

# Effect of *Boschniakia rossica* on expression of GST-P, p53 and p21<sup>ras</sup> proteins in early stage of chemical hepatocarcinogenesis and its anti-inflammatory activities in rats

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**Subject headings** *Boschniakia rossica*; liver neoplasms/chemically induced; glutathione transferases; protein p53; immunohistochemistry; anti-inflammatory agents; rats

Yin ZZ, Jin HL, Yin XZ, Li TZ, Quan JS, Jin ZN. Effect of *Boschniakia rossica* on expression of GST-P, p53 and p21<sup>ras</sup> proteins in early stage of chemical hepatocarcinogenesis and its anti-inflammatory activities in rats. *World J Gastroentero*, 2000;6(6):812-818

## Abstract

**AIM** To investigate the effect of *Boschniakia rossica* (BR) extract on expression of GST-P, p53 and p21<sup>ras</sup> proteins in early stage of chemical hepatocarcinogenesis in rats and its anti-inflammatory activities.

**METHODS** The expression of tumor marker-placental form glutathione S-transferase (GST-P), p53 and p21<sup>ras</sup> proteins were investigated by immunohistochemical techniques and ABC method. Anti-inflammatory activities of BR were studied by xylene and croton oil-induced mouse ear edema, carrageenin, histamine and hot scald-induced rat paw edema, adjuvant-induced rat arthritis and cotton pellet-induced mouse granuloma formation methods.

**RESULTS** The 500mg/kg of BR-H<sub>2</sub>O extract fractionated from BR-Methanol extract had inhibitory effect on the formation of DEN-induced GST-P-positive foci in rat liver (GST-P staining was 78% positive in DEN+AAF group vs 20% positive in DEN+AAF+BR group,  $P<0.05$ ) and the expression of mutant p53 and p21<sup>ras</sup> protein was lower than that of hepatic preneoplastic lesions (33% and 22% positive respectively in DEN+AAF group vs negative in DEN+AAF+BR group). Both CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O extracts from BR had anti-inflammatory effect in xylene and croton oil-induced mouse ear edema (inhibitory rates were 26%-29% and 35%-59%, respectively).

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Project supported by the National Natural Science Foundation of China, No.39660021

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Received 2000-05-05 Accepted 2000-06-09

**BR-H<sub>2</sub>O extract exhibited inhibitory effect in carrageenin, histamine and hot scald-induced hind paw edema and adjuvant-induced arthritis in rats and cotton pellet-induced granuloma formation in mice.**

**CONCLUSION** BR extract exhibited inhibitory effect on formation of preneoplastic hepatic foci in early stage of rat chemical hepatocarcinogenesis. Both CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O extracts from BR exerted anti-inflammatory effect in rats and mice.

## INTRODUCTION

*Boschniakia rossica* (BR) Fedtsch. et Flerov is a parasitic plant growing on the root *Alnus* plants (Betulaceae)<sup>[1]</sup>. It is one of the valuable medicinal plants growing mostly on the Changbai Mountain at 1450-1800 meters above sea level, Jilin, China. It is also distributed in the Democratic People's Republic of Korea (DPRK), Japan and Russia. *Boschniakia rossica* is named "Bu Lao Cao" (antisenile plant), because it has effects of tonifying the Kidney and strengthening Yang, and has been used as a tonic or invigorating medicine in China. Yin ZZ *et al* isolated four iridoid compounds from *Boschniakia rossica* of the Changbai Mountain by chromatographic techniques. Their structure was determined by means of the spectra of nuclear magnetic resonance (NMR) and mass spectra<sup>[2]</sup>. We discovered that Methanol extract of *Boschniakia rossica* exerted inhibitory effect on the formation of diethylnitrosamine (DEN) induced GST-P-positive foci in the liver of F344 rats<sup>[3,4]</sup> and BR also has antioxidative activities<sup>[5,6]</sup>. In the present study, we report the inhibitory effect of BR-water extract fractionated from BR-Methanol extract on the expression of GST-P, p53 and p21<sup>ras</sup> proteins in early stage of rat chemical hepatocarcinogenesis and its anti-inflammatory activities in rats and mice.

