Kode/Nama Rumpun Ilmu: 402/Farmakologi dan Farmasi Klinik

LAPORAN AKHIR

PENELITIAN HIBAH BERSAING

RINGKASAN DAN EXECUTIVE SUMMARY

PENGEMBANGAN MINYAK JAHE (*Zingiber officinale*) SEBAGAI PILIHAN TERAPI NYERI KRONIK PADA KEADAAN NEUROPATI DAN INFLAMASI

Tahun ke 1 dari rencana 2 tahun

TIM PENGUSUL

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RINGKASAN

Berdasarkan definisinya, nyeri kronik merupakan keadaan nyeri yang terjadi dalam jangka waktu yang lama, lebih dari 6 bulan yang umumnya menyertai penyakit seperti kanker, diabetes, *rheumatoid arthritis* dan masih banyak lagi. *International Association for the Study of Pain* (IASP) mendefinisikan nyeri sebagai pengalaman sensoris dan emosional tidak menyenangkan yang berhubungan dengan terjadinya kerusakan jaringan baik secara aktual maupun potensial.

Nyeri kronik menjadi suatu masalah yang serius sehubungan dengan tingginya angka prevalensi. Di Indonesia sendiri, pada penduduk dengan usia lanjut, dilaporkan bahwa 25-50% diantaranya mengalami nyeri. Kondisi nyeri kronik juga dihubungkan dengan fungsi mental dan sosial serta kemiskinan akibat kehilangan pekerjaan. Peningkatan kejadian nyeri kronik juga berhubungan dengan peningkatan biaya kesehatan.

Berdasarkan studi yang ada, 75% pasien menggunakan terapi komplementer menggunakan tanaman sebagai alternatif dalam mengatasi penyakitnya. Masyarakat beranggapan bahwa penggunaan tanaman memberikan efek samping yang lebih kecil dan hampir tidak toksik dibandingkan obat sintetik. Indonesia merupakan satu dari negara tropis yang kaya akan sumber alam. Biodiversivitas sumber alam memungkinkan untuk eksplorasi aktivitas potensial dari tanaman herbal, salah satunya sebagai terapi nyeri kronik. Jahe, merupakan tanaman yang awalnya hanya digunakan sebagai bumbu dapur, namun saat ini banyak penelitian yang memperlihatkan aktivitas tanaman jahe pada berbagai macam penyakit. Minyak jahe memperlihatkan aktivitas antiinflamasi, antinosiseptif dan imunomodulator. Minyak jahe dapat mempengaruhi memori dan tingkah laku hewan coba dan hal ini dihubungkan dengan sistem kolinergik. Minyak jahe juga memperlihatkan efek anastesi diperantarai dengan menurunkan aktivitas serotonin (5HT-3) dan memberikan efek analgesik dengan menghambat substansi P di otak. Pemberian minyak jahe meningkatkan jumlah GABA pada daerah hippokampus dan korteks otak hewan coba. Tujuan penelitian ini adalah mengembangkan minyak jahe sebagai terapi nyeri kronik disebabkan inflamasi dan neuropati yang dibuktikan secara ilmiah. Parameter yang diamati pada tahun pertama perubahan tingkah laku dari hewan coba seperti waktu ketahanan terhadap stimulus panas dan tebal plantar serta kadar 6-gingerol pada minyak jahe yang memberikan aktivitas sebagai antinyeri. Pada tahun kedua akan diamati histopatologi otak dan *spinal cord* mencit, mekanisme antinyeri secara imunohistokimia dan toksisitas akut dari minyak jahe.

Penelitian ini diperoleh bahwa minyak jahe merah yang diperoleh dengan destilasi telah sesuai dengan SNI 06-1312-1998 dalam bobot jenis (BJ), bilangan asam dan bilangan ester. Minyak jahe merah yang diberikan secara per oral pada mencit nyeri kronik juga dapat menurunkan hiperalgesia yang ditandai dengan peningkatan waktu ketahanan terhadap stimulus panas. Aktivitas ini meningkat dengan peningkatan dosis. Aktivitas ini diduga karena adanya senyawa 6-gingerol dalam jahe merah yang dapat berfungsi pada keadaan neuroprotektif.

