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A Hepatoprotective Activity of *Galium verum* L. Extracts against Carbon Tetrachloride-Induced Injury in Rats

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ABSTRACT

A hepatoprotective activity of the original polyphenolic phytosubstances derived from *Galium verum* herb (*G. verum* L.; Rubiaceae Juss. family), namely I and II dry extracts, was studied against carbon tetrachloride-induced acute hepatitis in rats. The hepatoprotective effect of I and II extracts at the dose of 25 mg/kg is characterized by a decreased activities of serum enzymes, with Serum Alanine Aminotransferase (ALT) decreasing from 2.7-3.5 fold, Serum Aspartate Aminotransferase (AST) decreasing from 2.4-3.4 fold and ALP decreasing from 1.2-1.3 fold respectively; whereas the activity of cholinesterase increases from 1.3-1.4 fold respectively. The administration of the I extract induces a decrease in Thiobarbituric acid Reactive Substances (TBARS) levels 1.4 fold in the serum and 1.8 fold in the homogenate of the liver tissue. The administration of the II extract induces a decrease in TBARS levels 1.6 fold in the serum and 2.0 fold in the homogenate of the liver tissue against the control group. The biochemical parameters taken into account, the possible mechanism of the hepatoprotective activity of the extracts under study can be explained by a reduction in the hepatocytes cytolysis and an oxidative stress. The histopathological analysis of the liver material of the experimental group showed neither degenerative-dystrophic changes nor significant hemodynamic changes in comparison with the control group. The hepatoprotective effect of the II extract is more pronounced than that of the I extract and is comparable to the hepatoprotective activity of the reference drug Silibor.

Keywords: *Galium verum* L., Carbon tetrachloride, Hepatoprotective activity, Extracts

INTRODUCTION

An impact of adverse environmental conditions and increased xenobiotic loads tend to result in an influx of the hepatobiliary system diseases [1-3]. Since pathological processes in liver are linked to the development of an oxidative stress and an inflammatory infiltration, the complete functional recovery of the liver takes a significant period of time. Hence, the indisputable requirements to hepatoprotective drugs [4] are low toxicity and antioxidant, membrane-stabilizing and anti-inflammatory properties. The original phytosubstances developed on the basis of Lady's Bedstraw herb (*Galium verum* L.; Rubiaceae Juss. family) raw material meet all the given criteria.

Species of *Galium* L. genus have long been used as choleric and anti-inflammatory agents in treatment of the hepatobiliary system diseases. Research in Biologically Active Substances (BAS) of various species of *Galium* L. genus and the development of medicinal drugs on their basis seem a promising direction in the search of effective hepatoprotective [5].

Previously, from *G. verum* L. herb we obtained two dry extracts, one by extraction with 70% alcohol followed by the purification from lipophilic compounds –I and the other by aqueous extraction at heating followed by the purification from polysaccharides –II. It was established that DL₅₀ of dry extracts exceed 5000 mg/kg, which, according to Konstantin K. Sidorov toxicity scale, allows classifying them as class VI, or relatively harmless substances [6]. The phenolic compounds, i.e., flavonoids and hydroxycinnamic acids of the extracts were studied [7]. The content of hydroxycinnamic acids in the extracts I and II was established at 10.12% and 9.93%, respectively and the content of flavonoids at 3.77% and 3.54%, respectively. High levels of hepatotropic BAS served as sufficient grounds for the further research in the hepatoprotective activity of the extracts obtained.

The aim of the present study was to evaluate the hepatoprotective activity of the I and II extracts from *G. verum* L. herb in carbon tetrachloride-induced acute hepatitis in rats.

MATERIALS AND METHODS

As the plant material for this study we used dry extracts of *Galium verum* L. herb, namely I and II. The phytochemical profiles of extracts under study are characterized by the presence of hydroxycinnamic acids such as caffeic acid and its derivatives, namely chlorogenic acid and neochlorogenic acid; and the presence of flavonoids such as apigenin, luteolin and quercetin [8,9].

Hepatoprotective activity of the extracts was evaluated in the model of carbon tetrachloride-induced acute liver injury in rats. The experiment was performed on 30 male white rats (weight range 180-250 g). The animals had been kept in compliance with the sanitary standards on a standard diet and under the principles of humane treatment to laboratory animals. The animals were divided into 5 groups of 6 animals each; the animals in the first and second groups were subcutaneously treated with I and II extracts at the dose of 25 mg/kg, respectively, whereas the third group was subcutaneously treated with the reference drug Silibor, at the dose of 25 mg/kg, the fourth group (untreated animals) was left as a control group, and the intact animals constituted as the fifth group.

The carbon tetrachloride acute injury was induced in rats of groups 1-4 with a single subcutaneous injection of carbon tetrachloride (dissolved in oil, 50% solution) at the dose of 0.8 ml/100 g per animal's body weight for 2 days with a 24 h interval. The animals of groups 1-2 were subcutaneously treated with extracts I and II and the animals of the third group were subcutaneously treated with Silibor 1 h before and 2 h after the carbon tetrachloride injection [10,11]. The rats were decapitated on the third day after the first injection of carbon tetrachloride.