## MATERIALS AND METHODS

### Chemicals

Diethylnitrosamine (DEN), 2-Acetylaminofluorene (AAF), determination kit for GGT and histamine

were purchased from Sigma Chemical Co.(USA). Vectastain ABC kit (pk 4001) was obtained from Vector Laboratories Inc. (USA); anti-GST-P antibody was kindly supplied by Professor Shigeki Tsuchida, Second Department of Biochemistry, Hirosaki University School of Medicine, Japan. p53 (DO-1) and pan ras (F-132) monoclonal antibody were purchased from Santa Cruz Biotechnology.

#### **Preparation of the extract of *Boschniakia rossica***

*Boschniakia rossica* harvested from the Changbai Mountain area was used and the plants were identified by the authors. They were dried, cut, made into powder and extracted for overnight with Methanol five times. The Methanol extract was fractioned with  $\text{CH}_2\text{Cl}_2$  and  $\text{H}_2\text{O}$ , and  $\text{H}_2\text{O}$  extract was vacuum-concentrated. The extract was dried by speed vacuum.

#### **Animals and treatment**

Male Wistar rats, aged 6 weeks and weighing 160g-180g were used in the experiments of hepatocarcinogenesis. The male Wistar rats (180g-200g) and Kunming strain mice (20g-22g) were used in the anti-inflammatory experiment. Animals were housed in groups of 5 animals in plastic cages with stainless-steel grid tops at room temperature with a 12h light/dark cycle.

#### **Induction of preneoplastic hepatic foci<sup>[7-11]</sup>**

Enzyme-altered hepatic foci and hyperplastic nodules were induced by the modified protocol of Solt and Farber. The animals were divided into 3 groups. The rats in groups B and C were given a single i. p. injection of DEN (200mg/kg body weight) dissolved in saline to initiate hepatocarcinogenesis. After 2 weeks on basal diet, the rats received 0.004% 2-AAF in the diet for the following 6 weeks. Group C, after 2 weeks of injection of DEN, was given the diet containing 0.004% 2-AAF+500mg/kg BR for the following 6 weeks as a BR treatment group. Group A, as a control group, was intraperitoneally injected with the saline instead of DEN and then maintained on basal diet for 8 weeks. All rats of experimental and control groups were subjected to two-thirds partial hepatectomy (PH) at the 3rd week. Rats in each group were killed for examination at the 8th week.

#### **Immunohistochemical staining for GST-P, p53 and p21<sup>ras</sup><sup>[12-16]</sup>**

Rat liver slices were fixed with ice-cold acetone and embedded in paraffin. Immunohistochemical staining for GST-P was performed by ABC method using anti-GST-P antibody; immunohistochemical staining for p53 and p21<sup>ras</sup> proteins was performed using p53 (DO-1) and pan ras (F-132) monoclonal antibody, respectively.

#### **Quantitative analysis**

The number and the area of GST-P-positive hepatic foci larger than 0.1mm in diameter were analyzed using the microscopic quantitative analyzer (OC.M 19m/m Square 10/10×10, Tokyo, Japan).

#### **Investigation of anti-inflammatory activities of BR extract<sup>[17-19]</sup>**

**Xylen or croton oil-induced mouse ear edema** An edema was induced on the right ear by topical application of xylene in mice 30 minutes after oral administration of 500mg/kg-1000mg/kg BR- $\text{H}_2\text{O}$  extract or BR- $\text{CH}_2\text{Cl}_2$  extract. The left ear was controlled. Ear edema was measured by comparing the difference in weight (mg) between the same size of left and right ears 30 minutes after xylene-induction and 4h after croton oil-induction of inflammation and swelling degree and inhibition rate were calculated.

**Carrageenin-induced rat paw edema** An edema was induced on the rat right hind paw by aponeurosis injection of 0.15mL of 1% carrageenin in 0.9% saline. Test drug (500mg/kg-1000mg/kg of BR- $\text{H}_2\text{O}$  extract) was given orally 30 minutes before the injection of carrageenin. The volume of the right paw was measured before injection and at 1, 2, 3, 4, 6 and 24h after induction of inflammation. The edema was expressed as an increase in paw volume due to carrageenin injection. The results were obtained by measuring the volume difference before and after injection of the right paw. The swelling degree of paw and inhibition rate of edema were calculated.

