5065.20±1914.72^{abc}	5246.40±1432.68^{ab}
HP-25	2742.00	1566.00
HP-50	2448.00±649.45^a	1194.00±250.71^a
HP-100	1164.00	474.00
F/p Value	5.000/0.001	8.170/0.000

The values represent the Mean±SEM. Post-hoc Tukey's HSD test: $^a p<0.05$ with respect to the ISS group, $^b p<0.05$ with respect to the CCl_4 group, $^c p<0.05$ with respect to the silibinin group

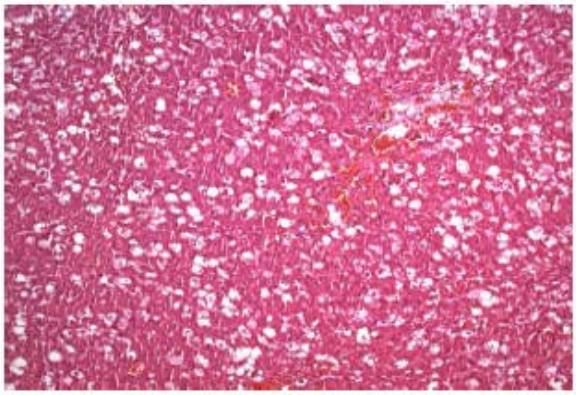

Fig. 1: Diffuse ballooning degeneration and an increase in connective tissue are seen in the liver of a rat from the CCl_4 group. (Hematoxylin-eosin stain, original magnification, x10)

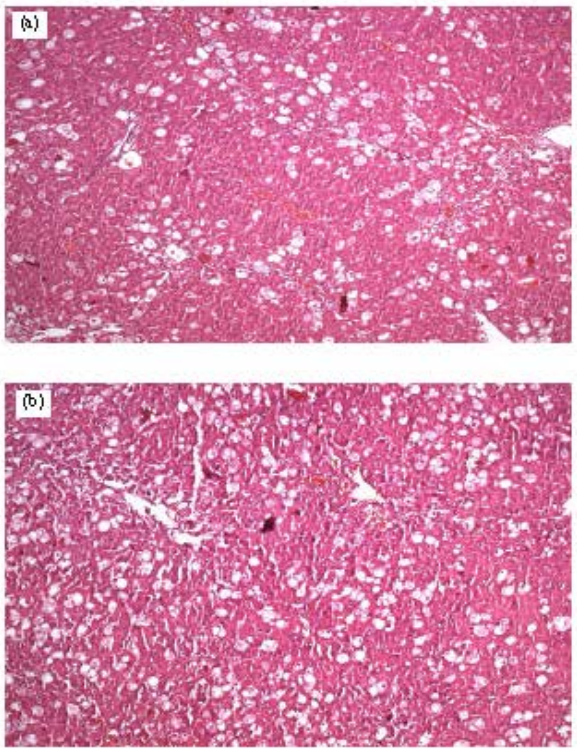

Fig. 2: Numerous ballooned hepatocytes are seen in the livers of a rat from (a) HP-50 group and (b) TB-50 group. (Hematoxylin-eosin stain, original magnification, x10)

DISCUSSION

In the model of acute liver damage used in this study 0.8 mg kg^{-1} CCl_4 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 CCl_4 were remarkably reduced by silibinin, 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 silibinin group was significantly lower compared to those in the CCl_4 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 CCl_4 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 CCl_4 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 CCl_4 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 toxicated rats, another model for hepatotoxicity. Rusu *et al.* (2005) showed that alcoholic extract of *Chrysanthemum balsamita* reversed the hepatotoxic effects of CCl_4 in rats and attributed the effect to its phenylpropanoids and flavones contents. The difference in the administration method of CCl_4 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 CCl_4 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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