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Hepatoprotective Activities of *Rosa davurica* Root Extract in Rats Intoxicated with Bromobenzene

Jong Cheol Park[1,*], Jong Moon Hur[1], Young Hee Hwang[2], Myeong Rak Choi[3], Suk Nam Kim[4] and Jong Won Choi[5]

[1]*Department of Oriental Medicine Resources and Research Institute of Korean Oriental Medicine, Sunchon National University, Suncheon 540-742, Republic of Korea*
[2]*Department of Forestry, Chonbuk National University, Jeonju 561-756, Republic of Korea*
[3]*Department of Biotechnology, Yosu National University, Yosu 550-749 Republic of Korea*
[4]*Rosa davurica Institute, Korea National Agricultural College, Hwasung 445-890, Republic of Korea*
[5]*Department of Pharmacy, Kyungsung University, Busan 608-736, Republic of Korea*

Abstract

To investigate hepatoprotective activities of the root extract of *Rosa davurica*, the activities of hepatic enzymes, aminopyrine N-demethylase, aniline hydroxylase, glutathione S-transferase and epoxide hydrolase in rats intoxicated with bromobenzene were studied. Pretreatment with the methanol extract from the roots of *Rosa davurica* did not show any significant effects on the increases of the activities of aminopyrine N-demethylase and aniline hydroxylase, enzymes forming toxic epoxide by bromobenzene. There was no change in glutathione S-transferase activity by *Rosa davurica*. However, the activity of epoxide hydrolase, an epoxide-removing enzyme, was increased 33% by the administration of 500 mg/kg of the methanol extract. From the results, the protection of *Rosa davurica* against bromobenzene-induced hepatotoxicity is thought to be *via* enhancing the activity of epoxide hydrolase, an enzyme removing toxic epoxide rather than through epoxide-producing system.

Key words – Bromobenzene, *Rosa davurica*, Hepatoprotective effect, Epoxide hydolase, Hepatic enzyme activity

Introduction

Rosa davurica Pall. (Rosaceae) is a shrub which is widely distributed in the northeast part of China. The fruits of *Rosa davurica* have been used as a traditional oriental medicine for treatment of dyspepsia, gastroenteralgia and menoxenia, and also as a folk medicine [1]. *Rosa davurica* was reported that the extract had a promoting action on learning and memory in mice [2], inhibition of immediate-type allergic reaction [3] and anti-HIV protease activity [4]. The isolation of triterpenoid [5] and tannin compounds [6,7] from this plant was previously reported. As a part of our continuing search for the antihepatotoxic components in the medicinal plants we have investigated the biological activity of *Rosa davurica* in rats intoxicated with bromobenzene.

Bromobenzene is a toxic industrial solvent that is known to produce centrilobular hepatic necrosis through the formation of reactive epoxides as the toxic intermediates. Epoxides inhibit the activities of various enzymes and act as a mutagen. They are often associated with cancer, aging and metabolic diseases. The toxins are converted to bioreactive intermediates which are harmful

*To whom all correspondence should be addressed
Tel : +82-61-750-3660, Fax : +82-61-750-3608
E-mail : flavonoid@empal.com

:o the body and undergo further metabolism to be :xcreted [8,9]. In the present paper we describe the effects)f methanol extract from *Rosa davurica* roots on bromo-)enzene-metabolizing enzyme system in rat.

Materials and Methods

Plant material

The roots of *Rosa davurica* were collected in Jeongseon, Kangwon-do, Korea on September 2, 1998 and authen-icated by the author (J.C.P.). The voucher specimen (No. NM0353) is deposited at the Herbarium of Department)f Oriental Medicine Resources, Sunchon National Uni-versity. The dried and powdered root of *Rosa davurica* was refluxed with MeOH for 3 hour. The extract was concentrated and freeze-dried.

Animals

Male Sprague-Dawley rats, 6 to 7 weeks old weighing about 180-200 g, were used for the study. Animals were fed with commercial standard rat diet and water *ad libitum*, and maintained at 20±2℃ and with a 12 hr light/dark cycle. Animals were orally administered daily with 250 or 500 mg/kg of the methanol extract from of *Rosa davurica* roots for one week. Samples were dissolved in 1% Tween 80. Normal group was given 0.2 ml of 1% Tween 80 solution per 200 g.

