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Protective effect of *Agrimonia eupatoria* L. against chemical-induced hepatotoxicity

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ABSTRACT

The hepatoprotective activities of *Agrimonia eupatoria* L. extract were assessed by measuring hematological and serological levels in SD rats following chemical-induced liver injury. The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were dose-dependently decreased in carbon tetrachloride (CCl_4) and D-galactosamine (GalN)/lipopolysaccharide (LPS)-induced liver injury in rats. These results revealed significant, dose-dependent hepatoprotective potential for *A.eupatoria* extract as it restored the majority of abnormal hepatic parameters in experimental liver damage in rats; it may therefore be useful in recovery of liver disorders.

KEYWORDS

Hepatoprotective activity;
Carbon tetrachloride;
D-galactosamine;
Lipopolysaccharide;
Agrimonia eupatoria L.

INTRODUCTION

The liver regulates many important metabolic functions and damage to the liver is associated with distortions of these metabolic functions. Despite tremendous advances in modern hepatology, many environmental toxins cause liver injury in humans, and there is currently no reliable hepatoprotective drug available for liver disease therapy. Therefore, a number of natural products have been studied to evaluate their hepato protective activity.

The *Agrimonia* species have been reported to possess several activities, including anti-viral[6], anti-tumor[7], diuretic[4], anti-diabetic[9] and anti-oxidant activities[10].

Recently, it has been reported that some *Agrimonia* species inhibit hepatitis surface antigen (HBsAg) secretion from HBV infected cells[5]. Based on this result, the present study was designed to evaluate whether administration of *A.eupatoria*, one of the *Agrimonia*

species, can reduce liver damage in CCl_4- and GalN/LPS-induced hepatotoxicity in SD rats.

MATERIALS AND METHODS

Animals

Six-week-old male SD rats were obtained from Central Laboratory Animal Inc. (Korea). All animals were maintained in plastic cages at 23±2°C on a 12 h light/dark cycle with free access to pellet food and water. All animal experiments complied with the NIH guidelines for the care and use of laboratory animals (NIH Publication No. 80-23; revised 1978).

Plant material and preparation of aqueous extract

The plant, *Agrimonia eupatoria* L. of genus *Agrimonia*, was collected in Youngdong (Korea) from May to August, 2005. The specimen was identified by Kyongnam Agricultural Research Extension Services (Jinju, Korea). The aerial parts of the plant were dried and then extracted with 40 volumes of distilled water. The extraction was performed for 1h at 60°C. This extract was filtered and the filtrate was centrifuged at 10.000 rpm using an A8.24 rotor in a Kontron T-324 centrifuge[5]. The extract was lyophilized and then weighed.

Induction of hepatic damage

CCl_4-induced hepatotoxicity

Liver injury was produced by IP administration of a single dose of CCl_4 mixed with olive oil (1:4v/v %; 5ml/kg). Four hours before and after CCl_4 administration, a dose of 25, 50 and 100mg/kg of extract was administered to rats by PO. Using the same procedure, 25mg/kg of ursodeoxycholate (UDCA) and silymarin were administered to rats as a positive control.

D-GalN/LPS-induced hepatotoxicity

Rats were given 200mg/kg of GalN and 60μg/kg of LPS by IP. Extract and other chemicals were administered to rats as in the CCl_4 experiment.

Hematological analysis

Whole blood was used to assay WBC, RBC, HGB and HCT using a hematology analysis system (JT Counter, Coulter Co, USA).

Serological analysis

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were measured in serum with a serum analyzer (Advia 1650, Jeol Co., Japan).

Statistical analysis

The data obtained were subjected to statistical analysis using Dunnett's test for multiple comparison with control. A p value of <0.05 or less was the criterion for significance.

RESULTS AND DISCUSSION

The results of this study demonstrate that *Agrimonia eupatoria* L. extract promotes liver recovery and might prevent CCl_4- and GalN/LPS-induced hepatotoxic activity.

CCl_4- and GalN/LPS-induced hepatic injury are experimental models that are widely used for hepatoprotective drug evaluation. CCl_4 is biotransformed by hepatic microsomal cytochrome P450 to produce trichloromethyl and peroxyl free radicals. These hepatotoxic metabolites react with proteins and lipids in cell organelle membranes, leading to membrane lipid peroxidation and necrosis of hapatocytes[1]. The altered membrane permeability that results from hepatic injury causes enzymes to be released from the cells into circulation; therefore, damage to hepatic cells is revealed by abnormally high serum levels of hepato specific enzymes, i.e., AST and ALT. GalN is an amino sugar that is selectively metabolized by hepatocytes. Uridine diphosphate (UDP)-GalN derivatives accumulate in liver, which depletes the uridine triphosphate (UTP) pool and thereby inhibits RNA and protein synthesis[2]. When GalN is given together with LPS, animals develop lethal liver injury that mimics fulminant hepatitis[3]. Therefore, the injection of GalN and LPS is a useful experimental model of acute hepatic damage, and the leakage of hepatic housekeeping enzymes such as AST, ALT and LDH is commonly used as a biochemical index of hepatocellular damage.

