Assessment of antioxidant and hepatoprotective potential of *Satureja montana* extracts against CCl_4 induced liver damage

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The influence of *Satureja montana* extracts on several liver biochemical parameters in mice administered with carbon tetrachloride was estimated. *In vivo* investigation of antioxidant properties of *S. montana* extracts encompassed monitoring of biochemical parameters (derived from liver homogenate and blood hydrolyzate) and examination of potential hepatoprotective effect after intoxication with carbon tetrachloride. Biochemical tests included determination of activity of several antioxidant enzymes: xanthine oxidase, catalase, peroxidase, glutathione peroxidase, amount of reduced glutathione and intensity of lipid peroxidation. In order to obtain data about the potent hepatoprotective effect of examined extracts, the following parameters were determined: aspartate aminotransferase, alanine aminotransferase, bilirubin, hepatic DNA and hydroxyprolin. Results of this study demonstrate that *S. montana* extracts inhibited the hepatotoxicity produced by carbon tetrachloride administration most probably through activation of physiological common defense mechanisms.

Key words: *Satureja montana; hepatoprotective; antioxidant*

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1. INTRODUCTION

Winter savory (*Satureja montana* L.) grows widely in the Balkan region and belongs to a very important family of medicinal plants, the Lamiaceae family. Winter savory is an aromatic herb which is traditionally used as a spice and natural food preservative and represents a common ingredient in folk medicines for therapy of different digestive, respiratory, and urinary ailments. Furthermore, it is quite often used as a tonic for strengthening the immune system. In addition, our previous results showed that it could be recommended for skin and mucous membrane inflammation (Vladić et al., 2017a). Apart from this, frequent use in traditional medicine and overall increase of interest in natural remedies has also been a basis for scientific research conducted for the purposes of confirming *S. montana*'s biological activities. Significant antioxidative potential of *S. montana* was confirmed for essential oil, extracts obtained by conventional extraction methods, as well as extracts obtained by modern extraction methods (Ćavar et al., 2008; 2014; Grosso et al., 2009; Serrano et al., 2011; Vidović et al., 2014; Vladić et al., 2017b). In addition to its antioxidative activity, winter savory possesses antimicrobial, diuretic, antiproliferative, angiotensin-converting enzyme (ACE) inhibitory, and anti-HIV-1 activity (Ćavar et al., 2014; Cetojević-Simin et al., 2004; Prieto et al., 2007; Serrano et al., 2011; Skočibušić and Bezić, 2004; Stanic and Samaržija, 1993; Vidović et al., 2014; Vladić et al., 2017a; 2016; Yamasaki et al., 1998).

Winter savory is a herb rich in carvacrol, a representative of oxidative monoterpenes which exhibits significant antioxidant potential, both *in vitro* and *in vivo* (Aeschbach et al., 1994; Aristatile et al., 2015; 2009; Guimarães et al., 2010; Slamenova et al., 2011). Also, it was confirmed that carvacrol demonstrated characteristics of a hepatoprotective agent in *in vivo* models (Aeschbach et al., 1994; Aristatile et al., 2009; Suntres et al., 2015). The hepatoprotective effect of carvacrol had been correlated with its capacity to preserve the integrity of hepatocellular membrane and oxidant/antioxidant balance in liver (Aristatile et al., 2009). In addition, studies were conducted where the hepatoprotective effect of species which belong to the genus *Satureja* (*S. macrostema* and *S. khuzestanica*) (Assaei et al., 2014; Gutierrez and Navarro, 2010). Based on the importance and role of carvacrol, as well as the antioxidant effect of *S. montana*, and confirmed beneficial activity of some species from genus *Satureja*, the starting point of our study was to evaluate pharmacological potency of *S. montana* extracts. In

our previous study, green extraction method, supercritical carbon dioxide extraction (SC-CO_2), was established for obtaining *S. montana* extracts (Vidović et al., 2014). In that study, process parameters of SC-CO_2 were determined with the aim to produce *S. montana* extract with the highest concentration of carvacrol. Considering the fact that carbon dioxide is a solvent with an affinity for non-polar compounds, in the other study water/ethanol mixture was chosen as a solvent because of its affinity for polar compounds. Liquid ethanol-water extract was then converted into dry form via spray drying technology (Vladić et al., 2016). Selected extracts of *S. montana* obtained in previously mentioned studies were selected for investigation in this study (Vidović et al., 2014; Vladić et al., 2016).
Therefore, the main aim of this study was to assess the hepatoprotective potential of *S. montana* extracts in a mouse model of carbon tetrachloride (CCl_4) induced liver toxicity.

