Hepatoprotective Properties of Aqueous Extract from *Pentaphylloides fruticosa* during Chronic Toxic Hepatitis

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Hepatoprotective properties of *Pentaphylloides fruticosa L.* aqueous extract were studied using rat model of chronic toxic hepatitis. The preparation normalized alanine aminotransferase activity and bilirubin concentration in the plasma. Aqueous extract of *Pentaphylloides fruticosa* produced a protective effect on microsomal metabolism of xenobiotics and lipid peroxidation activity in the plasma and microsomal liver fraction.

Key Words: *chronic toxic hepatitis, Pentaphylloides fruticosa L.*

Despite new advances in hepatology associated with application of new laboratory and instrumental diagnostic techniquies and better understanding of the etiology and pathogenesis of many liver diseases at the cellular and molecular levels, the therapy of patients with acute and chronic liver diseases remains a complex and sometimes unsolved problem [5]. Therefore, the search of the new drugs, in particular, phytopreparations possessing hepatoprotective activity is actual.

The purpose of the present study was to evaluate hepatoprotective properties of aqueous extract from the flowers and young shoots of *Pentaphylloides fruticosa* (PFE) during experimental chronic toxic hepatitis.

MATERIALS AND METHODS

Male Wistar rats weighing about 200 g were used. The animals were kept in individual cages under conditions of artificial illumination (12:12 light/dark regimen) and free access to water and food (standard forage) at 26±1°C.

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Chronic toxic hepatitis was modeled by enteral administration of CCl_4 in vegetable oil and ethanol as described elsewhere [4]. Control rats received vegetable oil without CCl_4. Group I rats were sacrificed on day 28 of toxic treatment. Rats receiving CCl_4 alone and sacrificed on days 14 and 28 after toxic treatment comprised groups II and III, respectively. Groups IV and V rats daily received 50 mg/kg PFE via a gastric tube for 14 and 28 days, respectively. Each group consisted of 7-9 animals.

The rats were sacrificed under ether anesthesia, alanine aminotransferase (ALT) activity and plasma concentrations of total and conjugated bilirubin were measured using standard kits (Vector-Best). Functional activity of the hepatic monooxygenase system was estimated by the rate of aniline, aminopyrine, erythromycine [10], and diazepam [6] metabolism in liver microsomal fraction isolated by differential centrifugation. The concentration of lipid peroxidation (LPO) products malonic dialdehyde (MDA) [1] and diene conjugates (DC) [2] and the content of α-tocopherol [12] in the plasma and microsomal fraction were determined. Protein content was determined by the method of Lowry and lipid concentration by the method described elsewhere [2]. The results were processed statistically using Student's *t* and Kolmogoroff—Smirnoff tests.

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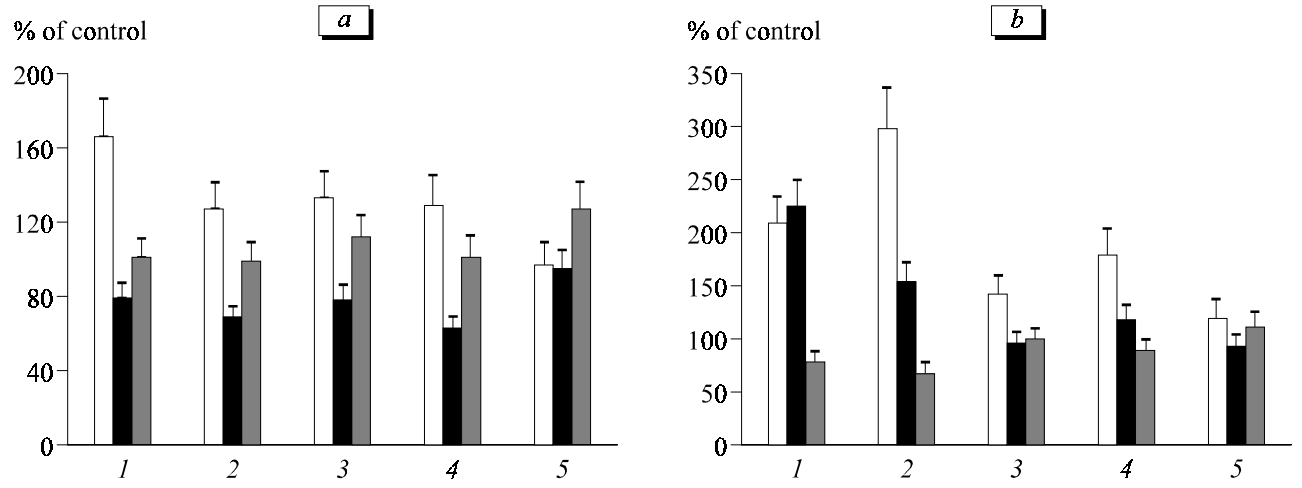

Fig. 1. Content of LPO products and α-tocopherol in blood plasma (*a*) and microsomal fraction (*b*) in rats with chronic toxic hepatitis during treatment with aqueous *Pentaphylloides fruticosa* extract. Open bars: MDA content; dark bars: diene conjugates; hatched bars: α-tocopherol. *1*) day 28 of CCl_4 treatment; *2* and *3*) days 14 and 28 after CCl_4 poisoning without correction; *4* and *5*) days 14 and 28 of treatment with *Pentaphylloides fruticosa* aqueous extract after CCl_4 poisoning.

