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Processed Xanthii fructus increases cell viability of mast cell line, RBL-2H3

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SUMMARY

The effect of aqueous extract of processed Xanthii fructus (PXF) on cell viability and histamine release from mast cell has been investigated. PXF showed higher cell viability than unprocessed Xanthii fructus (XF) at the concentrations of 5 and 10 mg/ml. Aqueous extract of PXF and unprocessed XF inhibited compound 48/80-induced systemic anaphylaxis in mouse. Both PXF and unprocessed XF dose-dependently inhibited histamine release from rat peritoneal mast cells by compound 48/80 to the similar extent at 0.01, 0.1 and 1 mg/ml. Our studies provide evidence that processing of XF may be beneficial to reduce cytotoxicity in high concentration (at 5 and 10 mg/ml) but does not affect on anti-allergic activity.

Key words: Processed Xanthii fructus; Systemic anaphylaxis, Histamine

INTRODUCTION

Xanthii fructus (XF) which is well known as "Chang-ih-jah" in Korea is the dried fruit of *Xanthium strumarium* L. (or *Xanthium sibiricum* PATR. Ex WIDD., Asteraceae). Traditionally water extract of this fruit has been used for treatment of various inflammatory diseases such as tympanitis, allergic rhinitis or ozena as an alternative therapy material usually by oral administration in far eastern countries.

Studies on *Xanthium strumarium L.* have been attempted in several different directions since 1965 (Chu *et al.*, 1965). They include investigations on characteristics of its substances, effects on photoperiod, relations with tocopherol, toxicologic studies, actions as an allergen, effects against microbial and human mesangial cell proliferation (Kuo *et al.*, 1998). This is lately reported to have the role to lower plasma glucose in diabetic rats (Hsu *et al.*, 2000). We have previously reported the inhibitory effects of XF on mast cell-mediated allergic reaction in murine model (Hong *et al.*, 2003).

XF is known to have some constituents such as saponin, flavone, caffeic acid, 1,4-dicaffeoylquinic acid, and sesquiterpene lactones. Sesquiterpene lactones are important secondary metabolites known to cause contact dermatitis. Including contact dermatitis, XF is known to have some harmful effect in folk remedy especially when it is used in the raw. Thus heat-processing is recommended for medicinal use to avoid possibly happening side effects.

The mast cells are thought to play a major role in the development of many physiologic changes during allergic responses (Kim *et al.*, 1999). Among the preformed and newly synthesized inflammatory substances released on degranulation of mast cells, histamine remains the best-characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity (Pertersen *et*

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al., 1996). Intracellular calcium, cAMP, and histamine release in murine mast cells were effectively stimulated by positively charged substances such as NaF or compound 48/80 (Alfonso *et al.*, 2000). It is usually supposed that the most potent secretagogues include the synthetic compound 48/80 and polymers of basic amino acids (Ennis *et al.*, 1980). An appropriate amount of compound 48/80 has been used as a direct and convenient reagent to study the mechanism of the anaphylactic reaction (Allansmith *et al.*, 1989). Compound 48/80 is a mixture of polymers synthesized by condensing N-methyl-p-methoxyphenyl ethylamine with formaldehyde (Baltzly *et al.*, 1949).

This study attempts to find pharmacological change by heat processing of XF. Viability of mast cell line, RBL-2H3, compound 48/80-induced systemic anaphylaxis, and histamine release from rat peritoneal mast cells were investigated to compare the difference of pharmacological effect between processed Xanthii fructus (PXF) and unprocessed XF.

MATERIALS AND METHODS

Reagents

Compound 48/80, *o*-phthaldialdehyde (OPA), and metrizamide were purchased from Sigma chemical Co. (St. Louis, MO). The α-minimal essential medium was purchased from Flow Laboratories (Irvine, UK). Fetal calf serum was purchased from Life Sciences (Grand Island, NY).

Animals

The original stock of male ICR mice (4 week age) and male Sprague-Dawley rats (7 week age) was purchased from the Dae Han Experimental Animal Center (Daejon, Korea), and the animals were maintained in the College of Pharmacy, Wonkwang University. The rats were housed five to ten per cage in a laminar air-flow room maintained at a temperature of 22±1°C and relative humidity of 55±10% throughout the study.

Heat processing of XF

50 g of raw XF was parched in the 20 cm (diameter) port at about 130°C for 5 min. Final weight of PXF was about 7% lighter than the raw XF. The appearance became dark brown, which is recommended color in the traditional oriental herbalogy.

Preparation of crude extract of PXF and unprocessed XF

PXF and unprocessed XF extraction was prepared by decocting the fruit with boiling distilled water (100 g/l). The extraction decocted for approximately 3 h has been filtered, lyophilized, and kept at 4°C. This plant material was purchased from an Oriental drug store, Choong-Ang Yakupsa (Iksan, Korea) and identified by Professor Kim HM. A voucher specimen was deposited at the Department of Oriental Pharmacy, College of Pharmacy, Wonkwang University.

