Hepatoprotective and Antioxidant Activity of Meadowsweet Extract during Experimental Toxic Hepatitis

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The extract of meadowsweet aerial parts exhibits hepatoprotective and antioxidant activity during experimental toxic CCl_4 hepatitis. This extract improved liver function. Meadowsweet extract in 70% ethanol (100 mg/kg) was most potent and exhibited low toxicity. By several parameters the effectiveness of this extract surpassed that of Carsil.

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

Liver and biliary diseases occupy an important place in the overall morbidity [4-6]. Despite the existence of numerous potential sources for hepatoprotectors, there are only small number of drugs of this group. They exhibit therapeutic efficacy only after long-term treatment, which is often associated with side effects. In Russia, much attention is paid to the search and synthesis of highly effective and safe hepatoprotectors.

The pathogenesis of liver damage induced by various hepatoprotectors is mainly determined by prooxidant activity of these drugs. Plant antioxidants are promising hepatoprotective agents. The therapeutic effect of these agents under conditions of toxic liver injury is primarily related to the presence of phenol compounds (*e.g.*, flavonoids, coumarins, and phenol carbonic acids). They have a wide range of biological activity, including antioxidant, membrane-stabilizing, antitoxic, and reparative properties [2,4,8].

Meadowsweet (*Filipendula ulmaria (L.) Maxim.*) of the *Rosaceae* family holds much promise in this respect. This plant contains various phenol compounds and is extensively used in traditional medicine for the treatment of digestive diseases [1].

Here we studied hepatoprotective and antioxidant activity of the extract from meadowsweet aerial parts (ME) during experimental toxic hepatitis.

MATERIALS AND METHODS

Experiments were performed on 112 male outbred albino rats weighing 220-240 g. The animals were kept in a vivarium under standard conditions and fed a standard diet. ME was obtained by treatment of meadowsweet aerial parts with solvents (water; 40, 70, and 95% ethanol) at 80-85°C for 2 h. The raw material/extracting agent ratio was 1:10. ME in 70% ethanol consists of various substances that are extracted from ground raw materials with 70% ethanol. The extract contains 3.93% flavonoids by the content of quercetin and its glycosides (dry residue ≥22%). Meadowsweet aerial parts contain simple phenols, flavonoids, organic acids, coumarins, tannins, saponins, polysaccharides, carotenoids, amino acids, macroelements, and trace elements. Toxic hepatitis was modeled by daily intragastric administration of 1 ml/kg CCl_4 in 20% oil solution for 6 days. Toxic hepatitis in rats was verified by aminotransferase activity and contents of bilirubin and its fractions. The suspension of ME in 1% starch gel

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(daily dose 100 mg/kg) was administered intragastrically for the next 5 days. 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. The liver was removed [6]. We measured the content of lipid peroxidation (LPO) products conjugated dienes and lipid hydroperoxides (LHP), spontaneous and Fe^{2+}-induced level of thiobarbituric acid-reactive substances (TBARS), and activity of key enzymes of antiradical and antiperoxide protection superoxide catalase (SOD) and catalase. Protein content was estimated by the microbiuret method [3].

The effect of ME on liver function was studied on the model of hexobarbital sleep. The test compound in a dose of 80 mg/kg was injected intraperitoneally after the formation of toxic hepatitis and therapy with ME and Carsil. We recorded sleep latency (time from administration of the test compound to the first signs of sleep onset) and sleep duration (period of lying on the side) [7].

Acute toxicity of ME in 70% ethanol was studied on 36 male outbred albino mice weighing 22-24 g. The suspension of ME in 1% starch gel was administered intragastrically in single doses of 0.5, 1.0, 2.0, 5.0, 7.0, and 10.0 g/kg. The survival rate, weight, physical activity, behavior, and appetite were recorded over 2 weeks [7].

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

RESULTS

The course treatment with ME was followed by shortening of hexobarbital-induced sleep in rats with toxic hepatitis. Sleep duration in rats with toxic hepatitis approached that in intact control animals (Table 1), which reflects the recovery of antitoxic liver function. Sleep duration decreased most significantly in animals receiving ME in 40 and 70% ethanol (by 1.7 times compared to the CCl_4 control group). Pharmacological activity of extracts was 1.1 times higher compared to that of Carsil.

ME in 70 and 95% ethanol improved the weight indexes of the liver in animals with toxic hepatitis (Table 1). The intensity of LPO increased after CCl_4 intoxication, but returned to normal under the influence of ME. ME probably stabilized the structure and function of liver cells. ME in 70 and 95% ethanol most significantly decreased the content of intermediate and end LPO products compared to the CCl_4 control group. The indexes observed in

TABLE 1. Sleep Duration, Weight Indexes of the Liver, LPO, and Antioxidant Enzymes in Liver Tissue of Rats with CCl_4 Intoxication after Course Treatment with ME ($X \pm m$, $n=8$)

