Hepatoprotective Properties of Fractions from Meadowsweet Extract during Experimental Toxic Hepatitis

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Fractions of the extract from meadowsweet aerial parts in 70% ethanol exhibited hepatoprotective properties during CCl_4-induced toxic hepatitis. This extract produced a normalizing effect on activity of enzymes, markers of cytolysis, lipid peroxidation, and antioxidant defense system in liver cells. Fractionation of the extract was accompanied by dissociation of the effect. These changes reflect specific action of a complex of bioactive substances. The ethyl acetate and chloroform fractions from this extract were most potent. The effectiveness of these fractions by several parameters surpassed that of Carsil.

Key Words: *meadowsweet; hepatoprotective properties; Carsil; CCl_4-induced toxic hepatitis*

Plant antioxidants hold much promise as hepatoprotective drugs [10-12]. Much attention is paid to meadowsweet plants (*Filipendula ulmaria (L.) Maxim.*) of the *Rosaceae* family. Published data show that extracts of meadowsweet aerial parts have hepatoprotective and antioxidant activity [8,9]. By several parameters the effectiveness of meadowsweet extract in 70% ethanol surpasses that Carsil [8].

Here we studied hepatoprotective activity of fractions from the extract of meadowsweet aerial parts in 70% ethanol during experimental toxic hepatitis.

MATERIALS AND METHODS

Experiments were performed on 63 male outbred albino rats weighing 220-240 g. The animals were kept in a vivarium under normal conditions and fed a standard diet. Plant extract was obtained by 3-fold treatment of meadowsweet aerial parts with 70% ethanol at 80-85°C for 30 min. The raw material/extracting agent ratio was 1:10. Meadowsweet extract consists of bioactive substances that are extracted from ground raw materials with 70% ethanol. The extract contains 4.3% flavonoids (by the content of quercetin and quercetin glycosides). The dry residue is not less than 22%. The extract contains simple phenols, flavonoids, phenyl carboxylic acids, coumarins, triterpene compounds, tanning agents, amino acids, macroelements, and microelements. The fractions were obtained by extraction of aqueous solution of meadowsweet extract with solvents of increasing polarity (chloroform, ethyl acetate, and butanol-1). These fractions were evaporated to dryness at 40°C under vacuum. Aqueous residue was not examined due to low antioxidant activity of its fractions (as shown by cathode voltammetry). Toxic hepatitis was induced by intragastric administration of 1 ml/kg CCl_4 in 20% oil solution (sunflower oil). This treatment was performed daily for 6 days [7].

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The development of toxic hepatitis in rats was verified by aminotransferase activity and contents of bilirubin and its fractions. The suspension of meadowsweet extract in 1% starch gel (daily dose 100 mg/kg) was administered intragastrically for the next 5 days [8]. The fractions were administered in equivalent doses (by the content of individual fractions). The rats were treated with 7 mg/kg chloroform fraction, 20 mg/kg ethyl acetate fraction, and 44 mg/kg butanol fraction. Reference hepatoprotector Carsil (Sopharma) was administered in a dose of 200 mg/kg. Control animals received an equivalent volume of 1% starch gel. The rats were decapitated under light ether anesthesia 1 day after the end of therapy [7]. Activities of alanine transaminase (ALT) and aspartate transaminase (AST) in blood plasma were measured with Lachema kits. Liver homogenates were examined for the content of lipid peroxidation (LPO) products [1]: conjugated dienes and lipid hydroperoxides (LHP), thiobarbituric acid-reactive substances (TBARS), and activity of key enzymes of antiradical and antiperoxide protection superoxide dismutase (SOD) and catalase. The amount of lipids was measured gravimetrically. Protein content was estimated by the microbiuret method [4].

The results were analyzed by Student's *t* test and nonparametric Wilcoxon—Mann—Whitney test.

