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The effect of Brotowali (*Tinospora crispa* (L) Miers ex Hook .f. & Thems) extracts on Degranulation Mast cell.

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ABSTRACT

Mast cell degranulation that induced by substance 48/80 at dose of 10 $\mu\text{g/ml}$ from Brotowali extract (*Tinospora crispa* (L) Miers ex Hook .f. & Thems) using invitro methode has been investigated. Extract of Brotowali as preparation was maceration using ethanol and fraction of petroleum ether, chloroform and water. The result of study showed that chloroform fraction of Brotowali with concentration of 5 $\mu\text{g/ml}$ to 625 $\mu\text{g/ml}$ inhibited the mast cell degranulation that induced by 10 $\mu\text{g/ml}$ substance 48/80 with significanty ($p \leq 0,01$).

Keywords: *Tinospora crispa* (L) Miers ex Hook .f. & Thems; Mast cell degranulation; substance 48/80; fraction.

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INTRODUCTION

The use of traditional medicine is very instrumental in disease prevention efforts and improving the standard of public health. The word everyday based on experience or knowledge that is passed on orally and have not been much researched scientifically about the pharmacological efficacy [1,2].

Tinospora crispa (L) Miers ex Hook .f. & Thems , known by the family Menispermaceae is one of the herbs that have been used traditionally as a community remedy, cure hives sores, fevers, malaria, rheumatism, jaundice, and diarrhea[3,4,5].

Studies about the efficacy of herbs has been much done Brotowali, among other analgetik effects, anti diabetic, anti inflammatory and anti-malaria[6,7] of research results has recently been reported that Brotowali extract can give effect as anaphylaxis and anti histamine[8,9].

Anaphylactic reaction is a form of hypersensitivity reaction that occurs soon after exposure to an antigen by the body. The entry of a foreign object (antigen) into the body of a person generally will be followed by the immune response. The specifications of the immune response directed against a particular antigen can provide protection against the effects of tissue known as immune processes (immunity), and can also cause damaging effects of tissue. Such a State is called the hypersensitivity. Immune components in this type of hypersensitivity quickly reaction is IgE (Imunoglobulin E)[10,11] . Imunoglobulin E in the body is bound on the surface of mastcell and basophils. When an antigen enters the body that have been exposed by the antigens it before then it will happend biochemical processes in cells and basophils or mast finally happened the release of mediator-mediator such as histamine, serotonin, bradykinin, arakhidonat acid and others. Mediator at cells stored in the granule-granule, so the release of mediators from granule in cells called degranulation[12,13].

Based of that, then try continued research of Brotowali extract fraction to the mast cell degranulation induced by compound 48/80. as a comparison to inhibition test cell mast degranulation by compound 48/80 is used teofilin, and chromoglikat sodium.

MATERIALS & METHODOLOGY

The samples of this research used extract and fraction of *Tinospora crispa* (L) Miers ex Hook .f. & Thems , distilled water , NaCl physiological solution , compound 48/80 (Sigma No. C-4257) , potasium chloride , calsium chloride , magnesium chloride , sodium dihydrogenfosfat , sodium bikarbonat , glucose , glacial , asetic acid , formaldehyde 37 % (v/v) , toluidin blue , heparin , gelatin , teofillin , and kromoglikat sodium. And this research used the rate males age 2,5 months with age 180 g - 200 g. The entire study was carried out from december 1996 to april 1997 [1,6].

Mast cell dergranulation test

The manufacture of suspension mast cell

Rat fasting for 18 hours,and then was sacrificed,immediately injected with 10 ml of tyrode solution that has been coupled with a gelatin 0,1% b/v and heparin with a concentration of 50 µg/ml in intraperitoneal. Massage the abdomen slowly for more or less 10 minutes. Surgical abdomen carefully and avoid bleeding. Peritoneal fluid taken as much as possible. Entered into the test tubes,disentrifuse for 10 minutes with a speed of 1500 RPM.supernatan aqueous deposite of discarded,wash twice with tyrode solution that has been on the add gelatin and heparin,then suspended with tyrode solution diluted 1 ml [7,13].

Calculate mast cell

Taken 50 µl suspension cell mast placed into test tubes,add 10 µl of solution blue toluidin and 40 µl tyrode solution. Stir slowly with vortices , incubation for 10 minutes at a temprature of 37°C. Mix a few drops of the solution given above haemositometer and the number of cell mast blue purple counted under a microscope.