**Histamine-induced rat paw edema** An edema was induced on the right hind paw of rat by subplantar injection of 200μg/0.1mL of histamine. Test drug (500mg/kg of BR- $\text{H}_2\text{O}$  extract) was given 30 minutes before the injection of histamine. The volume of the right paw was measured before injection and 0.5, 1, 2, 3 and 4h after induction of inflammation. The swelling degree of paw and inhibition rate of edema were calculated.

**Hot scald-induced rat paw edema** Edema was induced on the right hind paw of rat by hot scald. The right hind paw of rat soaked in thermostate water bath maintained at  $53^\circ\text{C} \pm 0.5^\circ\text{C}$  and cut-off time was 14 sec and test drug (500mg/kg of BR- $\text{H}_2\text{O}$  extract) was given 30 minutes before the hot scald test. The volume of the right paw was measured before test and 1, 2, 3, 4, 5, 6 and 24 h after induction of inflammation. The swelling degree of paw and inhibition rate of edema were calculated.

**Adjuvant-induced arthritis in rats** The Arthritis was induced by injection of 0.1mL complete Freund's adjuvant into the subplantar region of the right hind paw of rats. Five hundred mg/kg of BR- $\text{H}_2\text{O}$

extract was orally administrated 30 minutes before the injection of adjuvant and the BR extract was given daily for 3 days after induction of inflammation. From the 8th day the BR extract was given daily for 7 days more. The volume of the right paw was measured before injection and at 18h, and on day 3, 6, 9, 12, 15, 18, 21 and 24 after induction of inflammation. The swelling degree of paw and inhibition rate of edema were calculated.

**Cotton pellet-induced granuloma formation** Pellets of surgical aseptic cotton weighing 15mg were implanted in both scapular regions in mice. The test drug (250mg/kg-500mg/kg) was administered daily for 7 days, and on the 8th day, the granulomatous tissues were removed. The pellets were dried overnight at 60°C and weighed. The dry weight was considered the weight of the granuloma. The results of this subacute inflammation were compared with the control group.

**Statistical analysis** Statistical analysis was made using the  $\chi^2$  test and the Student's *t* test. Values of  $P < 0.05$  were considered statistically significant.

## Results

**Effect of *Boschniakia rossica* extract on expression of GST-P, p53 and p21<sup>ras</sup> proteins during chemical hepatocarcinogenesis in rats** Immunohistochemical investigation of expression of

GST-P, p53 and p21 protein in DEN-induced preneoplastic hepatic foci (group B), in administration of BR extract in the Solt-Farber protocol of rats (group C) and control (group A) are summarized in Table 1. GST-P staining was 78% positive in group B and 20% positive in group C, while in group A it was negative (B vs C,  $P < 0.05$ ). Expression of oncogene products p53 and p21<sup>ras</sup> protein in group B was 33% and 22% positive, while in groups A and C it was negative. The number (no/cm<sup>2</sup>) and area (mm<sup>2</sup>/cm<sup>2</sup>) of GST-P-positive hepatic foci in group C given DEN-AAF+BR was significantly decreased as compared with the values of group B given DEN-AAF (B vs C,  $P < 0.001$  and  $P < 0.05$ ) and these quantitative values are shown in Table 1 and Figure 1.

## Effect of extract from BR on the anti-inflammatory activities in rats and mice

Both CH<sub>2</sub>Cl<sub>2</sub> and water extract from BR have inhibitory effect in the xylene and croton oil-induced mouse ear edema, its inhibitory rate was 26%-29% and 35%-59% respectively (Tables 2 and 3) and exert inhibitory effect in the cotton pellet-induced granuloma formation in mice (Tables 8 and 9). BR-H<sub>2</sub>O extract fractionated from BR-Methanol extract exhibited inhibitory effect in carrageenin, hot scald and histamine-induced rats hind paw edema (Tables 4, 6 and 7) and adjuvant-induced arthritis in rats (Table 5).