RESULTS

The concentrations of bilirubin and MDA and ALT activity in the plasma peaked and the decrease in the rate of microsomal oxidation was most pronounced on day 28 of toxic treatment. These changes coincided with MDA and DC accumulation in the microsomal fraction. In groups 2 and 3 the examined parameters tended to decrease, but even after 4 weeks the concentrations of total and conjugated bilirubin and ALT activity in the plasma remained above the control. The concentration of MDA in the plasma and microsomal fraction surpassed the control, while low plasma content of DC attested to intensive LPO and rapid utilization of intermediate LPO [3]. In group II the content of α-tocopherol in the microsomal fraction significantly decreased (by 33.3%) compared to the control.

In rats receiving aqueous PFE for 2 weeks ALT activity and total and conjugated bilirubin were markedly decreased, while after 4-week treatment these parameters returned to normal (Table 1). PFE restored the rate of microsomal oxidation of all examined substrates to the initial level (Table 1). The rate of diazepam metabolism in group IV was 1.4-fold higher than in group II. The effect of PFE on aminopyrine-N-demethylase activity was most pronounced: activity of this enzyme in groups VI and V was 76.6 and 40.2% higher than in group II, respectively, and 1.5-fold surpassed the control.

TABLE 1. Effect of Aqueous ERF on Biochemical Parameters in Plasma and Rate of Xenobiotic Metabolism (per min per mg protein) in Microsomal Liver Fraction in Rats with Experimental Chronic Toxic Hepatitis (*M*±*m*)

Parameter	Control	CCl_4, 28 days	Days after CCl_4 poisoning			
			14		28	
			without correction	+PFE	without correction	+PFE
ALT activity, mmol/l	1.08±0.09	3.20±0.09*	2.06±0.11*	1.28±0.06*+	1.86±0.14*	1.12±0.09+
Bilirubin, μmol						
total	7.50±2.04	27.15±1.79*	16.65±0.49*	14.96±0.94*	11.59±2.72*	9.85±1.77
conjugated	1.49±0.15	11.50±2.50*	11.94±1.11*	8.30±0.86*+	3.84±0.47*	2.07±0.53
Rate of xenobiotic metabolism						
aminopyrine, nmol HCHO	2.41±0.27	0.66±0.07*	1.24±0.08*	2.19±0.11+	2.51±0.22	3.52±0.13*+
aniline, nmol p-nitrophenol	0.32±0.03	0.07±0.01*	0.25±0.04	0.26±0.03	0.31±0.04	0.27±0.02
erythromycine, nmol HCHO	3.44±0.35	1.30±0.19*	2.21±0.15*	2.27±0.35*	3.26±0.28*	3.87±0.29
diazepam, nmol	1.85±0.21	0.64±0.07*	0.92±0.11*	1.28±0.11*+	1.53±0.15	1.49±0.16

Note. $p<0.05$ compared to the control (*), compared to the same day in the group without correction (+).

We assume that the observed changes are determined by the state of the pro- and antioxidant systems. The dynamics of LPO parameters attested to a pronounced normalizing effect of PFE after 14-day application, while after 28-day treatment the concentration of LPO products in the plasma (Fig. 1, *a*) and microsomal fraction (Fig. 1, *b*) returned to the control values. Plasma concentration of α-tocopherol on day 28 of PFE treatment increased by 27.1 and 26.2% compared to control and group III, respectively.

The observed hepatoprotective effect of PFE can be atributed to the presence of water-soluble antioxidants, in particular ascorbic acid, thiol and polyphenol compounds [9]. Reduced bioflavonoids and ascorbic acid reduce α-tocopheryl radicals, thus restoring their antioxidant properties [8,13]. Moreover, ascorbic acid reduces thio- and thioperoxyl radicals of glutathione [11], that participate in the reduction of polyphenol compounds, thus preserving antioxidant properties of *Pentaphylloides fruticosa.* It was shown that LPO resistance in liver microsomes is mediated by glutathione-dependent vitamin E reductase [3,7].

Thus, we demonstrated hepatoprotective properties of aqueous PFE and the possibility of using this preparation for correction of toxic liver diseases.

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