RESULTS

Activity of marker enzymes of hepatocyte injury (ALT and AST) in blood plasma from control animals increased by 8.6 and 3.0 times, respectively, compared to intact specimens (Table 1), which attested to the development after CCl_4 intoxication. Course treatment with meadowsweet extract and its fractions reduced activity of enzymes, which were released into the blood during liver injury, which attests to hepatoprotective properties of the test compounds. Activities of ALT and AST in blood plasma from rats receiving the ethyl acetate fraction decreased to a level observed in intact animals. Meadowsweet extract was less effective in this respect (enzyme activities decreased by 5.5 and 2.4 times, respectively, compared to the control); its effectiveness was comparable to that of the reference preparation. Administration of the chloroform and butanol fraction was followed by less significant decrease in ALT and AST activities.

CCl_4 primarily damages lipids of the endoplasmic reticulum of hepatocytes [7]. The pathological process involves all membrane structures in cells of the target organ. CCl_4 intoxication was accompanied by activation of LPO in the liver tissue. The contents of conjugated dienes, LHP, and TBARS after CCl_4 intoxication increased by 2.1, 2.0, and 3.4 times, respectively, compared to intact animals (Table 1). Meadowsweet extract and its fractions had a normalizing effect on LPO, which was related to direct antioxidant activity (interaction with reactive radicals) [9] and, probably, to membrane-stabilizing properties. Course treatment with meadowsweet extract and fractions was followed by a decrease in the content of conjugated dienes by 1.4-1.6 times compared to control animals. Meadowsweet extract and its fractions decreased the content of LHP to a level typical of intact control specimens. The chloroform fraction was most potent in modifying the content of intermediate LPO products. The effectiveness of this fraction was comparable with that of the reference preparation. Administration of ethyl acetate fraction was followed by maximum decrease in the content of LPO end-products

TABLE 1. Effect of Fractions from Meadowsweet Extract in 70% Ethanol on Aminotransferase Activity in Blood Plasma, Content of LPO Metabolites, and Antioxidant Enzyme Activity in Liver Tissue of Rats with CCl_4 Intoxication ($X \pm m$, n=9)

Group	ALT, mmol/liter/h	AST, mmol/liter/h	Conjugated dienes, U/mg lipids	LHP, U/mg lipids	TBARS, nmol/mg protein/min	SOD, U/mg protein	Catalase, μmol/mg protein/min
Intact animals	0.84±0.09	2.10±0.15	0.37±0.07	0.170±0.010	0.62±0.11	6.36±1.70	31.82±2.01
Control animals	7.20±0.30*	6.30±0.50*	0.80±0.06*	0.340±0.060*	2.13±0.17*	1.02±0.33*	13.22±1.22*
Treatment							
extract	1.30±0.10+	2.60±0.08+	0.56±0.03+	0.124±0.078+	1.10±0.17+	4.20±1.98+	29.55±1.30+
chloroform fraction	3.50±0.40+	3.10±0.20+	0.50±0.04+	0.140±0.033+	1.22±0.44+	3.47±1.31+	27.87±2.20+
ethyl acetate fraction	0.80±0.07+	2.40±0.05+	0.54±0.04+	0.168±0.078+	1.04±0.15+	4.56±1.60+	36.91±1.60+
butanol fraction	2.10±0.05+	3.70±0.07+	0.58±0.04+	0.170±0.059+	1.43±0.28+	2.15±0.40+	20.49±0.40+
Carsil	1.34±0.10+	2.60±0.11+	0.52±0.03+	0.131±0.040+	1.38±0.23+	3.57±1.20+	23.30±1.90+

Note. $p<0.05$: *compared to intact animals; +compared to control animals.

(TBARS) in rats with CCl_4 intoxication (by 2 times). The extract and chloroform fraction were less potent in this respect (decrease by 1.9 and 1.7 times, respectively). The amount of TBARS in animals with toxic hepatitis receiving Carsil was higher than in rats receiving the extract and fractions of meadowsweet plants.

