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Anti-Inflammatory Activities of Some Traditional Indonesian Crude Drugs

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Hot water extracts of 8 Indonesian crude drugs, Cabe jawa, Daun luntas, Iler, Ketepeng, Klabet, Mesoyi, Patikan kebo and Temu giring, were assayed for their anti-inflammatory effects. All the extracts were found to have an inhibitory effect on compound 48/80-induced histamine release from mast cells, and all the extracts excepting that of Daun luntas, to have an inhibitory effect on carrageenan-induced paw edema in rats. Moreover, Iler, which showed the strongest anti-inflammatory effect of the tested materials, had a dose-dependent inhibitory effect on cotton pellet granuloma formation in rats. Iler also showed a superoxide-scavenging activity on the superoxide anion produced by the xanthine oxidase system.

Keywords——Indonesian crude drugs; anti-inflammatory; histamine release

The traditional Indonesian medicine, "Jamu," includes many crude drugs, from more than 1,000 plant species. These drugs are produced by many Indonesian companies, and easily available commercially.[1,2] However, many of these plants, which are native to Indonesia, have not been studied for their pharmaceutical effects. In this paper, we examined the anti-inflammatory effects of 8 crude drug extracts which have been used externally for eye diseases, deodorant, itch, ointment or skin care. Inhibitory activities on compound 48/80-induced histamine release from mast cells, and on carrageenan-induced paw edema in rats were used in the present anti-inflammatory effect screening. Of these materials, Iler was found to have the strongest anti-inflammatory effects. We further studied its activities by using some inflammatory experimental models.

MATERIALS AND METHODS

Animals Male Wistar rats purchased from Charles River Japan Inc. were used. They were kept at room temperature (24±1℃) and humidity (50-55%) under 12 h of light.

Drugs Eight crude drugs; Cabe jawa, Daun luntas, Iler, Ketepeng, Klabet, Mesoyi, Patikan kebo and Temu giring, were purchased in an Indonesian market. Each of the cut dried materials (100 g) was extracted twice with 2,000 ml of hot water (95℃) for 2 h. The aqueous solution was filtered, concentrated and lyophylized. The drug origins, their local uses and the yields of extracts are shown in TABLE I.[3,4] The assay results and test drugs used (mg freeze-dried extracts) are given in TABLES II-VI. For *in vivo* experiments, each test drug or indomethacin (Sigma) used as a positive control was suspended in an aqueous solution containing 0.1% carboxymethyl-cellulose (Katayama Chemical), before injection.

Xanthine oxidase (XOD, Sigma), superoxide dismutase (SOD, Sigma), compound 48/80 (Sigma) and carrageenan (Tokyo Kasei) were obtained commercially.

Assay for inhibitory activity on histamine release Inhibitory activity on histamine release was studied by using mast cells harvested from the abdominal cavity of male Wistar rats (weight 250-300 g) by Sullivan's method.[5] Briefly, a mast cell suspension (10^5/ml) was incubated with a test drug at 37℃ for 10 min. Then, 1 μg/ml of compound 48/80 was added. After 10 min, the reaction was stopped by cooling the mixture in ice. The reaction solution was centrifuged at 3,000 rpm for 5 min, and the histamine in the supernatant was measured by May's method.[6]

Assay for inhibitory activity on carrageenan-induced paw edema[7] Right hind paw volume of male Wistar rat (6-week-old) was measured by a volume meter (Volume Meter TK105, Muromachi Kikai). One hour after subcutaneous injection of a test drug in the back, 0.1 ml of 1% carrageenan suspension in physiologi-