Mast cell degranulation by compound 48/80

Mast cell suspensions taken 50 µl entered into test tubes and added 30 µl tyrode solution. 10 µl solutions of compound 48/80 and 10 µl aqueous solution blue toludin. Puree slowly with a vortex and incubated for 10 minutes at temperature of 37 °c , and given a few drops above haemositometer and calculate the number of mast cell that are still logs behind . solution of compound 48/80 made with a different concentrations so that the concentration can be obtained dergranulation maximum mast cells [14].

Testing with the sample solution

Mast cell suspension 50 µl is placed in the test tubes , add 30 µl of the sample solution . shake slowly by vortex , added 10 µl solution of compound 48/80 dose 10 µg/ml and 10 µl of solution touidin blue. Puree with a vortex, and incubated for 10 minutes at a temprature of 37 °c , give a few drops above haemosithometer and count the number of cells of the mast which is still lagging

Testing with a another samples

As comparision to the inhibitory test of mast cell degranulation by compound 48/80 used kromoglikat sodium and teofillin.

RESULTS

In this research about the influence of the Brotowali extract fraction to mast cell degranulation the first is from the chemical contents of ethanol extract , petroleum ether fraction, the fraction of chloroform . and the fraction of water from the stem brotowali. These are represented in Table 1.

Table No.1 The chemical content in a Brotowali.

Chemical Content	Reagent	Ethanol	Pethrol	CHCL3	Water
Alkaloid	Mayer	+	-	+	+
Flavonoid	HCL/Mg	-	-	-	-
Phenol	FeCl3	+	-	-	+
Saponins	H2O	-	-	-	+
Triterpenoid / steroids	Liebermannbourchad	+	+	+	-

Key : + (have a chemical contents)
 - (dont have a chemical contents)

Secondary , after the chemical content test , in a preliminary test, namely whether can inhibit mast cell degranulation. It turns out from the test results, ethanol extract may inhibit degranulasi cell mast, then performed well against each such fraction of petroleum ether and chloroform, only a fraction of chloroform that can give the effect of mast cell degranulation. Third, the determination of the optimal concentration of compound 48/80 which mast cell degranulation obtained concentrate 10 µg/ml . These are represented in Table 2.

Table No.2 The average presentage mast cell degranulation by 48/80 compound.

SAMPLE	DEGRANULATION (%)			DEGRANULATION AVERAGE (%)
	PET I	PET II	PET III	
F	0,00	0,00	0,00	0,00
S1	64,28	40,95	54,76	53,33 ± 11,73
S2	71,42	52,38	74,23	66,01 ± 11,88
S3	73,81	69,05	80,95	74,60 ± 5,99
S4	90,48	92,86	93,19	92,17 ± 1,48

Key : F (mast cell suspension)
 S1 (concentration 1 from 48/80 compound) µg/ml
 S2 (concentration 2 from 48/80 compound) µg/ml
 S3 (concentration 5 from 48/80 compound) µg/ml
 S4 (concentration 10 from 48/80 compound) µg/ml

Fourth, testing of the extract ethanol, chloroform fraction, the fraction protelem ether, and water fraction of *Tinospora crispa* (L) Miers ex Hook .f. & Them s stem with various concentrations of 48/80 compound induced by 10 µg/ml. These represented in Table 3 , 4, 5, 6 .

Table No.3 The average presentage mast cell degranulation of 48/80 compound 10 µg/ml after add ethanol extracts of *Tinospora crispa* (L) Miers ex Hook .f. & Them s.

SAMPLE	DEGRANULATION (%)			DEGRANULATION AVERAGE (%)
	PET I	PET II	PET III	
F	0,00	0,00	0,00	0,00
F + S	78,53	88,57	74,97	80,69 ± 7,05
E1 + S	72,48	68,24	65,66	68,79 ± 3,44
E2 + S	59,42	52,09	46,77	52,76 ± 6,35
E3 + S	54,19	39,14	34,55	42,63 ± 10,27
E4 + S	30,63	38,48	25,39	31,50 ± 6,59

Key : F (Mast cell suspension)
 S (concentration of 48/80 compound)
 E1 (the concentration ethanol extracts of 5 on *Tinospora crispa*) µg/ml
 E2 (the concentration ethanol extracts of 25 on *Tinospora crispa*) µg/ml
 E3 (the concentration ethanol extracts of 125 on *Tinospora crispa*) µg/ml
 E4 (the concentration ethanol extracts of 625 on *Tinospora crispa*) µg/ml

Table No.4 The average presentage mast cell degranulation of 48/80 compound 10 µg/ml after add petroleum ether fraction of *Tinospora crispa* (L) Miers ex Hook .f. & Them s.