In CCl_4-intoxicated rats, treatment with the *A.eupatoria* extract decreased the serum AST, ALT and LDH activity in a dose-dependent manner(TABLE 1). It suggests that the *A.eupatoria* extract may scavenge

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TABLE 1: Hematological and serum biochemical changes following oral treatment with *Agrimonia eupatoria* L. extract in CCl_4-induced liver injury

Group/Item	VC	Con (-)	Con (+)	Low dose (25 mg/kg)	Middle dose (50 mg/kg)	High dose (100 mg/kg)
AST	182.0±12.0	349.7±19.2	256.3±12.3[a]	252.5±27.3[a]	234.3±22.2[a]	201.1±15.3[a]
ALT	46.2±4.7	178.0±15.0	124.8±6.7[a]	129.2±13.1[a]	102.9±10.8[b]	99.5±12.3[b]
LDH	2136.6±88.2	2688.2±108.9	2822.2±140.1	2232.4±122.4[a]	1769.6±201.5[a]	1722.5±109.9[a]
WBC	9.44±1.22	9.49±1.32	9.52±1.28	10.11±1.22	10.01±0.79	9.53±1.28
RBC	6.51±0.12	6.56±0.47	6.76±0.41	6.58±0.33	6.53±0.32	6.59±0.24
Hb	14.50±0.33	14.60±0.36	14.76±0.32	14.68±0.29	14.62±0.38	14.79±0.41
HCT	42.16±1.33	41.41±1.23	42.36±1.55	44.32±1.51	41.42±1.41	42.41±1.48

VC: Olive oil, Con (-):CCl_4, Con (+): CCl_4 + UDCA, Silymarin 25mg/kg, Treatment groups: CCl_4 + *Agrimonia eupatoria* L. extract [a]p < 0.05 vs. CCl_4 control group; [b]p < 0.01; Values represent the mean±SD.

TABLE 2: Hematological and serum biochemical changes following oral treatment with *Agrimonia eupatoria* L. extract in GalN/LPS-induced liver injury

Group/Item	VC	Con (-)	Con (+)	Low dose (25 mg/kg)	Middle dose (50 mg/kg)	High dose (100 mg/kg)
AST	211.4±13.5	1211.8±66.2	342.6±25.31[b]	252.0±25.6[b]	248.8±14.9[b]	221.8±18.3[b]
ALT	50.8±3.3	608.9±10.7	272.8±22.3[b]	156.4±18.9[b]	75.8±16.8[b]	65.5±14.9[b]
LDH	1123.4±102.3	1321.0±123.7	952.6±108.3[a]	1111.2±95.3[a]	920.8±105.6[a]	868.6±142.3[a]
WBC	9.75±1.45	12.06±2.63	12.01±2.25	12.01±2.89	10.84±2.14	9.58±3.84
RBC	7.12±0.15	8.01±0.33	7.74±0.41	7.50±0.32	7.18±0.35	7.36±0.42
Hb	14.69±0.41	16.98±0.56	16.80±0.57	15.46±0.55	15.68±0.29	15.00±0.69
HCT	46.23±1.10	52.02±2.84	50.32±1.98	47.13±1.87	47.65±0.41	46.25±2.02

VC: Olive oil, Con (-):GalN/LPS, Con (+): GalN/LPS + UDCA, Silymarin 25mg/kg, Treatment groups: GalN/LPS + *Agrimonia eupatoria L.* extract; [a]p < 0.05 vs. GalN/LPS control group; [b]p < 0.01; Values represent the mean±SD.

CCl_4-derived radicals, thereby depressing the toxicity.

GalN/LPS induced liver injury was protected by treatment with the extract of *A.eupatoria* as evidenced by reduction in the increased serum AST, ALT and LDH levels compared with group Con(-) (TABLE 2). The level of ALT, AST and LDH is the functional parameter in acute hepatic injury. Therefore, the attenuation of these levels indicates *A.eupatoria* have hepatoprotective activity. There were no differences in body weight and body weight: liver weight ratios between the treatment and non-treatment groups. Also, hematological indices did not show detectable changes in all groups (TABLES 1, 2).

The abnormal higher levels of serum AST, ALT and LDH observed in our study are the consequence of CCl_4- and GalN/LPS-induced liver dysfunction and denote the damage to hepatic cells[8]. The administration of *A. eupatoria* extract reduced the enhanced level of serum AST, ALT and LDH in CCl_4- and GalN/LPS-treated rats. These results indicate that treatment with *A.eupatoria* extract offers protection against hepatotoxicity induced by CCl_4 and GalN/LPS in rats, and also maintains the functional integrity of hepatic cells. In conclusion, although this study was limited to the SD rat model, the results suggest that *A.eupatoria* extract could be a good source for a protective drug for liver disorders.

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