MTT assay

The MTT assay, an index of cell viability, is based on the ability of viable cells to reduced MTT from a yellow water-soluble dye to a dark blue insoluble formazan product (Mosmann, 1983). MTT dye, suspended in phosphate-buffered saline (PBS), was added to each well for the last 4 h of treatment. Media was removed with needle and syringe. 200 μl of dimethyl-sulfoxide was added to each well. Dissolved crystals were transferred to plate reader, and absorbance was measured at 550 nm.

Compound 48/80-induced systemic anaphylaxis

Mouse was given an i.p. injection of 8 mg/kg of the mast cell degranulator compound 48/80. XF was dissolved in distilled water and administered orally from 10 to 1000 mg/kg 1 h before the compound 48/80 injection. Mortality was monitored for 1 h after the induction of anaphylactic shock.

Preparation of RPMC

RPMC were isolated as previously described (Jippo *et al.*, 1993). In brief, rats were anesthetized by ether, and injected with 20 ml of Tyrode buffer B (137 mmol/l NaCl, 5.3 mmol/l glucose, 12 mmol/l $NaHCO_3$, 2.7 mmol/l KCl, 0.3 mmol/l NaH_2PO_4, pH 7.4) containing 0.1% gelatin (Sigma Chemical Co.) into the peritoneal cavity; the abdomen was gently massaged for about 90 s. The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells was aspirated by Pasteur pipette. Then the peritoneal cells were sedimented at 150×g for 10 min at room temperature and resuspened in

Tyrode buffer B. Mast cells were separated from the major components of rat peritoneal cells (i.e. macrophages and small lymphocytes) according to the method described by *Yurt et al.* (1977). In brief, peritoneal cells suspended in 1 ml of Tyrode buffer B were layered onto 2 ml of 22.5% (w/v) metrizamide (density 1.120 g/ml; Sigma) and centrifuged at room temperature for 15 min at 400×g. The cells remaining at the buffer-metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1 ml of Tyrode buffer A (10 mmol/l HEPES, 130 mmol/l NaCl, 5 mmol/l KCl, 1.4 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$, 5.6 mmol/l glucose, pH 7.4) containing 0.1% bovine serum albumin (Sigma Chemical Co.). Mast cell preparations were about 95% pure as assessed by toluidine blue staining. More than 97% of the cells were viable as judged by trypan blue uptake.

Histamine release

Purified RPMC were resuspended in Tyrode buffer A containing calcium for the treatment with compound 48/80. RPMC suspensions (1×10^6 cells/ml) were pre-incubated for 10 min at 37°C for stabilization before adding XF. After that the cells were pre-incubated with the XF for 20 min, and then incubated for 15 min with compound 48/80 (6 μg/ml). The reaction was stopped by cooling the tubes in ice. The cells were separated from the released histamine by centrifugation at 400×g for 5 min at 4°C. Residual histamine in the cells was released by disrupting the cells with perchloric acid and centrifugation at 400×g for 5 min at 4°C.

The histamine content was measured by the OPA spectrofluorometric procedure (Shore *et al.*, 1959). The fluorescent intensity was measured at 438 nm (excitation at 353 nm) in a spectrofluorometer.

The inhibition percentage of histamine release was calculated using the following equation:

$$\% \text{ Inhibition} = (a - b) \times 100/a$$

Where 'a' is histamine release without the sample and 'b' is histamine release with the sample. 'a' and 'b' can be considered as amount of histamine expressed by florescent intensity.

Statistical analysis

The results were expressed as mean±S.E. for the number of experiments. Statistical significance was compared between each treated group and control by the Dennetts test. Results with $P<0.05$ were considered statistically significant.

RESULTS

Effect of PXF and unprocessed XF on cell viability

The effect of the exposure to PXF and unprocessed XF on the cell viability of RBL-2H3 cells, as assessed by the MTT test, is shown in Fig. 1. Both PXF and unprocessed XF did not severely affect cell viability up to 1 mg/ml concentration. But at the concentration of 5 and 10 mg/ml, unprocessed XF significantly lowered the cell viability. On the other hand cell viability has been improved about 1.3 fold (10 mg/ml) when PXF was applied with the same concentration. These data indicate that heat processing of raw XF can reduce cytotoxic effect on the mast cell line.

Effect of PXF and unprocessed XF on compound 48/80-induced systemic anaphylaxis

To assess the difference of PXF and unprocessed XF in allergic reactions, we used the in vivo model of systemic anaphylaxis. Compound 48/80 was used as a systemic fatal anaphylaxis inducer. After the injection of compound 48/80, the mouse was monitored for 1 h, after which the mortality rate was determined. When both samples were pretreated

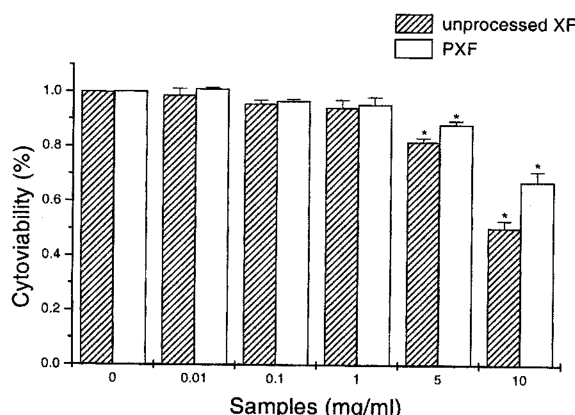

Fig. 1. Effect of PXF and unprocessed XF on the cell viability in RBL-2H3 cells. The cells (1×10^6 cells/ml) were incubated for 24 h and 48 h in DMEM (control) with various concentrations of the samples. Cell viability was assessed by MTT test. Each data value indicates the mean±S.E. of the three separated experiments. *$P<0.05$ versus medium alone control.