2. MATERIALS AND METHODS

2.1. Plant material

Aerial parts of winter savory (*Satureja montana*) was collected at the Institute of Field and Vegetable Crops, Bački Petrovac, Republic of Serbia, in July 2012. Collected plant material has been naturally dried and then stored in paper bags at the room temperature.

2.2. Chemicals

Carbon tetrachloride (>99.5%) was purchased from Kemika (Croatia) and methanol (>99.9%) were purchased from Sigma-Aldrich (Germany). All other chemicals were of analytical reagent grade.

2.3. Preparation of extracts

The detailed procedure of obtaining *S. montana* extract (SME1) by SC-CO_2 extraction had been described by (Vladić et al., 2016). Briefly, the extraction of *S. montana* herbal material was conducted using supercritical carbon dioxide at a pressure of 350 bar and temperature of 50 °C. The extraction time was 4.5 hours. The separator conditions were 15 bar and 23 °C (Vladić et al., 2016).
The detailed procedure of obtaining *S. montana* extract via spray drying is described by Vidović et al. (2014). Spray-dried extract (SME2) is produced by the drying of liquid extract via spray drying technology (inlet temperature 120 °C, outlet temperature 80 °C). *S. montana* liquid extract was obtained using 50% ethanol/water mixture as an extraction solvent. Extraction was carried out for five days at room temperature in a dark place. Maltodextrin (DE16) in the percentage of 10% (calculated on extract dry weight) was used as a carrier material (Vidović et al., 2014).

2.4. Animals and treatments

Animal care and all experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animal Resources edited by the Commission of Life Sciences, National Research Council. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution (University of Novi Sad; EK: II-2013-03; 01-160-5). Animals were fed with standard mouse chow (LM2, Veterinarski zavod, Subotica, Serbia) had free access to tap water at a temperature of 25 °C and controlled humidity (30-50%), in the animal house under 12 h light/day cycles. SME1 was dissolved in olive oil in a concentration of 1%. The SME2 was dissolved under sonication in water (1%; w/v) and filtered through 0.45 mm membrane filter. The resulting solutions were kept refrigerated at a temperature of 4 °C. The extracts were administered intraperitoneally (i.p.). The variability in the volume of administered doses was managed by adjusting the concentration to ensure a constant volume (2 mL/kg body weight). The carbon tetrachloride (CCl_4) was also administered intraperitoneally (2 mL/kg) 24 h before sacrifice. The animals were randomly allocated to six groups of six in each group, under the following conditions and treatments: control group: – the mice received 1 mL/kg (i.p.) distilled water for 7 days; control + CCl_4: the mice were treated with 1 mL/kg (i.p.) distilled water for 7 days and 2 mL/kg (i.p.) of CCl_4 (day 7) 24 hours before sacrifice; SME1, SME2: the mice were treated with the investigated *S. montana* extracts (SME1 and SME2), receiving 2 mL/kg (i.p.) for 7 days; SME1 + CCl_4, SME2 + CCl_4 – the mice were treated with the same dosage of extracts, followed by 2 mL/kg (i.p.) of CCl_4 (day 7) 24 h before sacrifice.
At the end of the experiment (day 8), the animals were anesthetized with isofluorane, decapitated and exsanguinated. Livers were excised immediately after the animals were sacrificed. Liver homogenates were prepared in the following way: 1 g of liver tissues were homogenized with Tris–HCl: saccharose solution (50 mmol/L; 0.25 mol/L; pH 7.40), 1:3, 4 °C, using a glass Potter-Elvehjem homogenizer set. The resulting homogenate was centrifuged at 3000 rpm for 10 min, and the concetration of proteins was determined by biuretic method using bovine serum albumine as a standard (Wood, 2012). Samples of blood and liver were used for determination of biochemical parameters of oxidative stress.