The hepatoprotective activity of I and II extracts was evaluated by biochemical parameters, i.e., the activity of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Cholinesterase (CE), Alkaline Phosphatase (ALP) and Thiobarbituric acid Reactive Substances (TBARS) levels measured after 24 h from the last carbon tetrachloride injection as well as by the results of histopathological analysis.

Determining ALT and AST activity was performed by the Reitman-Frankel method (standard reagents of "SIMKO, Ltd.") whereas determining the cholinesterase and alkaline phosphatase activity was carried out spectrophotometrically ("Lachema" standard diagnostic kits, Czech Republic) [12]. TBARS levels were quantified spectrophotometrically through the Korobeynikova method (reaction with 2-thiobarbituric acid) with the use of the biochemical reagent kit, "Filisit-Diagnostic" Company, Ukraine [13]. The histopathological analysis was performed with the use of microscope, Micros 400. Photomicrographies were made with the use of Nikon Cool Pix 4500 digital camera. Digital processing of the photos was performed on a Pentium 4 GH computer (Nikon View 5 Software).

All results are expressed as mean \pm SEM. Comparison between groups was drawn with the use of Student's *t*-test. A $P \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

In the study of hepatoprotective activity of substances the survival rate of the animals was considered as one of the criteria of the efficacy [10]. The mortality rate in the control group constituted 16.7%, in the other groups; the animals were alive throughout the experiment.

The comparative study of biochemical parameters of the control group and the intact animals indicated the development of the acute carbon tetrachloride induced hepatic injury in the control group. In the control group, a significant increase in serum ALT and AST activity (5.7 and 5.4 times respectively) was indicative of the toxic effects of carbon tetrachloride manifested in the development of hepatocytes cytolysis, the oxidative stress was evidenced by the increase in TBARS levels (1.65 and 2.37 fold in the serum and the homogenate of the liver tissue, respectively). The activity of serum cholinesterase decreased by 1.46 times and ALP activity increased by 1.3 times in comparison with those of the intact animals.

Extracts I and II displayed manifested hepatoprotective effects against carbon tetrachloride-induced injury, which is evidenced by the improvement of all assessed biochemical parameters (Table 1).

Table 1: Effects of I and II extracts on carbon tetrachloride-induced liver injury in rats

Biochemical and hematological parameters	Research materials				
	I	II	Silibor	CCl ₄ (dis. in oil, 50% solution)	Intact animals
Dose, mg/100 g	2.5	2.5	2.5	0.8 ml	-
Blood serum					
ALT, $\mu\text{mol/h.ml}$	0.50 \pm 0.02	0.39 \pm 0.02**	0.27 \pm 0.01**	1.36 \pm 0.04**	0.24 \pm 0.02**
AST, $\mu\text{mol/h.ml}$	0.44 \pm 0.02*	0.32 \pm 0.02**	0.23 \pm 0.01**	1.08 \pm 0.04**	0.20 \pm 0.02**
CE, $\mu\text{kat/l}$	73.2 \pm 1.68*	79.5 \pm 1.24**	72.3 \pm 1.45**	57.8 \pm 1.54**	84.5 \pm 1.89
ALP, $\mu\text{kat/l}$	1.67 \pm 0.07*	1.60 \pm 0.08**	1.85 \pm 0.07**	2.08 \pm 0.13**	1.60 \pm 0.08
TBARS, nmol/ml	4.11 \pm 0.10*	3.64 \pm 0.06**	3.86 \pm 0.05**	5.84 \pm 0.02**	3.52 \pm 0.07
Homogenate of the liver tissue					
TBARS, $\mu\text{mol/g}$	34.3 \pm 1.14*	30.7 \pm 0.88**	33.5 \pm 0.87**	62.2 \pm 1.51**	26.2 \pm 0.63

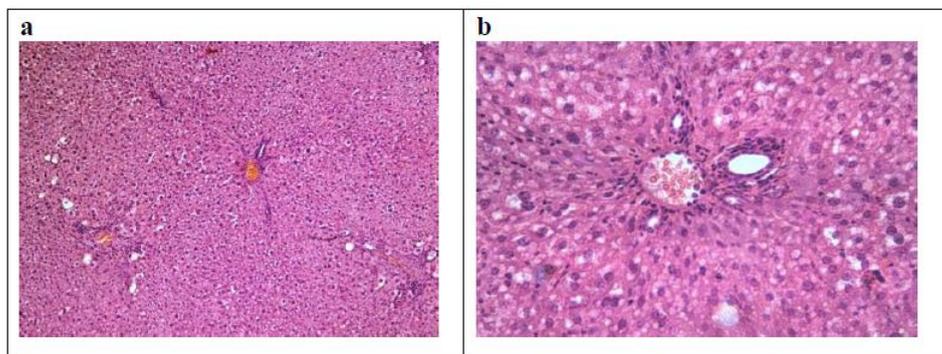
Note: animals subjected to no substances, *Mean estimated error for the intact animals ($P \leq 0.05$); **Mean estimated error for the control group ($P \leq 0.05$)

Blood samples from the animals treated with the I dry extract showed a decrease in serum ALT and AST activity (by 2.7 and 2.4 times, respectively) and ALP activity (by 1.2 times) and an increase in serum cholinesterase activity (by 1.3 times), as well as a decrease in TBARS levels (by 1.4 and 1.8 times in the serum and the homogenate of the liver tissue, respectively) in comparison with those in the control group.