**Table 1 Effect of *Boschniakia rossica* on the expression of GST-P, p53 and p21 protein in early stages of rat chemical hepatocarcinogenesis**

Groups	Treatment (8 weeks)	n	GST-P positive(%)	No.of foci (No/cm <sup>2</sup> ) <sup>*</sup>	Area of foci (mm <sup>2</sup> /cm <sup>2</sup> ) <sup>*</sup>	p53 positive (%)	p21 positive (%)
A	Saline-BD-PH	10	Negative	0	0	Negative	Negative
B	DEN-AAF-PH	9	7(77.8)	18.9±1.54 <sup>△</sup>	0.27±0.32	3(33.3)	2(22.2)
C	DEN-AAF-BR-PH	10	2(20.0) <sup>a</sup>	0.30±0.67 <sup>b</sup>	0.03±0.07 <sup>a</sup>	Negative	Negative

<sup>a</sup> $P < 0.05$ , vs B( $\chi^2$  test);  $P < 0.05$ , <sup>b</sup> $P < 0.01$ , vs B(*t* test).

<sup>\*</sup>Foci more than 0.1nm in diameter were quantified; <sup>△</sup>Values  $\bar{x} \pm s$

**Table 2 Effect of BR-extract on xylene-induced mouse ear edema**

Groups	Dose (mg/kg)	Mouse (n)		BR-H <sub>2</sub> O fraction (I)		BR-CH <sub>2</sub> Cl <sub>2</sub> fraction (II)	
		I	II	Edema degree(mg)	Inhibitory rate(%)	Edema degree(mg)	Inhibitory rate(%)
NS(A)	0.85%	10	20	19.1±3.6 <sup>*</sup>		11.2±4.0	
Ind(B)	20	10	20	15.5±1.8 <sup>a</sup>	18.8	8.8±2.6 <sup>a</sup>	21.5
BR(C)	500	10	20	17.4±3.1 <sup>b</sup>	8.9	8.3±3.9 <sup>a</sup>	26.2
BR(D)	1000	10	20	13.6±3.1 <sup>a</sup>	28.8	8.1±3.7 <sup>a</sup>	27.4

<sup>\*</sup> $\bar{x} \pm s$ , <sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ , vs NS (*t* test);

NS: normal saline; Ind:indomethacin; BR: *Boschniakia rossica*

**Table 3 Effect of BR-extract on Croton oil-induced mouse ear edema**

Groups (mg/kg)	Dose	Mouse (n)		BR-H <sub>2</sub> O fraction (I)		BR-CH <sub>2</sub> Cl <sub>2</sub> fraction (II)	
		I	II	Edema degree(mg)	Inhibitory rate(%)	Edema degree(mg)	Inhibitory rate(%)
NS(A)	0.85%	10	12	8.6±2.5 <sup>*</sup>		7.6±3.3	
Ind(B)	20	10	12	6.6±4.0 <sup>a</sup>	23.3	5.0±2.1 <sup>a</sup>	33.5
BR(C)	500	10	12	5.4±3.4 <sup>b</sup>	45.3	4.9±1.7 <sup>a</sup>	35.1
BR(D)	1000	10	12	4.7±1.7 <sup>c</sup>	59.3	4.5±2.8 <sup>a</sup>	40.0

<sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ ; <sup>c</sup> $P < 0.001$ ; vs A (*t* test);