Induction of hepatic injury

Bromobenzene (460 mg/kg) [10] was *i.p.* injected four times with 12 hr interval during final two days of the oral treatment of *Rosa davurica* extracts. The animals were starved overnight before sacrificed in order to reduce the variation of hepatic metabolism. Animals were sacrificed by exsanguination under anesthesia with CO_2 after 12hr injected final bromobenzene injection.

Enzyme source

The liver was immediately removed and weighed after exhaustive perfusion with ice-cold saline through portal vein until uniformly pale. After trimmed and minced, the liver was homogenized with 4 volumes of ice cold 0.1 M potassium phosphate buffer (pH 7.5). The homogenate was centrifuged at 600×g for 10 min. The resulting supernatant was recentrifuged at 10,000×g for 20 min, and the supernatant was further centrifuged at 105,000×g for 60 min to obtain the upper fraction as cytosol. The pellet was resuspended in the same volume of 0.1 M potassium phosphate buffer and centrifuged at 105,000×g for 60 min to obtain the microsomal fractions. The cytosolic fraction was used as the enzyme source of glutathione S-transferase. And the microsomal fraction was used for the measurement of the activities of aniline N-demethylase, aniline hydroxylase and epoxide hydrolase.

Enzyme assays

Aminopyrine N-demethylase activity was assayed by measuring the production of formaldehyde formed by the demethylation of aminopyrine [11]. The reaction mixture consisted of 300-400 μg microsomal protein, 0.1 M Na^+/K^+ phosphate buffer (pH 7.5) and 2.0 mM aminopyrine in a total volume of 2.0 ml. The mixture was preincubated for 3 min at 37℃ and 0.5 mM NADPH was added to initiate the reaction. After 30 min the reaction was stopped by the addition of 0.5 ml of 15% $ZnSO_4$ and saturated $Ba(OH)_2$, and cooled at room temperature. After centrifugation at 1,000 x g for 10 min, 1 ml of the supernatant was mixed with 5 ml of Nash reagent. Then the tubes were capped and heated at 60℃ for 30 min. After cooling in tap water, the absorbance was read at 415 nm against a water blank. The activity was expressed as nmol of formaldehyde per mg protein per min.

Aniline hydroxylase activity was assayed by determining *p*-aminophenol formation from aniline [12]. The basic incubation system was same as described above, except that 1 mM aniline was used as the substrate. The reaction was initiated by the addition of 0.5 mM NADPH. After shaking for 30 min at 37℃, the reaction was

terminated by the addition of 0.5 ml of 20% trichloroacetic acid. The mixture was centrifuged at 1,000×g for 10 min and 1 ml of the supernatant was added to 1 ml of 0.2 N NaOH containing 2% phenol. After mixing, 1 ml of 10% Na_2CO_3 was added. After 20 min at room temperature the absorbance was read at 640 nm against a water blank. The activity was expressed as nmol of *p*-aminophenol per mg protein per min.

Glutathione S-transferase activity was assayed by conjugated glutathione 2,4-dinitrobenzene formation from 1-chloro-2,4-dinitrobenzene [13]. The reaction mixture consisted of 100 μl cytosol fraction, 0.1 M potassium phosphate buffer (pH 6.5), 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM glutathione in a total volume of 3.5 ml. The mixture was incubated at 25℃ for 2 min. The measurement was carried out by spectrometric changes at maximal absorbance wavelength (340 nm) per unit time and calculated with molar extinction coefficient (9.6 $mM^{-1}cm^{-1}$). The activity was expressed as nmol of 1,2-dinitro-4-nitrobenzene per mg protein per min.

Epoxide hydrolase activity was measured spectrophotometically by monitoring the rate of decrease in transstilbene oxide (TSO) at 229 nm [14]. The reaction mixture was consisted of 100-200 μg microsomal protein and 3.0 mM TSO in 0.05 M potassium phosphate buffer (pH 7.0) with a total volume of 3.0 ml. The mixture was incubated for 20 min at 37℃. The activity was defined as nmol of TSO per mg protein per min.