SAMPLE	DEGRANULATION (%)			DEGRANULATION AVERAGE (%)
	PET I	PET II	PET III	
F	0,00	0,00	0,00	0,00
F + S	82,02	81,56	80,14	81,24 ± 0,98
FP1 + S	76,67	73,38	78,26	76,10 ± 2,49
FP2 + S	75,38	79,98	77,25	77,54 ± 2,31
FP3 + S	74,63	78,71	72,96	75,43 ± 2,96
FP4 + S	72,59	74,07	75,01	73,89 ± 1,22

Key : F (cell mast suspension)
 S (48/80 compound on 10 µg/ml)
 Fp1 (concentration 5 of petroleum ether fraction) µg/ml
 Fp2 (concentration 25 of petroleum ether fraction) µg/ml
 Fp3 (concentration 125 of petroleum ether fraction) µg/ml
 Fp4 (concentration 625 of petroleum ether fraction) µg/ml

Table No.5 The average presentage mast cell degranulation of 48/80 compound 10 µg/ml after add Chloroform fraction of *Tinospora crispa* (L) Miers ex Hook .f. & Them s.

SAMPLE	DEGRANULATION (%)			DEGRANULATION AVERAGE (%)
	PET I	PET II	PET III	
F	0,00	0,00	0,00	0,00
F + S	87,95	83,93	89,96	87,28 ± 3,07
Fk1 + S	73,87	79,91	77,90	77,23 ± 3,08
Fk2 + S	69,20	67,86	61,89	66,32 ± 3,89
Fk3 + S	49,27	56,47	57,14	54,29 ± 4,36
Fk4 + S	29,05	17,02	32,61	26,23 ± 8,17

Key : F (cell mast suspension)
 S (48/80 compound on 10 µg/ml)
 Fk1 (concentration 5 of Chloroform fraction) µg/ml
 Fk2 (concentration 25 of Chloroform fraction) µg/ml
 Fk3 (concentration 125 of Chloroform fraction) µg/ml
 Fk4 (concentration 625 of Chloroform fraction) µg/ml

Table No.6 The average presentage mast cell degranulation of 48/80 compound 10 µg/ml after add water fraction of *Tinospora crispa* (L) Miers ex Hook .f. & Thems.

SAMPLE	DEGRANULATION (%)			DEGRANULATION AVERAGE (%)
	PET I	PET II	PET III	
F	0,00	0,00	0,00	0,00
F + S	86,08	83,76	88,40	86,08 ± 2,32
FA1 + S	80,85	89,65	85,26	85,25 ± 4,40
FA2 + S	84,67	83,48	82,61	83,59 ± 1,03
FA3 + S	80,18	82,53	81,02	81,24 ± 1,19
FA4 + S	79,59	80,38	81,93	80,63 ± 1,19

Key : F (cell mast suspension)

S (48/80 compound on 10 µg/ml)

FA1 (concentration 5 of Water fraction) µg/ml

FA2 (concentration 25 of Water fraction) µg/ml

FA3 (concentration 125 of Water fraction) µg/ml

FA4 (concentration 625 of Water fraction) µg/ml

Table No.7 The average presentage mast cell degranulation of 48/80 compound 10 µg/ml after add sodium cromoglikat of *Tinospora crispa* (L) Miers ex Hook .f. & Thems.

SAMPLE	DEGRANULATION (%)			DEGRANULATION AVERAGE (%)
	PET I	PET II	PET III	
F	0,00	0,00	0,00	0,00
F + S	82,97	84,92	87,57	85,15 ± 2,31
NC1 + S	62,16	59,46	56,76	59,46 ± 2,70
NC2 + S	29,73	24,32	18,92	24,32 ± 5,41
NC3 + S	27,02	21,62	16,22	21,62 ± 5,40

Key : F (cell mast suspension)

S (48/80 compound on 10 µg/ml)

NC1 (concentration 5 of sodium cromoglikat) µg/ml

NC2 (concentration 16 of sodium cromoglikat) µg/ml

NC3 (concentration 50 of sodium cromoglikat) µg/ml

Table No.8 The average presentage mast cell degranulation of 48/80 compound 10 µg/ml after add teofillin of *Tinospora crispa* (L) Miers ex Hook .f. & Thems.