TABLE I. List of Tested Indonesian Crude Drugs

Crude drugs (Local names)	Scientific names	Family	Part* used	Use in Indonesia	Yield of water extract (%)
Cabe jawa	*Piper retrofuractum* L.	Piperaceae	S	Eye diseases	10.4
Daun luntas	*Pluchea indica* L.	Compositae	H	Deodorant	19.4
Iler	*Coleus scutellarioides* BENTH.	Labiatae	H	Eye diseases	19.1
Ketepeng	*Cassia alata* L.	Leguminosae	L	Itch	22.0
Klabet	*Trigonella foenum-graecum*	Leguminosae	S	Ointment	24.9
Mesoyi	*Cryptocarya aromatica* KOSTEM	Lauraceae	B	Ointment	3.4
Patikan kebo	*Euphorbia pilulifera* L.	Euphorbiaceae	H	Eye diseases	23.3
Temu giring	*Curcuma heyneana* VAL. *et* v. ZIJP	Zingiberaceae	R	Skin care	13.0

*B: bark, H: herb, L: leaf, R: rhizome, S: seed.

cal salt solution was injected subcutaneously in the right hind paw. The paw volume was measured again 3 h later. The edema increase (%) was calculated as compared with the initial volume, and its inhibition (%) was calculated as compared with the increase of control.

Assay for inhibitory activity on cotton pellet granuloma[8] Male Wistar rats (6-week-old) anesthetized with pentobarbital were clipped on the back. The skin was slightly cut along the median line on the back. A cotton pellet (30±1 mg) was inserted subcutaneously into both right and left shoulders. Test drugs were injected subcutaneously at the abdomen once a day for 6 days, starting from the day of pellet insertion. Two days after the final injection, the granuloma was excised. The surgically removed granuloma was weighed after drying at 60℃ for 48 h and the weight of granuloma was calculated by reducing the weight of the cotton pellet from the weight of the excised granuloma.

Assay for superoxide-scavenging activity Superoxide-scavenging activity was studied by using the modified Oyanagi's method.[9] After 6.3×10^{-2} U/ml XOD was added, a mixture of a test drug, 0.1 mM xanthine, hydroxylamine-*O*-sulfanilic acid, hydroxylammonium chloride and borate buffer (pH 8.3) was incubated for 30 min at 37℃. A solution of sulfanilic acid, *N*-1-naphthylethlendiamine and acetic acid was added, and the optical density at 550 nm was measured after 30 min at room temperature. SOD from bovine erythrocytes was used as a positive control.

RESULTS AND DISCUSSION

Hot water extracts of 8 drugs; Cabe jawa, Daun luntas, Iler, Ketepeng, Klabet, Mesoyi, Patikan kebo and Temu giring, were found to inhibit histamine release from mast cells significantly. Of them, the effect of Iler was the strongest and Iler give 98% inhibition at 0.1 mg/ml (TABLE II).

These extracts, except that of Daun luntas, were also found to have a significant inhibitory effect on paw edema in rats induced by subcutaneous injection of carrageenan. Iler showed the strongest inhibition in them, too (TABLE III).

In order to investigate the anti-inflammatory action of

TABLE II. Inhibitory Effect of Indonesian Crude Drug Extracts on Compound 48/80-Induced Histamin Release from Mast Cells

Drugs	Conc. (mg/ml)	No.	Histamine release (% of control)	Inhibition (%)
Control		3	100.0±5.8	
Cabe jawa	0.1	3	89.0±2.7*1	11
	1.0	3	41.6±5.7*3	58
Daun luntas	0.01	3	88.6±2.4*1	11
	0.1	3	11.1±1.4*2	89
	1.0	3	1.2±0.2*3	99
Iler	0.01	3	81.7±0.4*2	18
	0.1	3	1.8±0.5*3	98
	1.0	3	1.0±0.6*3	99
Ketepeng	0.1	3	90.2±6.7	10
	1.0	3	2.5±0.5*3	98
Klabet	0.1	3	82.9±8.3*1	17
	1.0	3	66.8±9.4*2	33
Mesoyi	0.1	3	60.8±3.1*3	39
	1.0	3	4.1±0.4*3	96
Patikan kebo	0.01	3	91.9±2.0	8
	0.1	3	30.8±4.2*3	69
	1.0	3	2.6±1.9*3	97
Temu giring	0.1	3	78.3±8.4*1	22
	1.0	3	8.4±1.1*3	91

*1 $p<0.05$, *2 $p<0.01$, *3 $p<0.001$, significantly different from control.