NS: normal saline; Ind:indomethacin; BR: *Boschniakia rossica*

**Table 4** Inhibitory effect of BR-H<sub>2</sub>O extract on carrageenin-induced paw edema in rats ( $\bar{x} \pm s$ )

Groups	Dose (mg/kg)	(n) Rats	Swelling degree (inhibitory rate)					
			1h	2h	3h	4h	6h	24h
NS		9	39.5±16.4	57.1±19.3	63.6±26.1	67.9±22.6	67.5±19.5	28.2±13.0
Ind	20	9	25.9±13.8 (34.2)	30.6±19.2 <sup>a</sup> (46.5)	32.6±18.6 <sup>a</sup> (47.2)	38.6±22.1 <sup>a</sup> (43.1)	42.2±34.6 (37.5)	16.9±12.3 (39.9)
BR	500	9	30.6±13.8 (22.7)	28.7±8.3 <sup>b</sup> (49.8)	39.4±12.2 <sup>a</sup> (38.0)	40.1±14.2 <sup>a</sup> (36.2)	39.5±20.0 <sup>a</sup> (41.5)	9.6±8.5 <sup>b</sup> (65.8)
BR	1000	9	31.8±15.7 (19.6)	26.3±18.9 <sup>b</sup> (54.0)	36.5±19.9 <sup>a</sup> (40.6)	37.0±28.8 <sup>a</sup> (45.5)	23.5±16.4 <sup>c</sup> (65.2)	6.8±10.4 <sup>b</sup> (76.5)

<sup>a</sup>*P*<0.05; <sup>b</sup>*P*<0.01; <sup>c</sup>*P*<0.001, vs NS(*t* test)NS: normal saline; Ind: indomethacin; BR: *Boschniakia rossica***Table 5** Effect of BR-H<sub>2</sub>O extract on adjuvant arthritis in rats

Groups	Dose (mg/kg)	Rats (n)	Swelling degree (inhibitory rate)							
			18h	3d	6d	9d	12d	15d	18d	21d
NS		6	108.2 ±43.8	74.7 ±33.0	66.9 ±47.9	58.0 ±39.0	77.8 ±39.6	77.1 ±28.0	99.6 ±34.9	78.6 ±31.3
BR	500	6	79.1 ±22.3 <sup>b</sup> (26.9)	33.9 ±17.2 <sup>a</sup> (54.7)	51.8 ±25.0 (22.6)	52.6 ±23.7 (9.3)	67.5 ±21.1 (13.2)	75.7 ±23.7 (1.8)	61.7 ±19.0 <sup>a</sup> (38.1)	53.4 ±14.9 <sup>b</sup> (32.1)

<sup>a</sup>*P*<0.05; <sup>b</sup>*P*<0.01, vs NS (*t* test)NS: normal saline; Ind: indomethacin; BR: *Boschniakia rossica***Table 6** Effect of BR-H<sub>2</sub>O extract on hot scald-induced paw edema in rats

Groups	Dose (mg/kg)	Rats (n)	Swelling degree (inhibitory rate)						
			1h	2h	3h	4h	5h	6h	24h
NS		8	67.2±4.7	59.5±3.9	74.2±6.5	81.5±23.7	77.7±17.1	69.8±19.1	63.7±14.5
BR	500	8	40.4±11.9 <sup>b</sup> (39.9)	23.3±10.1 <sup>b</sup> (60.8)	33.5±16.8 <sup>b</sup> (68.6)	39.7±10.5 <sup>a</sup> (51.3)	24.8±8.2 <sup>b</sup> (68.1)	18.0±16.1 <sup>b</sup> (74.2)	23.4±7.0 <sup>b</sup> (63.3)

<sup>a</sup>*P*<0.05; <sup>b</sup>*P*<0.01, vs NS (*t* test) NS: normal saline; Ind: indomethacin; BR: *Boschniakia rossica***Table 7** Effect of BR-H<sub>2</sub>O extract on histamine-induced rat hind paw edema

Groups	Dose (mg/kg)	Rats (n)	Swelling degree (inhibitory rate)				
			30min	1h	2h	3h	4h
NS		9	48.0±11.1	34.8±12.6	22.9±8.1	18.3±3.8	12.7±5.9
BR	500	9	28.5±7.5 <sup>a</sup> (36.2)	18.4±7.5 <sup>a</sup> (31.1)	9.0±6.5 <sup>a</sup> (31.7)	9.6±6.7 <sup>a</sup> (36.8)	2.3±3.9 <sup>b</sup> (71.3)