2.5. Biochemical assays

The activity of xanthine oxidase (XOD) was determined using the Lück (1965) method, catalase (CAT) according to Beers and Sizer (1952), peroxidase (Px) according to Simon et al. (1974) glutathione peroxidase (GSH-Px) according to Beutler et al. (1963) and glutathione reductase (GR) according to Goldberg and Spooner (1983). Amount of reduced glutathione (GSH) was also determined Beutler (1984), as well as the intensity of lipid peroxidation (LPx) using the Buege and Aust (1978) protocol. The content of hepatic DNA was determined according to Schulte-Hermann et al. (1988). Contents of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin were determined following the Reitman and Wootton method. The concentration of hydroxyproline was also determined using Woessner (1961) protocol. All the measurements were performed in triplicate.

2.6. Statistical analysis

The activities are expressed as mean ± standard deviation. Mean values between the groups in biochemical analyses were considered significantly different at the $p<0.05$ confidence level, after performing a one-way single factor ANOVA, followed by Tukey and Bonferroni multiple comparison post hoc tests.

3. RESULTS AND DISCUSSION

Different agents can be responsible for liver damage and disease, including viruses, hepatotoxic chemicals, as well as pollution. The hepatotoxicity produced by CCl_4 administration is frequently used as a model for examination of the liver (Ćebović and Maksimović, 2012; Manna et al., 2006; Gutierrez and Navarro, 2010; Sreelatha et al., 2009; Yang et al., 2010). The efficacy of any hepatoprotective agent depends on its capability to reduce effects of CCl_4 or to maintain normal physiological function, which was disrupted due to hepatotoxin application. The results of an investigation of *S. montana* extracts' influence on parameters of oxidative stress induced by CCl_4 are presented in Table 1. Several activities were analyzed, such as xanthine oxidase (XOD), catalase (CAT), peroxidase (Px), glutathione reductase (GR) and glutathione peroxidase (GSHPx),

Table 1. Effects of SC-CO_2 (SME1) and spray-dried (SME2) extracts of winter savory (*S. montana*) on oxidative stress biochemical parameters.

Group	Biochemical parameters						
	XOD[a]	CAT	Px	GR	GSH-Px	GSH	LPx
Control	1.93 ± 0.02	9.56 ± 0.37	10.68 ± 1.38	6.13 ± 0.07	8.70 ± 0.38	4.98 ± 0.16	3.04 ± 0.07
Control+CCl_4	1.89 ± 0.02	3.32 ± 0.05 a	4.87 ± 0.28 a	1.2 ± 0.10 a	21.75 ± 1.35 a	0.57 ± 0.28 a	9.79 ± 1.46 a
SME1	1.87 ± 0.03 a	13.4 ± 0.59 a,b	14.88 ± 0.38 a,b	7.03 ± 0.46 b	7.54 ± 2.23 b	4.99 ± 0.17 b	2.62 ± 0.54 b
SME1+CCl_4	1.93 ± 0.01	9.02 ± 0.16 b	9.52 ± 1.15 b	5.84 ± 2.11 b	10.94 ± 1.73 b	3.34 ± 0.75 a,b	3.97 ± 0.11 b
SME2	1.86 ± 0.03 a	12.37 ± 0.18 a,b	14.57 ± 0.80 a,b	6.53 ± 1.61 b	6.67 ± 1.06 b	4.92 ± 0.39 b	2.21 ± 0.24 b
SME2+CCl_4	1.92 ± 0.03	8.72 ± 0.29 a,b	9.30 ± 0.72 b	5.41 ± 0.40 b	12.42 ± 0.64 a,b	2.9 ± 0.73 a,b	3.94 ± 0.98 b

[a] Values are expressed as mean ± standard deviation of six mice. Activities of xanthine oxidase (XOD), catalase (CAT), peroxidase (Px), glutathione reductase (GR) and glutathione peroxidase (GSHPx) are expressed in nmol/mg of protein min^{-1}. Content of hepatic reduced glutathione (GSH) is expressed in nmolGSH/mg of protein. Intensity of lipid peroxidation (LPx) is expressed in nmol/MDA/mg of protein; MDA, malonyldialdehyde. Letters a and b denote statistically significant difference from the Control group and from the Control+CCl_4 group based on *post-hoc* Bonferroni test at P<0.05 level.

along with glutathione content (GSH) and intensity of lipid peroxidation (LPx). Evaluation of the hepatoprotective influence of *S. montana* extracts on mice with chemically induced toxicity is expressed by measurement of hepatic DNA content, hydroxyproline, and bilirubin, as well as activities of serum parameters AST and ALT, before and after CCl_4 intoxication (Table 2).