Blood samples from the animals treated with the II dry extract showed a significant decrease in serum ALT and AST activity (by 3.5 and 3.4 times, respectively) and ALP activity (by 1.3 times), and increase in serum cholinesterase activity (by 1.4 times); a decrease in TBARS levels (by 1.6 and 2.0 times in the serum and the homogenate of the liver tissue, respectively) in comparison with those in the control group.

The biochemical parameters taken into account, the possible mechanism of the hepatoprotective activity of the extracts under study can be explained through an inhibited cytolysis of hepatocytes and reduced oxidative stress, which is indicative of the membrane-stabilizing and anti-inflammatory properties antioxidant of the given phytochemicals.

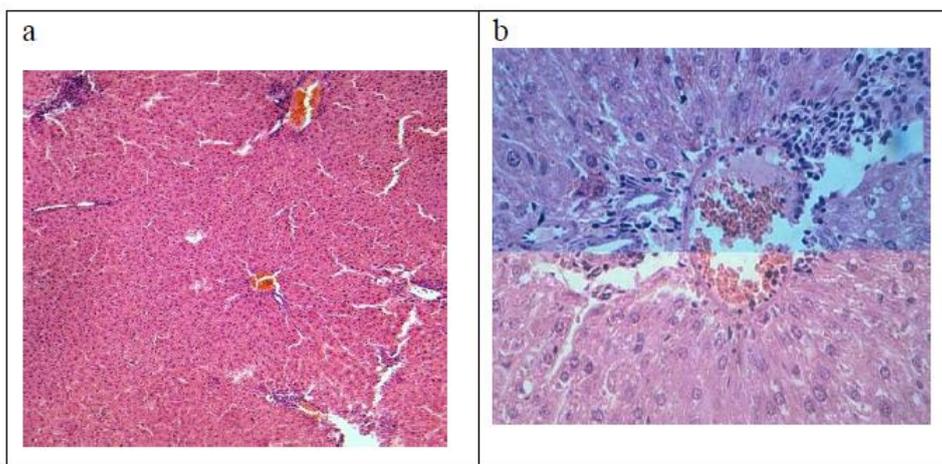
The histopathological analysis showed no degenerative-dystrophic changes and no significant hemodynamic changes in the livers of the treated animals in comparison with the intact animals. In the animals treated with the I extract (Figure 1) the liver lobules were normal with their cytoarchitectonics preserved, the hepatocytes with sharply-margined nuclei, the cytoplasm transparent with an odd granular degeneration and hyaline-drop dystrophy. Scattered lymphocytes and macrophages were detected mostly in the perivascular space and fibrotic tissue in the periphery of bile ducts.



(a) Uniformly distributed hypertrophic hepatocytes with transparent cytoplasm (magnification $\times 10$); (b) Granular and hyaline-drop dystrophy (magnification $\times 40$). Staining: haematoxylin and eosin

Figure 1: Liver tissue of animals treated with I extract

In the animals treated with the II extract (Figure 2) hepatic beams consisted of two rows of hepatocytes with normal and hyperchromic nuclei with radially directed, unevenly-filled sinusoidal capillaries converging into the vein. In central parts of the lobules, full-fledged central veins were detected. The periphery of the lobules featured portal tracts formed by the interlobular artery, the interlobular vein and the interlobular bile duct. The walls of the bile ducts and blood capillaries were well-circumscribed. In some hepatocytes, granular dystrophy and hydropic degeneration were detected. Scattered hepatocytes had an enlarged nucleus and binuclear hepatocytes were observed. Liver lobules were normal, with no necrotic changes in hepatocytes detected, and no cholestasis observed. The histological picture of the liver was normal.



(a) Hepatocytes with granular dystrophy and hydropic degeneration (magnification $\times 10$); (b) Radially directed, unevenly-filled sinusoidal capillaries converging into the vein (magnification $\times 40$). Staining: haematoxylin and eosin

Figure 2: Liver tissue of animals treated with II extract

CONCLUSION

It was established that the I and II extracts from *G. verum* L. herb at the dose of 25 mg/kg display a hepatoprotective activity against carbon tetrachloride-induced acute hepatitis in rats. This was evidenced by the improvement of all assessed biochemical parameters and histopathological analysis. The hepatoprotective effect of II extract is more pronounced than that of I extract and is comparable with the hepatoprotective activity of the reference drug, Silibor. The results of the preclinical studies give grounds for further in-depth pharmacological research in the hepatoprotective activity of the II extract.

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