Protein assay

Protein content was determined by the method of Lowry *et al.* [15] using bovine serum albumin as a standard.

Statistical analysis

The statistical differences between the experimental group were determined by Duncan's multiple range test.

Results and Discussion

Bromobenzene, a xenobiotic liver toxin, is converted to the nontoxic 2,3-oxide, which readily forms 2-bromophenol, or the toxic 3,4-oxide by mixed function oxidase system in the liver. Bromobenzene 3,4-oxide acts as a liver toxin. The harmful epoxide is metabolized to a nontoxic bromobenzene 3,4-dihydrodiol by epoxide hydrolase. Bromobenzene 3,4-oxide can also be converted to bromobenzene glutathione by the action of glutathione S-transferase and excreted thereafter. When more 3,4-epoxide is produced than can readily be detoxified, cell injury increases [16].

The effects of methanol extract from *Rosa davurica* roots on bromobenzene-metabolizing enzyme system in rat were investigated. Rats were orally administered daily with 250 or 500 mg/kg of the methanol extract from *Rosa davurica* roots for one week. During final two days of the oral treatment, rats were *i.p.* injected with bromobenzene (460 mg/kg) four times with 12 hr interval. The changes in the activities of hepatic aminopyrine N-demethylase and aniline hydroxylase, 3,4-epoxide-producing enzymes are shown in Tables 1 and 2. The treatment of bromobenzene increased the activities of aminopyrine N-demethylase and aniline hydroxylase compared with the control groups. Oral pretreatments with the methanol extract did not show any significant effects on the

Table 1. Effects of methanol extract from *Rosa davurica* roots on the hepatic microsomal aminopyrine N-demethylase activity in bromobenzene-treated rats[1]

Group	Aminopyrine N-demethylase*
Normal	4.79 ± 0.30^b
BB	6.27 ± 0.57^a
ME250+BB	5.90 ± 0.42^a
ME500+BB	5.78 ± 0.55^a

[1]Rats were orally administered with methanol extract at a dose of 250 or 500 mg/kg body weight for one week, and bromobenzene was *i.p.* injected four times at 12 hr intervals during final two days of the oral treatments. Values are mean±SD of 5 animals. Values followed by the same letter are not significantly different ($p<0.05$).

*unit: formaldedhyde nmol/mg protein/min

Table 2. Effects of methanol extract from *Rosa davurica* roots on the hepatic microsomal aniline hydroxylase activity in bromobenzene-treated rats[1]

Group	Aniline hydroxylase*
Normal	0.49 ± 0.12^{b}
BB	1.03 ± 0.18^{a}
ME250+BB	0.92 ± 0.15^{a}
ME500+BB	0.82 ± 0.16^{a}

Treatments are as described in Table 1. Values are mean±SD of 5 animals. Values followed by the same letter are not significantly different ($p<0.05$).
*unit: *p*-aminophenol nmol/mg protein/min

increases of the aminopyrine N-demethylase and aniline hydroxylase activities by bromobenzene. Glutathione S-transferase catalyzes the reaction of a wide variety of electrophiles with glutathione. Bromobenzene 3,4-oxide can be detoxified by glutathione S-transferase, a hepatic enzyme containing glutathione. The injection of bromobenzene decreased glutathione S-transferase activity to 239.2 nmol/mg protein from a normal value of 251.1 nmol/mg protein. There was no change of glutathione S-transferase activity by the methanol extract from *Rosa davurica* (Table 3). Bromobenzene-3,4-oxide is also metabolized to a nontoxic bromobenzene 3,4-dihydrodiol by epoxide hydrolase. The activity of hepatic epoxide hydrolase of the extract group were decreased to 4.17 nmol/mg protein from 14.8 nmol/mg protein, the enzyme activity of bromobenzene-treated group. Oral administration

Table 3. Effects of methanol extract from *Rosa davurica* roots on the hepatic glutathione S-transferase activity in bromobenzene-treated rats[1]

Group	Glutathione S-transferase*
Normal	251.1 ± 13.27^{a}
BB	239.2 ± 10.26^{a}
ME250+BB	254.6 ± 18.37^{a}
ME500+BB	247.2 ± 14.15^{a}

[1]Treatments are as described in Table 1. Values are mean±SD of 5 animals. Means sharing the same superscript letter are not significantly different ($p<0.05$).
*unit: 1,2-dinitro-4-nitrobenzene nmol/mg protein/min.