Regarding XOD activity, no change was noticed after CCl_4 administration nor upon administration of extracts+CCl_4 (SM1+CCl_4 and SM2+CCl_4). The observation that CCl_4 administration did not influence strongly XOD activity was reported previously in the literature (Ćebović and Maksimović, 2012). CAT represents one of the most efficient enzymes and its key role is to dissolve hydrogen peroxide to water and oxygen, in that way performing cell protection from oxidative damage induced by hydrogen peroxide (Blokhina, 2003). Intraperitoneal CCl_4 administration led to a significant reduction of CAT activity (2.9-fold) in comparison to untreated control group, while pretreatment with extracts led to a significant reduction of CCl_4 influence and an increase of enzymatic activity to a value close to the control group. Investigated extracts (SME1 and SME2) increased Px activity in comparison to the control group, while CCl_4 administration reduced its activity almost 2-fold. Similar as in the case of CAT, pretreatment with extracts led to a decrease of hepatotoxic CCl_4 effect, i.e. enzymatic activity maintained close to Px activity measured in the untreated control group, which can be an indicator of increased production of hydrogen peroxide, partially converted into the water via both CAT and Px.

Administration of CCl_4 led to a crucial drop in GSH levels, which indicates that significant damage of hepatocyte appeared. The application of extracts with CCl_4 significantly increased the amount of GSH (by comparison with the negative control group), but significantly lower than the value of the untreated group, i.e. they failed to completely eliminate the negative CCl_4 effect.

Significant differences in the effects of applied extracts, which succeeded to suppress CCl_4 influence, were not found. It is a statistically important observation having in mind the multiple decreases of enzymatic activity via CCl_4 administration. Regarding the function of GR to maintain a high level of GSH activity, it can be concluded that reductions of activities of both GSH and GR after CCl_4 application, were correlated. Therefore, extracts' influences exerted on GR and GSH activities indicate a protective effect of extracts.

GSHPx is responsible for cell protection from the harmful effect of endogenously formed hydroperoxide through degradation into water and oxygen, and can also be responsible for detoxification of lipid peroxide generated by reactive oxygen species (Blokhina, 2003). CCl_4 administration led to a significant increase of enzymatic activity, while it was significantly decreased (compared to the negative control group) in the groups treated with extracts. Application of SME1 led to a decrease of enzymatic activity up to a basal value of the control group. Therefore, extracts succeeded in suppressing the toxic effects of excessive production of free radicals caused by CCl_4 application.

CCl_4 application led to a significant increase in intensity of lipid peroxidation. Pretreatment with extracts, managed to prevent oxidative damage, caused by CCl_4, without a difference in activity. Pretreatment with extracts can potentially prevent loss of integrity of the hepatocellular membrane because of their capability to inhibit lipid peroxidation and in that way protect lipid membranes from oxidative damage. Hence, it is possible to evaluate their antioxidant potential, as well as hepatoprotective, by inducing damage of hepatocyte membrane.

Regarding the control group, significant reductions of several activities CAT, Px and GR, and level of GSH, as well as an increase of LPx intensity and activity of GSHPx were observed in animals treated with CCl_4. Extracts, without a relevant difference in their effects, succeeded in suppressing toxic CCl_4 effects, protecting the liver from oxidative damage, and maintaining levels of enzymatic and non-enzymatic antioxidants.

Results of investigation of *S. montana* extracts showed that intoxication with CCl_4 led to a significant decrease of hepatic DNA, with regard to the control group. Extracts (SME1 and SME2) entirely inhibited the decline of the amount of hep. DNA. There were no notable differences between the concentration of hep. DNA when SME1 and SME2 were administered. Hydroxyproline, one of the parameters of liver damage, was determined in liver tissue hydrolyzate of mice. The groups treated with extracts (SME1 and SME2) did not differ in the amount of hydroxyproline in comparison with the control group, while it considerably increased under CCl_4 treatment. According to obtained results, extracts applied before a single CCl_4 dose showed a positive effect, that is, a significant decrease of hydroxyproline concentration towards its concentration in the untreated group.

The increased levels of serum bilirubin, AST and ALT are conventional indicators of liver injury (Thabrew et al., 1987). The amount of serum bilirubin was not significantly different

Table 2. Hepatoprotective influence of SC-CO_2 (SME1) and spray-dried (SME2) extracts of winter savory (*S. montana*) on liver function biochemical parameters.