SAMPLE	DEGRANULATION (%)			DEGRANULATION AVERAGE (%)
	PET 1	PET 2	PET 3	
F	0,00	0,00	0,00	0,00
F + S	85,91	80,83	83,33	83,35 ± 2,54
Tf1 + S	55,56	58,33	62,50	58,79 ± 3,49
Tf2 + S	37,50	41,00	40,28	39,82 ± 1,85
Tf3 + S	16,67	29,17	33,33	26,39 ± 8,67

Key : F (cell mast suspension)

S (48/80 compound on 10 µg/ml)

Tf1 (concentration 5 of teofillin) µg/ml

Tf2 (concentration 16 of teofillin) µg/ml

Tf3 (concentration 50 of teofillin) µg/ml

CONCLUSION

After an examination of the mast cell degranulation test of fractions extracts can be summarized as follows Brotowali, brotowali chloroform fraction of the plants at a concentration of 5 µg/ml to 625 µg/ml can reduce mast cell degranulation by compound 48/80 10 µg/ml invitro and with an increase in the concentration of chloroform fraction Brotowali the mast cell degranulation induced by compound 48/80 10 µg/ml of the smaller ($p \leq 0,01$).

REFERENCES

- [1] Departemen Kesehatan Republik Indonesia , *Tanaman Obat Indonesia* , Direktorat Jendral Pengawasan Obat dan Makanan , Jakarta , 1985.
- [2] Gunawan , D.J. Wahyono Donatus I. A. , Tareno dan Mulyono *Risalah Simposium Penelitian Tumbuhan Obat III* , Fakultas Farmasi , Universitas Gajah Mada , Jakarta , 1983.
- [3] Sudarma , M dan R. Harsono , *Cabe Puyeng Warisan Nenek Moyang* , cetakan kedua , PT Karya Weda , Jakarta , 1975.
- [4] Salim , S dan Gani . H . , *Pengembangan Obat Tradisional Dalam Rangka Pemakaian Secara Medis* , Majalah Kedokteran UNAND , Padang , 1985.
- [5] Departemen Kesehatan Republik Indonesia , *Penelitian Tanaman Obat di Beberapa Perguruan Tinggi di Indonesia* , I – III , Jakarta , 1988 , pp.2-5.
- [6] Taruna , Hilwan , “ Uji Efek Analgetik dari Infusa Batang *Tinospora crispa* (L) *Miers ex Hook .f. & Thems* pada Mencit Putih Jantan dengan Metoda Tail Flick “ , *Skripsi* , Sarjana , Farmasi , FMIPA , UNAND , Padang , 1987 , pp.20.
- [7] Rivai , Yutiardy , “Uji Efek Anti Inflamasi (Anti Radang) Infusa Batang Brotowali (*Tinospora crispa* (L) *Miers ex Hook .f. & Thems*) Pada Tikus Putih Jantan” , *Skripsi* , Sarjana Farmasi , FMIPA , UNAND , PADANG , 1987 , pp.23-24.
- [8] Sri Rahyuni , “Uji Aktivitas Senyawa *Tinoscrisposid* terhadap Histamin Secara *Invitro*” , *Skripsi* Sarjana Farmasi FMIPA , UNAND , padang , 1996.
- [9] Elfanetti , “Aktivitas Anti Anafilaksis Kutan Aktif dari Ekstrak Tumbuhan Brotowali (*Tinospora crispa* (L) *Miers ex Hook .f. & Thems*)” , *Skripsi* Sarjana Farmasi , FMIPA , UNAND , Padang , 1995 , pp. 14.
- [10] Bellanti , J.A. , *Imunologi* , Edisi III , Terjemahan A.S.Wahab , Gajah Mada University Press , Yogyakarta , 1993.
- [11] Kresna , S.B. , *Imunologi* , Edisi II , Fakultas Kedokteran Universitas Indonesia , Jakarta , 1991 , pp.34.
- [12] Roitt , I.M. , *Pokok-pokok Ilmu Kekebalan* , Terjemahan G. Bonang, E. Sulistijowati dan K. Tanzil, PT Gramedia Pustaka , Jakarta , 1990.
- [13] Aldi , Yufri , “Uji Efek Antialergi Dari Tumbuhan *Andrographis Paniculata* Ness. Dan *Blumea Balsamifera* (L) DC. , Thesis Magister Program Studi Farmasi , Program Pasca Sarjana , ITB , Bandung , 1994.
- [14] Kimura , M.I. , Waki and M.Kokubo , "Inhibition of Coumpound 48/80 Mediated Histamine Release from Isolated Rat Mast Cell by Oosponol Related Coumpound (4 - Acyl - Isocoumarins) " , *Japan J . Pharmacol* 1978;28:3-5.