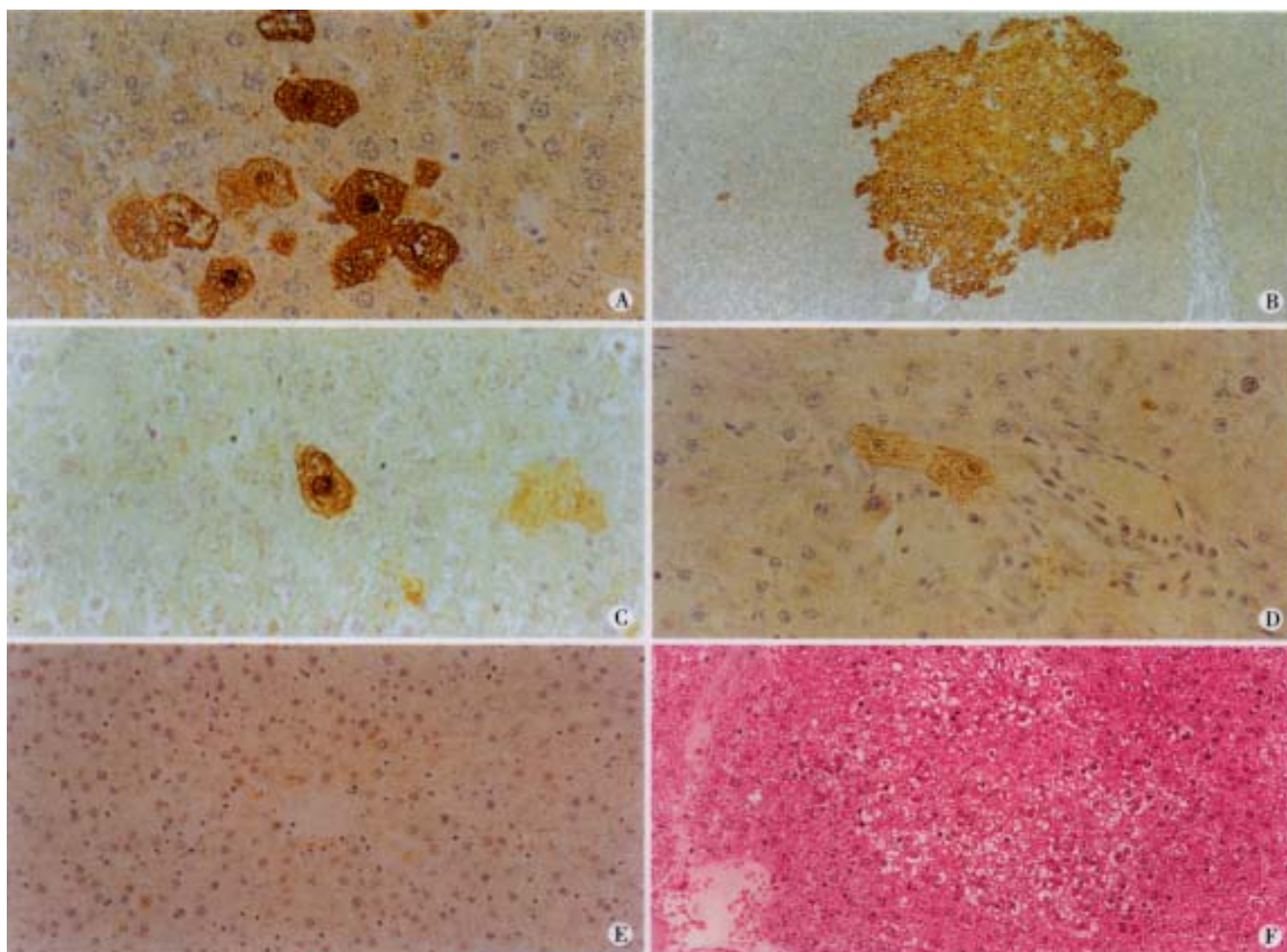
<sup>a</sup>*P*<0.05; <sup>b</sup>*P*<0.01, vs NS (*t* test)**Table 8** Effect of BR-HO extract on proliferation of granuloma caused by cotton pellet in mice

Groups	Dose (mg/kg)	Mouse (n)	Weight of granuloma		Inhibitory rate (%)	
			Wet w.(mg)	Dry w.(mg)	Wet w.(mg)	Dry w.(mg)
NS(A)		10	615.2±119.1	152.3±54.8		
BR(C)	250	10	507.0±41.1 <sup>a</sup>	103.7±14.4 <sup>a</sup>	17.6	31.9
BR(D)	500	10	463.5±49.6 <sup>b</sup>	101.0±15.1 <sup>a</sup>	24.7	33.7