Activities of SOD and catalase in the liver of control animals were much lower than in intact rats (by 6.2 and 2.4 times, respectively; Table 1). The more pronounced increase in SOD activity compared to catalase activity produces toxic effect on cells, since superoxide anion radical is not utilized under these conditions [3]. Course treatment with meadowsweet extract and fractions was followed by an increase in antioxidant enzyme activity in the liver of rats with CCl_4-induced hepatitis. Ethyl acetate fraction and extract most significantly increased antiradical activity of SOD (by 4.5 and 4.1 times, respectively) and antiperoxide activity of catalase (by 2.8 and 2.2 times, respectively). These changes contribute to the recovery of antioxidant activity in the liver. Activities of SOD and catalase increased in animals receiving the chloroform fraction (by 3.4 and 2.1 times, respectively). The effectiveness of meadowsweet extract and fractions surpassed that of Carsil.

Experiments on the model of CCl_4-induced toxic hepatitis showed that fractions of the extract from meadowsweet aerial parts in 70% ethanol exhibit hepatoprotective activity and produce a normalizing effect on activity of plasma enzymes (hepatocyte injury markers ALT and AST), LPO, and antioxidant defense system in liver cells. Dissociation of the hepatoprotective and antioxidant effects of meadowsweet extract reflects the specific action of a complex of bioactive substances. The ethyl acetate and chloroform fractions have the highest hepatoprotective activity, which is probably related to lipophilicity of phenol constituents (simple phenols, flavonoids, phenyl carboxylic acids, coumarins, *etc*.) [5,9]. These compounds inhibit the formation of LPO products in lipid-rich hepatocyte membranes, prevent cytolysis and, therefore, improve liver detoxification function. They interact with peroxide and alkoxyl radicals that are formed during LPO. This property is related to the presence of a mobile hydrogen atom in phenol groups of the molecule, which constitutes a major mechanism of the antioxidant effect. Moreover, phenol compounds chelate metal cations and play a role of antioxidant complex-forming agents [2,3, 6]. The effectiveness of ethyl acetate and chloroform fractions was comparable to or surpassed that of the reference preparation Carsil. Our results suggest that the hepatoprotective effect of meadowsweet extract and fractions is associated with antioxidant and membrane-stabilizing properties.

REFERENCES

1. A. V. Arutyunyan, E. V. Dubinina, and N. N. Zybina, *Methods to Study Free Radical Oxidation and Antioxidant System in the Organism* [in Russian], St. Petersburg (2000).
2. A. D. Gordienko, *Farmatsiya*, **39**, No. 3, 75-78 (1990).
3. V. G. Zaitsev, O. V. Ostrovskii, and V. I. Zakrevskii, *Eksper. Klin. Farmakol.*, **66**, No. 4, 66-70 (2003).
4. V. S. Kamyshnikov, *Handbook of Clinical and Biochemical Laboratory Diagnostics* [in Russian], Minsk (2000), Vol. 1, pp. 171-184.
5. E. A. Krasnov, V. A. Raldugin, I. V. Shilova, and E. Yu. Avdeeva, *Khim. Prirod. Soed.*, No. 2, 122-124 (2006).
6. L. D. Luk'yanova, E. L. Germanova, and A. I. Lysko, *Vestn. Ros. Akad. Med. Nauk*, No. 2, 55-61 (2007).
7. A. S. Saratikov, A. I. Vengerovskii, and V. S. Chuchalin, *Salsola Extract (Lochein) as an Effective Hepatoprotector* [in Russian], Tomsk (2000).
8. I. V. Shilova, T. V. Zhavoronok, N. I. Suslova, *et al*., *Byull. Eksp. Biol. Med*., **142**, No. 8, 181-184 (2006).
9. I. V. Shilova, E. A. Krasnov, E. I. Korotkova, *et al*., *Khim.-Farm. Zh*., **140**, No. 12, 22-24 (2006).
10. Yu. O. Shul'pekova, *Rus. Med. Zh*., **14**, No. 4, 337-340 (2006).
11. Y. Aniya, T. Koyama, C. Miyagi, *et al*., *Biol. Pharm. Bull*., **28**, No. 1, 19-23 (2005).
12. H. Nagata, S. Takekoshi, T. Takagi, *et al*., *Tokai J. Exp. Clin. Med*., **24**, No. 1, 1-11 (1999).