Group	Biochemical parameters				
	Hepatic DNA[a]	Hydroxyprolin	Bilirubin	AST	ALT
Control	6.222 ± 0.021	28.300 ± 0.141	0.118 ± 0.053	72.117 ± 0,496	20.667 ± 1.531
Control+CCl_4	4.631 ± 0.015 a	91.533 ± 0.979 a	2.217 ± 0.306 a	590.667 ± 3,777 a	327.833 ± 15.250 a
SME1	6.248 ± 0.091 b	30.883 ± 0.483 b	0.113 ± 0.016 b	70.533 ± 2.925 b	19.633 ± 4.361 b
SME1+CCl_4	6.413 ± 0.036 a,b	64.133 ± 7.156 a,b	1.028 ± 0.102 a,b	281.000 ± 19.910 a,b	106.667 ± 10.801 a,b
SME2	6.218 ± 0.015 b	29.267 ± 1.593 b	0.125 ± 0.019 b	67.000 ± 4,026 b	20.483 ± 3.596 b
SME2+CCl_4	6.417 ± 0.089 a,b	62.650 ± 2.054 a,b	1.100 ± 0.126 a,b	219.000 ± 12.474 a,b	115.667 ± 12.832 a,b

[a] Values are expressed as mean ± standard deviation of six mice. Content of hepatic DNA is expressed in mg DNA/100 g liver. Contents of bilirubin, AST and ALT are expressed in mg/dL, U/L and U/L, respectively. Concentration of hydroxyprolin is expressed in mg/100 mg of liver hydrolyzate. Letters a and b denote statistically significant difference from the Control group and from the Control+CCl_4 group based on *post-hoc* Bonferroni test at P<0.05 level.

from the control group when extracts were applied. After CCl_4 was applied, the amount of serum bilirubin was 18.8-fold increased. Administration of SME1 and SME2 notably decreased the concentration of serum bilirubin towards its concentration in the untreated group. After exposure to CCl_4, serum AST and ALT were 8.2 and 15.9- fold increase, respectively. Although both extracts notably reduced the negative effect of CCl_4 and significantly decreased serum AST activity, SME2 was more efficient in reduction than SME1. Also, both extracts considerably reduced the negative effect of CCl_4 and notably decreased serum ALT activity. Application of hepatotoxic agents led to membrane damage and release of enzyme from cytosol. In that context, reduction of levels of AST and ALT by applied extracts indicates that stabilization of plasma membrane and recovery of hepatic tissues damaged by CCl_4 occurred. This effect is in compliance with the widespread opinion that recovery of hepatic parenchyma and regeneration of hepatocyte bring back the levels of transaminases within physiological limits (Merlin and Parthasarathy, 2011; Thabrew et al., 1987).

