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Effects of Bergenia crassifolia lyophilized water extract on carbon tetrachloride-induced chronic liver injury in rats

Wpływ liofilizowanego wodnego ekstraktu *Bergenia crassifolia* na przewlekłe uszkodzenie wątroby indukowane czterochlorkiem węgla

INTRODUCTION

Bergenia crassifolia is used in clinical practice as astringent, anti-inflammatory, antimicrobial, haemostatic agent. Decoction and fluid extract made of the rhizome of *Bergenia* are recommended for the treatment of colitis, enterocolitis, stomatitis, gingivitis, uterine bleeding. Drug dosage forms made of leaves of this plant were shown to produce antibacterial, antioxidant, cerebroprotective, choleretic, diuretic effects.

Bergenia crassifolia contains a complex of biologically active substances like flavonoids, coumarins, phenolglycosides, polysacchirides, vitamins, organic acids, aminoacids et. al [6]. One of the most important active substance of *Bergenia crassifolia* is bergenin. Recently it has been shown to have hepatoprotective activity and flavonoids have been shown to possess antioxidant properties [3, 7].

The aim of this study was to investigate the ability of *Bergenia crassifolia* lyophilized water extract (BCLWE) to prevent chronic CCl_4 -induced liver injury.

MATERIAL AND METHODS

BCLWE was prepared by original technology as described previously [2]. Adult male rats weighing 220–280 g were used. Animals were maintained on a standard diet, given water *ad libitum*, and housed in a temperature- and humidity-controlled room, under a constant 12-h light/dark cycle.

The Local Ethics Committee approved the study. The procedures involving the animals and their care conformed to the institutional guidelines and were in compliance with national and international laws and guidelines for the Use of Animals in Biomedical Research.

Animals were randomly divided into three experimental groups: 1st – control group; 2nd – animals receiving CCl_4; 3rd – animals receiving CCl_4 and BCLWE. Each group consisted of 10 animals.

Chronic liver injury was induced by repeated intragastric administration of 20% CCl_4 solution in olive oil (2 ml/kg b.wt.) two times a week for 30 days. BCLWE (80 mg/kg b.wt.) was administered daily in the form of suspension intragastrically throughout the experimental period. The control group received an equivalent amount of water intragastrically every day throughout the experimental period.

Three days after the last CCl_4 administration, the rats, were sacrificed under ether anesthesia; the liver was removed for examination and blood samples were taken. Serum albumin, bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma-glutamyltranspeptidase activities, cholesterol were determined using biochemical analyzer BTS-370 (Spain).

For histology, the right lobe of each liver was fixed in 10% buffered formalin for 24 h and embedded in paraffin. For each staining, to prevent any bias because of the sampling error, the quantification was performed by the analysis of at least 10 different regions and examined by the same experienced pathologist who was blinded to laboratory data. The sections were stained with haematoxylin and eosin, chromotrope aniline blue. Haematoxylin and eosin slides were used for morphologic evaluation, assessment of steatosis, infiltration and hepatocellular ballooning. Collagen fibers were demonstrated by chromotrope aniline blue. Following the previously described methods, the hepatocyte injury (alteration) was semi-quantitatively assessed using a scoring system of 0 = microvesicular steatosis in the perivenular region (no injury); 1 = microvesicular steatosis in the perivenular region and single swollen hepatocytes (mild injury); 2 = microvesicular steatosis in the perivenular region and mid-zone, periseptal hepatocyte balooning (moderate injury); and 3 = diffuse microvesicular steatosis, periseptal hepatocyte balooning, apoptosis and coagulation (severe injury).

Fibrosis was also assessed using semiquantitave scoring system: 0 = normal portal tracts (no fibrosis); 1 = sclerosis of portal tracts and single short connective tissue septa (mild fibrosis); 2 = multiple long connective tissue septa (porto-central) (moderate fibrosis) 3 = cirrhosis (nodularity) (severe fibrosis).

The data are expressed as means ± SD. One-way analysis of variance, followed by Newman-Keuls test, were performed for multiple comparison among the groups. Values of $P< 0.05$ were considered statistically significant.

RESULTS

The serum biochemical and histological changes in three groups of rats are summarized in Table 1.