Table 1. Effect of PXF and unprocessed XF on Compound 48/80-Induced Systemic Allergic reaction

Dose [a] (g/kg)	Compound 48/80 [b] (8 mg/kg)	Mortality[c] (%)	
		PXF	unprocessed XF
None (Saline)	+	100	100
0.01	+	100	100
0.1	+	67	63
1	+	33	37
1	-	0	0

[a]Saline and samples were orally administered 1 h before (n=8 group) the compound 48/80 injection.
[b]The compound 48/80 solution was intraperitoneally given to the group of mice.
[c]Mortality (%) within 1 h following the compound 48/80 injections is presented as the Number of dead mice × 100/total Number of experimental mice.

at concentrations ranging from 0.01 to 1 g/kg for 1 h, the mortality induced by compound 48/80 was dose-dependently reduced. Table 1 shows the inhibitory effect of PXF and unprocessed XF on compound 48/80-induced systemic anaphylaxis. Dose of 1 g/kg for both samples did not alter physiological status by appearance. Number of mouse employed in each group was 24 (8/each group×3 independent experiment).

Effect of XF on histamine release from RPMC

The inhibitory effects of PXF and unprocessed XF on compound 48/80-induced histamine release from RPMC are shown in Fig. 2. Both samples (0.01 to 1 mg/ml) inhibited the compound 48/80-induced histamine release in dose dependent manner and showed little difference in their inhibitory effects. The doses applied in this study did not significantly affect the mast cell numbers or viability as shown in Fig. 1, indicating that the inhibitory effect on histamine was not due to a toxic effect on the mast cells.

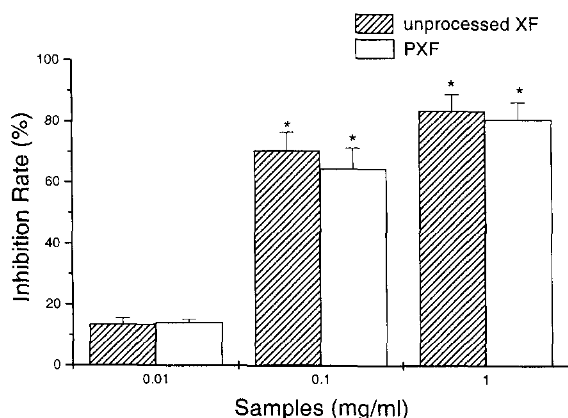

Fig. 2. Effect of PXF and unprocessed XF on compound 48/80-mediated histamine release from RPMC. RPMC (2×10^5 cells/ml) were preincubated with XF at 37°C for 10 min prior to incubation with compound 48/80 (5 μg/ml). Each data point represents the mean±S.E. of three experiments. $^*P<0.05$: significantly different from the saline value.

DISCUSSION

The present study showed that unprocessed XF could cause cytotoxic effect on mast cell line at the concentration of 5 and 10 mg/ml, and this effect may be reduced by heat processing of the fruit, XF. Pretreatment of PXF or unprocessed XF profoundly affected compound 48/80-induced systemic anaphylactic reaction. Both of them also inhibited the histamine release from RPMC. These results suggest heat processing of XF may reduce cytotoxic effect on mast cell without affecting anti-anaphylactic reaction of XF.

Previously we have reported that XF might act on the lipid bilayer membrane affecting the prevention of the perturbation being induced by compound 48/80 and regulate the degranulation of the mast cells in mouse skin by stabilizing membrane fluidity (Hong *et al.*, 2003). Even before this result had been reported, XF has already been broadly used as folk remedy for treating tympanitis, allergic rhinitis or ozena. But traditional recordes and folk remedies warned side effect of raw XF and advised to use it after heat processing. There are no report about the influence of heat processing of XF on the side effect and pharmacological effect against allergic inflammation.

As is mentioned before XF includes some compound causing contact dermatitis, which is inconsistent with the results of anti-inflammatory effects achieved in the present investigation. The discrepancy is not able to elucidate clearly without further study. We just assume at this step that there could be some effect differences between specific compound and total water extract.

In the present stage there are no clue to explain how PXF showed reduced cytotoxicity on mast cell line and why there are no change in pharmacological

effect against histamine release or systemic anaphylaxis. One possible speculation is some chemical modification caused by heat in harmful constituent of raw XF. But further study should be followed to clear the mechanism.

Conclusively the cytotoxicity of unprocessed XF in higher dose on mast cell line can be lowered by heat processing. And processed XF can still maintain its anti-allergic effect. Thus heat processing is recommended before medicinal use of XF especially when high dose is required. Efforts should be carried to find the cytotoxic agent among the compounds in XF.

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