<sup>a</sup>*P*<0.05; <sup>b</sup>*P*<0.01, vs NS (*t* test)**Table 9** Effect of BR-CH<sub>2</sub>Cl<sub>2</sub> extract on proliferation of granuloma caused by cotton pellet in mice

Groups	Dose (mg/kg)	Mouse (n)	Weight of granuloma		Inhibitory rate (%)	
			Wet w.(mg)	Dry w.(mg)	Wet w.(mg)	Dry w.(mg)
NS(A)		10	613.4±160.4	130.3±42.0		
BR(C)	250	10	445.8±37.0 <sup>b</sup>	97.9±12.8 <sup>a</sup>	27.3	24.9
BR(D)	500	10	422.8±33.2 <sup>b</sup>	84.8±7.8 <sup>a</sup>	31.1	34.9

<sup>a</sup>*P*<0.05; <sup>b</sup>*P*<0.01, vs NS (*t* test)



**Figure 1** Immunohistochemical staining for GST-P in rat hepatic preneoplastic lesions induced by Solt-Farber protocol (A and B), in rat liver treated with DEN and AAF plus BR for 6 weeks (C and D), and in normal rat liver (E).

A, GST-P-strongly positive minifoci in group B  $\times 400$ ; B, GST-P-strongly positive large foci in group B,  $\times 100$ ; C, GST-P-positive single cell in group C,  $\times 400$ ; D, GST-P-weakly positive two cells in group D,  $\times 400$ ; E, GST-P was negative in group A, normal rat liver,  $\times 200$ ; F, Hematoxylin and eosin staining for preneoplastic hepatic foci in group B,  $\times 200$ .

## DISCUSSION

### *Changes in GST-P, p53 and p21<sup>ras</sup> proteins during chemical hepatocarcinogenesis in rats*

Placental form of glutathione S-transferase (GST-P) was first isolated from rat placenta as a sensitive marker enzyme in chemical hepatocarcinogenesis of rat by Sato *et al* in 1984<sup>[20-24]</sup>. GST-P is considered to be an accurate marker for very early “initiated cells”<sup>[21,24]</sup>. GST- $\pi$ , purified from human term placenta, is related to rat GST-P in many properties and is grouped into the class Pi. It is of important clinical value. GST- $\pi$  is also a useful human tumor marker for hepatoma, esophageal, gastric and colonic carcinomas and its preneoplastic lesions<sup>[12-15,25-32]</sup>. Thereafter, using DEN as a initiator and AAF as a promoter, modified system based on Solt-Farber method was designed to screen the medium-term bioassay of chemical-induced carcinogenesis by Ito *et al*<sup>[33]</sup>. This screening system was used in this study and successfully induced the preneoplastic GST-P-positive hepatic foci and nodules. Recently Tsuda H *et al* developed a trial

for an initiation bioassay system. Initiation potential was assayed on the basis of significant increase in values of preneoplastic GST-P positive foci. This protocol may be useful for detection of the initiation potential of carcinogens irrespective of their mutagenicity<sup>[34]</sup>.

We investigated the effect of *Boschniakia rossica* on formation of GST-P positive preneoplastic hepatic foci during chemical hepatocarcinogenesis. The results demonstrated that BR-H<sub>2</sub>O extract fractionated from BR-Methanol extract with CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O has inhibitory effect on DEN-induced GST-P positive preneoplastic hepatic foci in early stage of rat chemical hepatocarcinogenesis. We consider that it is related to its antioxidative action of *Boschniakia rossica*<sup>[4]</sup>. Recently there are reports that GST-P is as a sensitive marker for preneoplastic hepatic foci during chemical hepatocarcinogenesis in rats<sup>[35-40]</sup>. It indicated that GST-P and GST- $\pi$  are very sensitive tumor markers for basic research, prevention and cure of cancer.