Table 1. Biochemical and histological variables in rats

	Group 1 Control	Group 2 CCl_4	Group 3 CCl_4 + BCLWE
Albumin	23.81±1.47	23.51±1.31	23.85±1.38
Bilirubin	2.93±0.55	3.07±0.46	2.74±0.44
Cholesterol	2.09±0.24	2.01±0.41	2.19±0.25
AST U/L	173.01±19.12	389.61±120.22*	275.76±42.01**
ALT	160.07±24.54	329.67±94.87*	224.43±41.22**
GGTP	40.53±14.59	43.49±8.95	46.16±11.39
ALP	279.91±38.19	770.38±104.38*	743.16±108.67*
Alteration (n(%))		*	**
0	8 (80)	-	-
1	2 (20)	2 (20)	8 (80)
2	-	4 (40)	2 (20)
3	-	4 (40)	-
Fibrosis (n(%))		*	
0	9 (90)	-	-
1	1 (10)	-	1 (10)
2	-	8 (80)	9 (90)
3	-	2 (20)	-

*p< 0.05 vs control group, **p< 0.05 vs CCl_4 group

Chronic administration of CCl_4 led to the increase of serum activity of AST, ALT, ALP, while serum albumin, bilirubin, cholesterol and GGTP levels were unchanged. Most rats showed a high degree of alteration and fibrosis: diffuse or focal microvesicular steatosis, multiple foci of necrosis and apoptosis; porto-portal and porto-central septa with nodule formation – cirrhotic transformation. Morphological findings were statistically significant for alteration and for fibrosis.

Compared with the CCl_4 group, in the CCl_4 + BCLWE group serum enzymatic activity had a tendency to improve. Serum AST and ALT levels were lower in the CCl_4 + BCLWE group, while serum ALP level was about the same in both groups. There were no significant differences in serum albumin, bilirubin, cholesterol and GGTP levels among three groups of rats. In rats receiving CCl_4 + BCLWE degrees of parenchymal injury and fibrosis are lower. The histological examination in most animals shows microvesicular steatosis in the perivenular region and mid-zone; signs of cellular death are not prominent. Fibrosis is manifested by enlarged portal tracts, several short and porto-portal connective tissue septa associated with indistinct nodularity. However, these differences are not statistically significant.

DISCUSSION

In the present study administration of BCLWE partially prevented elevation of serum ALT and AST, it attenuated parenchymal injury and fibrosis in rat liver induced by chronic administration of CCl_4. Hepatoprotective effect was confirmed by a statistically significant decrease in cell injury score – signs of necrosis and apoptosis were less prominent in animals treated with BCLWE. Antifibrotic effect was not so distinct. The signs of fibrosis were of moderate degree, but these differences were not statistically significant. These results coincide with that of M.W. Chung [3] who established that bergenin and acetyl bergenin ameliorated the liver damage induced by bile duct ligation in rats. In this study, bergenin and acetyl bergenin prevented elevation of serum AST and ALT, reduced accumulation of hydroxyproline, decreased the damaged area. The mechanisms underlying these effects are not known. Based on the results of Lim et al. [5], hepatoprotective effects of bergenin and acetyl bergenin are related to glutathione-mediated detoxification and their free radical suppressing activity. Antifibrotic activity of BCLWE may be attributed to bergenin-induced stimulation of prostaglandins E1 and E2 synthesis which inhibit the growth of hepatic stellate cells that are essential for the development of liver fibrosis [1].

CONCLUSIONS

BCLWE exerts a hepatoprotective effect against chronic CCl_4-induced lesions of liver tissue.

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SUMMARY

BCLWE exerts a hepatoprotective effect against chronic CCl_4-induced lesions of liver tissue in rats, which is confirmed by prevention of serum ALT and AST activity elevation and progression of liver cell injury and fibrosis.

Keywords: Bergenia crassifolia, hepatic fibrosis, carbon tetrachloride

STRESZCZENIE

Liofilizowany ekstrakt wodny *Bergenia crassifolia* wykazuje działanie hepatoprotekcyjne na tkankę wątroby z przewlekłym uszkodzeniem spowodowanym CCl_4, co potwierdzono brakiem wzrostu ALT i AST w surowicy oraz postępu uszkodzenia komórek i włóknienia wątroby.

Słowa kluczowe: Bergenia crassifolia, zwłóknienie wątroby, czterochlorek węgla