Group	Sleep duration, min	Liver weight index	Conjugated dienes, U/g protein	LHP, U/g protein	TBARS, µmol/liter	Fe^{2+}-TBARS, µmol/liter	SOD, U/mg protein	Catalase, mcat/liter/mg protein
Intact control	74.2±4.7	0.0402±0.0030	0.31±0.01	0.040±0.003	0.66±0.06	0.11±0.02	9.46±1.13	27.90±2.23
CCl_4 control	95.8±1.8*	0.0462±0.0011*	0.52±0.03*	0.130±0.004*	1.84±0.10*	0.66±0.05*	2.57±0.45*	14.88±1.69*
Aqueous ME	73.3±6.4+	0.0500±0.0018	0.40±0.02	0.058±0.003*+	0.98±0.03*+	0.10±0.01+	3.91±0.44*	16.20±1.74*
ME in 40% ethanol	57.8±2.5*+	0.0493±0.0010+	0.38±0.01+	0.060±0.003*+	0.81±0.02+	0.31±0.05*+	4.30±0.61*	17.72±1.90*
ME in 70% ethanol	57.3±5.4*+	0.0425±0.0013+	0.34±0.02+	0.050±0.002+	0.60±0.02+	0.16±0.03+	9.11±1.09+	29.30±2.02+
ME in 95% ethanol	77.7±4.8+	0.0450±0.0036	0.34±0.02+	0.050±0.003+	0.59±0.02+	0.15±0.04+	10.44±1.33+	30.98±2.76+
Carsil	63.2±1.8+	0.0454±0.0009	0.43±0.02	0.090±0.002*+	1.47±0.05*+	0.59±0.06*	5.32±0.76*+	21.37±2.13+

Note. $p<0.05$: *compared to intact control; +compared to CCl_4 control.

treated rats approached those in intact animals. The content of conjugated dienes in treated rats decreased by 1.5 times and did not differ from that in the intact control. Carsil therapy decreased the rate of conjugated diene formation by 1.2 times compared to the CCl_4 control group. However, the rate of conjugated diene formation in treated rats was 39% higher than in intact animals. The course treatment with ME in 70 and 95% ethanol decreased LHP content by 2.6 times compared to the CCl_4 control group. LHP content in treated rats approached that in intact animals. Carsil decreased LHP content only by 1.4 times. The content of TBARS decreased most significantly (by 3.1 times) and did not differ from that the intact control. However, TBARS content in Carsil-treated rats with toxic hepatitis remained high and differed from the CCl_4 control group only by 1.3 times. During Fe^{2+}-induced activation of liver LPO *in vitro*, ME in ethanol (70 and 95%) or water decreased TBARS content by 4 times or greater compared to the CCl_4 control group. The intensity of TBARS formation in treated rats practically did not differ from that in intact animals.

In rats with CCl_4 hepatitis, activity of key enzymes of antioxidant protection (SOD and catalase) detoxifying peroxide and superoxide anion radical retuned to normal after administration of ME in 70 and 95% ethanol (Table 1): antiradical activity of SOD increased by 3.5 and 4.0 times, respectively, and antiperoxide activity of catalase by 2 and 2 times, respectively. compared to the CCl_4 control group. In rats with toxic hepatitis receiving Carsil, enzyme activity did not return to normal. Carsil increased activities of SOD and catalase only by 2 and 1.4 times, respectively, compared to the CCl_4 control group.

These results suggest that ME in 70 and 95% ethanol has high antioxidant activity. ME in 70% ethanol (100 mg/kg) produced a strong protective effect on liver function and was more potent than Carsil. This extract had a stabilizing effect on the liver parenchyma, which was associated with inhibition of LPO in hepatocyte membranes, prevention of cytolysis, and increase in detoxification function of the liver.

Analysis of acute toxicity of ME in 70% ethanol revealed the absence of toxic effects; external appearance and appetite of animals receiving ME in doses of 0.5, 1.0, 2.0, and 5.0 g/kg did not differ from the control. Animal survival was 100%. Intragastric administration of ME in doses of 7 and 10 g/kg caused death of 50 and 80% animals, respectively. Therefore, ME in 70% ethanol has low acute toxicity.

Our findings show that ME has high hepatoprotective and antioxidant activity. The optimal concentration of ethanol is 70% (Table 1). Decreasing the concentration of ethanol is followed by reduction of hepatoprotective and antioxidant activity. However, increasing the concentration of ethanol decreases hepatoprotective properties and contributes to lower activation of SOD and catalase. ME in 70% ethanol exhibits low toxicity and more pronounced hepatoprotective and antioxidant activities compared to Carsil. These specific features are probably associated with chemical structure and content of phenol components (flavonoids, phenol carbonic acids, coumarins, *etc*.). These compounds contain hydroxyls with labile hydrogen atoms and neutralize hydroperoxides of polyenic fatty acids with the formation of nontoxic hydroxy acids. Lipophilic phenol compounds are integrated into hepatocyte membranes and inhibit the formation of primary and secondary LPO products. It should be emphasized that the pathogenesis of hepatitides includes inflammation and LPO imbalance, which is accompanied by hepatocyte destruction and impairment of specific functions. Probably, hepatoprotective activity of ME is associated with the membrane-stabilizing and antioxidant effects.

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