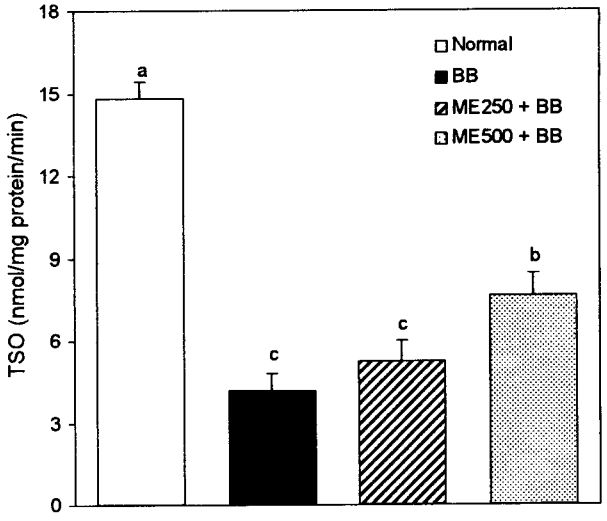

Fig. 1. Effects of methanol extract from *Rosa davurica* roots on the hepatic epoxide hydrolase activity in bromobenzene-treated rats[1]
[1]Rats were orally administered with methanol extract (ME) at a dose of 250 or 500 mg/kg body weight for one week, and bromobenzene (BB) was *i.p.* injected four times at 12 hr intervals during final two days of the oral treatments. Each bar represents mean±SD of 5 animals. The values sharing a common superscript letter are not significantly different ($p<0.05$).

of 500 mg/kg of the extract increased the epoxide hydrolase activity to 33% when compared with bromobenzene-treated group (Fig. 1). The results suggest that the protective effects of *Rosa davurica* in bromobenzene-induced hepatotoxicity is thought to be through enhancing the activity of epoxide hydrolase, an enzyme removing bromobenzene epoxide rather than through acting to epoxide-producing system. Investigation of the active biological components from *Rosa davurica* is now in progress.

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초록 : 브로모벤젠으로 유도된 간독성 흰쥐에서 생열귀나무 뿌리의 간보호활성

박종철[1,*] · 허종문[1] · 황영희[2] · 최명락[3] · 김석남[4] · 최종원[5]
([1]순천대학교 한약자원학과 및 한의약연구소, [2]전북대학교 산림과학부, [3]여수대학교 생명화학공학부, [4] 생열귀산업화연구소, [5]경성대학교 약대)

Bromobenzene으로 간독성을 유발한 흰쥐에 생열귀나무 뿌리를 투여하여 bromobenzene대사계에 미치는 효소활성을 관찰하였다. Bromobenzene은 bromobenzene 2,3-oxide와 bromobenzene-3,4-oxide로 전환되며, 3,4-oxide는 독성물질로서 epoxide hydrolase에 의해 무독성 bromobenzene-3,4-dihyrodiol로 대사 또는 glutathione S-transferase에 의하여 배설되기도 한다. 중국 민간에서 강장제로 사용하는 생열귀나무는 한방에서 소화불량, 위통 등의 치료에 사용되는 약용식물이다. 생열귀나무의 뿌리 추출물은 흰쥐의 간 epoxide 생성계에 관여하는 aminopyrine N-demethylase, aniline hydroxylase 활성과 epoxide를 대사시키는 glutathione S-transferase 활성에는 변화를 주지 않았다. 그러나 생열귀추출물 500 mg/kg 경구투여군은 epoxide를 무독화시키는 epoxide hydrolase 활성에서 bromobenzene 투여로 효소활성이 저하된 대조군보다 33% 활성을 회복시켰다. 따라서 생열귀나무 뿌리는 간독성물질인 bromobenzene 대사에 관여하는 epoxide hydrolase 활성 증가로 인해 간보호작용이 일어나는 것으로 판단된다.