As it was previously described, the activity of the extracts in the *in vivo* conditions was not significantly different although their chemical profile was different. In our previous studies (Vidović et al., 2014; Vladić et al., 2016) the chemical composition of *S. montana* extracts obtained by SC-CO_2 and essential oil isolated from spray dried extract (SME2eo) was established. In investigated SME2eo nine compounds were detected, which was two-fold less than the number of compounds detected in SME1. The most dominant component was phenolic compound: carvacrol, with relative content of 78.61 and 71.82%, in SME1 and SME2eo, respectively. In the sample obtained by SC-CO_2, p-cymene (3.96%) and trans-caryophyllene (2.4%) were detected in a notably higher percentage in comparison to SME2eo (0.36 and 0.24%), while caryophyllene oxide is present in a higher percentage in SME2eo (1.31%) than SME1 (1.26%). Linalool was also detected in both samples as well as terpinen 4-ol. The concentration of carvacrol in SME1 was 67-fold higher than in SME2eo. The content of carvacrol in obtained SME1 was 60.82 g/100 g, while in SME2 it was 0.903 g/100 g (Vidović et al., 2014; Vladić et al., 2016). Taking into account that the extract more abundant in carvacrol (SME1) did not show dominant antioxidant influence in *in vivo* model, it is possible to assume that both extracts activated the common antioxidant mechanism. Also, it is possible that the synergistic activity of all constituents was responsible for *in vivo* effect. Apart from this, it was already established that some effects of carvacrol can be improved by the presence of less dominant components. For instance, in combination with carvacrol *in vitro*, p-cymene is incorporated in the cytoplasmic membrane and led to a more intensive transport of carvacrol through the membrane (Ultee et al., 2002). Consequently, the antimicrobic effect of carvacrol is improved with the presence of p-cymene. Considering confirmed carvacrol potential in preserving the physiological function of the liver, manifested hepatoprotective and antioxidant activities of *S. montana* extracts can be at least partially subscribed to the presence of carvacrol. On the contrary, examined extracts were obtained by different extraction methods, and antioxidant activities of such extracts as well as their carvacrol contents differed from one another. For obtaining SM2 extract, a mixture of ethanol/water was used as a solvent. Considering the polarity of this solvent, the main compounds in extracts had a polar character and are most likely responsible for antioxidant activity of SM2 *in vivo*. Although both extracts showed high antioxidant activity *in vitro*, SME2 exhibited 3-fold higher antioxidant activity than SME1 (IC_{50} value of SME1: 17.40 µg/mL; IC_{50} value of SME2: 5.24 µg/mL (Vidović et al., 2014). Due to a significantly low share of carvacrol in SME2, it is highly likely that the antioxidant effect of SME2 is related to the high content of polar polyphenols (content of total phenols 153.61 mg/g and total flavonoids 118.69 mg/g (Vidović et al., 2014). Abd El Tawab et al. (2014) determined the presence of caffeic, syringic, and rosmarinic acid in *S. montana* extract. Also, they identified gallic, ferulic, cinnamic, and vanillic acids, and three flavonoids: luteolin, rutin, and quercetin. In extracts obtained from non-volatile fractions of *S. montana*, after hydrodistillation and extraction by supercritical carbon dioxide, the presence of gallic, caffeic, syringic, gentisic, ferulic, chlorogenic, coumaric, and vanillic acids, epicatechin, and catechin (Silva et al., 2009). Inhibition of free radicals is significant in terms of protection of the damaged liver. DPPH radical is a stabile radical that is known to remove labile hydrogen. Scavenging of free radicals is one of the main antioxidant mechanisms which inhibits the chain reaction of lipid peroxidation (Yang et al., 2010). The results of DPPH assay indicate that *S. montana* extracts show high scavenging effect towards free radicals. Moreover, this can protect the liver from damage caused by the generated free radical CCl3 induced by CCl_4. Despite the fact that *in vitro* results confirmed direct antioxidant activity of examined extracts, it is not transparent whether this might also be the case *in vivo*. In addition to *in vitro* antioxidant effect, a possible mechanism of *S. montana* extracts as hepatoprotective agents are the reduction in activation of CCl_4 into the reactive form. Gutierrez and Navarro (2010) investigated antioxidant

and hepatoprotective effects of the methanol extract of the *S. macrostema* on CCl_4- and paracetamol-induced damage in rats. Authors assumed that *S. macrostema* extract elicits hepatoprotective effect through antioxidant activity (Gutierrez and Navarro, 2010). Hepatoprotective activity of essential oil of *S. khuzestanica* was evaluated. Within that study the hepatoprotective activity of essential oil was confirmed, especially in combination with vitamin E. It also suggested that potential reason for antioxidant and hepatoprotective effects can be found in the high content of carvacrol (Assaei et al., 2014). Looking at the significant effect the examined antioxidant enzymes, it can be inferred that SME1 and SME2 have a potent hepatoprotective action through activation of common physiological defense mechanisms. Reported changes in various enzyme activities may also indicate an indirect antioxidant effect due to the engagement of both CCl_4 and extract component(s). There is a demand for further investigation in order to identify the active component(s) of extracts and their biochemical mechanism responsible for the treatment of oxidative stress-induced liver damage as well as other disorders caused by oxidative stress.

CONCLUSION

In this study, *S. montana* extracts inhibited hepatotoxicity induced by CCl_4, protected liver from oxidative damage, and preserved the antioxidant level of protection. Both extracts obtained by applying different technologies exhibited a protective effect without significant differences in their activities, although a significant difference between the extracts and antioxidant capacity *in vitro* was determined. Therefore, the most probable mechanism of action of extracts is activation of physiological defense mechanism and consequently the protective activity. In addition, this finding indicates that the *S. montana* extracts produced by applying modern green technologies could be implemented in prevention and/or treatment of acutely damaged liver. The next step in the research will be the identification of active ingredients, investigation of mechanisms of action, and evaluation of concentration-dependency of extracts.

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