TABLE III. Effect of Indonesian Crude Drug Extracts and Indomethacin on Carrageenan-Induced Paw Edema in Rats

Drugs	Dose (mg/kg, s.c.)	No.	Increase (%)	Inhibition (%)
Control		8	56.4±3.3	
Cabe jawa	500	4	41.1±6.0*1	27
Daun luntas	500	4	62.7±3.3	−11
Iler	500	4	12.7±5.4*3	78
Mesoyi	500	4	21.8±1.7*3	61
Ketepeng	500	4	43.6±2.5*1	23
Klabet	500	4	37.4±4.2*2	34
Patikan kebo	500	4	39.2±2.4*2	31
Temu giring	500	4	23.4±1.5*3	59
Indomethacin	10	8	30.6±3.0*3	46

*1 $p<0.05$, *2 $p<0.01$, *3 $p<0.001$, significantly different from control.

TABLE IV. Effect of Iler Extracts on Carrageenan-Induced Paw Edema in Rats

Drugs	Dose (mg/kg, s.c.)	No.	Increase (%)	Inhibition (%)
Control		12	56.1±2.6	
Iler	31	6	46.2±2.8	18
	63	6	29.7±4.9*	47
	125	6	25.3±2.6*	55
	250	6	25.9±2.6*	54
	500	6	15.1±1.4*	73

* $p<0.001$, significantly different from control.

TABLE V. Effect of Iler Extracts and Indomethacin on Cotton Pellet Granuloma in Rats

Drugs	Daily dose (mg/kg, s.c.)	No.	Dry weight of granuloma (mg)	Inhibition (%)
Control		10	40.8±2.4	
Iler	31	5	33.2±2.5	19
	63	5	32.2±2.9*1	21
	125	5	30.6±0.9*1	25
	250	5	26.2±1.5*2	36
Indomethacin	4	5	28.8±0.4*2	29

*1 $p<0.05$, *2 $p<0.01$, significantly different from control.

Iler further, the dose-activity relationship was assayed by using two experimental animal models; carrageenan-induced paw edema in rats was used as an acute inflammation model, and cotton pellet granuloma in rats as a subacute inflammation model. A hot water extract of Iler showed a dose dependent inhibition of carrageenan-induced paw edema within the dose range of 63 to 500 mg/kg, s.c. in rats (TABLE IV), and also a dose dependent inhibition of granuloma formation induced by subcutaneous insertion of 30 mg cotton pellet within the dose range of 63 to 250 mg/kg, s.c. in rats (TABLE V).

Recently, superoxide has been suspected as a mediator of inflammation. And one of the superoxide-scavenging enzymes, SOD, is studied for its anti-inflammatory action, and possible use for treatment of rheumatism.[10] Iler showed a dose dependent superoxide-scavenging activity within the concentration range of 25 to 200 μg/ml (TABLE VI). These results show that Iler has a possibility to be effective widely on various inflammation models.

TABLE VI. Superoxide-Scavenging Activity of Iler Extracts and SOD on Superoxide Anion Produced by Xanthine Oxidase System

Drugs	Concentration (μg/ml)	No.	Optical density (% of control)	Inhibition (%)
Control		3	100.0±4.7	
Iler	25	3	84.4±4.7*1	16
	50	3	74.0±4.5*1	26
	100	3	68.0±5.6*2	32
	200	3	47.4±2.1*3	53
SOD*4	1.3	3	6.5±0.4*3	94

*1 $p<0.05$, *2 $p<0.01$, *3 $p<0.001$, significantly different from control. *4 superoxide dismutase.

Iler is a Labiatae plant which is orally or externally used for treatment of eye diseases, convulsion and menstrual disorder in Indonesia. It is known that many Labiatae plants contain rosmarinic acid, a kind of tannin, which has an anti-inflammatory effect.[11] Since the acid-insoluble fraction of Iler showing an inhibitory effect on histamine release and also a superoxide-scavenging activity (data not shown), was positive to the ferric chloride reagent, it is possible that the effective component of Iler is a tannic acid, too.

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