Rat chemical hepatocarcinogenesis can be

divided into several steps: initiation, promotion, and progression stages. During the initiation stage, alterations of specific genes and in particular the activation of cellular proto-oncogenes occurs. During the promotion stage, groups of preneoplastic cells have been observed in many organs prior to appearance of malignant cancers, and in rat chemical hepatocarcinogenesis enzyme-altered foci and hyperplastic nodules have been excited<sup>[21]</sup>. Recently many scientists reported expression of ras, jun oncogene<sup>[42,47-49]</sup>, p53 tumor suppressor gene<sup>[41,43,45,52-54]</sup>, and nm23 tumor metastasis suppressor gene<sup>[44,46,50,51]</sup> in hepatocellular carcinoma, esophageal, gastric, and colonic carcinomas. Smith *et al.*<sup>[41]</sup> reported a p53 gene mutation occurring in foci of enzyme-altered hepatocytes induced by diethylnitrosamine in F 344 rats. We observed the relation to the expression of GST-P, ras gene product p21<sup>ras</sup> protein and suppressor gene product p53 protein in preneoplastic hepatic foci of rat liver. The results suggest that one-third of GST-P positive foci were positive for p53 protein and approximately one-fourth of GST-P positive foci were also positive for p21<sup>ras</sup> protein in group B, while in rat liver of group C treated with DEN and AAF plus BR for 6 weeks p53 protein and p21<sup>ras</sup> were not immunohistochemically detectable. Our result is similar to the report by Smith *et al.*, and also is similar to that GST-P appearing at an early stage of chemical hepatocarcinogenesis, when oncogene product c-jun was not immunohistochemically detectable, reported by Suzuki *et al.*<sup>[42]</sup>. These results indicate that BR-H<sub>2</sub>O extract fractionated from BR-Methanol extract exhibited an inhibitory effect on DEN-induced preneoplastic hepatic foci in rats administered with BR for 6 weeks during chemical hepatocarcinogenesis.

#### **Anti-inflammatory activities of *Boschniakia rossica***

Results of the present study demonstrated that BR-extract exerted significant anti-inflammatory activities. An inhibitory effect of H<sub>2</sub>O extract and CH<sub>2</sub>Cl<sub>2</sub> extract from BR was observed in the acute inflammatory process, such as xylene and croton oil-induced mouse ear edema, carrageenin induced rat hind paw edema and inflammatory factors such as histamine and hot scald-induced rat hind paw edema. Anti-inflammatory action of BR was also observed in the chronic inflammation process, such as cotton pellet-induced granuloma formation in mice and immune inflammation process, such as adjuvant-induced arthritis in rats. The experimental model of inflammation induced by carrageenin is highly sensitive to non-steroidal anti-inflammatory drugs, and it has long been accepted as a useful pharmacological tool for investigating new anti-inflammatory drugs. The oral administration of the BR-H<sub>2</sub>O extract (500mg/kg) inhibited the edema

formation by 49.8 % and 65.8% in 2 and 24 hours, respectively, after the administration of carrageenin in rats. Indomethacin, the standard anti-inflammatory drug used in this experiment, inhibited the edema by 46.5% and 40% in 2 and 24 hours. The anti-inflammatory effect of extract from BR was also observed in the cotton pellet-induced granuloma formation in mice. The daily oral administration of 250mg/kg and 500mg/kg of BR-CH<sub>2</sub>Cl<sub>2</sub> extract, using this model, showed on the eighth day an inhibitory effect of 25% and 35%, and 250mg/kg and 500mg/kg of BR-H<sub>2</sub>O extract 35% and 34%, respectively. The mechanism of anti-inflammatory action of *Boschniakia rossica* can be related to the chemical structure of BR. Recio *et al.*<sup>[55]</sup> reported that Iridoids is an anti-inflammatory agent. The results obtained in present study suggest that CH<sub>2</sub>Cl<sub>2</sub> extract and H<sub>2</sub>O extract from BR have anti-inflammatory effects. We isolated four iridoid compounds, a group of cyclopentano[c]pyran monoterpenoids, from *Boschniakia rossica*, and among them 8-epideoxyloganin acid was shown to exhibit a strong anti-inflammatory activity. Based on the results obtained in this study, we conclude that extract from BR exerted an anti-inflammatory effect.

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