Antihyperalgesia of Red Ginger (*Zingiber officinale* var. *rubrum*) Oil Activity in Male Mice with Completed Freud's Adjuvants (CFA)-Induced Chronic Pain

Abstract

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This study aims to prove antihyperalgesia activity of red ginger oil in chronic pain because of chronic inflammation. Twenty five mice were divided into 5 groups (sham, CFA, red ginger oil doses 100,200 and 400 mg/kbBW). Each group was induced chronic inflammation by CFA 40 µl intraplantar and sham groups was induced by normal saline. At days-7, all groups were given by treatment once daily for seven days. At days-14, all mice were sacrificed. Hyperalgesia and plantar thickness were measured at day 0,1,3,5,7,8,10,12 and 14. Red ginger oil was qualified by SNI 06-1312-1998 as standar and Camphene was the highest compound by GC/MS analysis. Red ginger oil administration in mice prolonged latency time with thermal stimulus and the activity was dose dependent and decreased the plantar thickness but was not significantly different with CFA groups. The conclusion of this research is red ginger oil have antihyperalgesia activity in mice with CFA-induced chronic pain.

Key word: Red ginger oil, chronic pain, hyperalgesia, CFA

Introduction

Chronic pain is a pain with duration more than 3 months. Chronic pain usually follows chronic diseases or degenerative diseases such as diabetes mellitus, cancer, chronic inflammation, infection and many else. Until now, treatment for chronic pain is still a chalengge because of their effectiveness and side effect.

Pathophysiology of chronic pain involves increasing of stress oxidative. After peripheral nerves injury by chronic inflammation cause increasing of superoxides (SO) and then activate peroxynitrite (PN). Induction of PN will activate some pathways, such

as Gamma Amino Butyric Acid (GABA), Cyclooxygenase (COX), glutaminergic neurotransmission, Protein Kinase C (PKC), Transient Receptor Protein (TRP) channel, cytokine and Mitogen-Activated Protein Kinase (MAPK) and stimulate central sensitization in chronic pain.

Red ginger is one of the species from ginger that have been used by Indonesian people as treatment in many disease. Red ginger have an antioxidant effect higher than ginger. Essential oil from red ginger have been known as a compound that responsible for this effect.

Red ginger oil composition, have been known consisted by essensial oil, such as cineol and zingiberen. This compound have antioxidant activity. Based on relationship between pathophysiology of chronic pain with stress oxidative, it is possible to block central sensitization by antioxidant effect. This research was done to prove activity of red ginger oil in reducing hyperalgesia in mice with Completed Freud's Adjuvants (CFA)-induced chronic pain

Material and Method

Twenty 8-week-old mice (males) were used for the experiment. They were purchased and kept in the Animal House, College of Pharmacy, University of Jember, East Java, Indonesia. The temperature of the room was maintained at 26-28 C with a 12-hour light/12-hour dark cycle.

Red ginger was puchased from farmer group "PeciFarm" in Kencong, Jember, East Java, Indonesia. Red ginger that was choosen in this research is red ginger that reached 10 months aged.

This research was done in Faculty of Pharmacy University of Jember and got the ethical clearance from committe of ethics from Faculty of Medicine University of Jember

Red Ginger Oil Preparation

100-200 g fresh red ginger were prepared in destilator with aquadest (1:2). Destilation process was done in 100-121 ^{0}C, for 5-6 hours. Red ginger oil was emulted with tween 0,5% at dose 100, 200, and 400 mg/kgBW.

Red Ginger Oil Specification (SNI 06-1312-1998)

Distillates were analyzed for physico-chemical characteristics, including density, the acid number and the ester number. The analytical method used to follow the national standard in accordance with the quality standards referred, namely SNI 06-1312-1998 (Oil of ginger). Experiments were performed with three replications.

GC/MS Analysis for Red Ginger Oil

GC/MS analysis was carried out on GCMS-QP2010 Plus. The sample was detected on a coloumn oven temperature 80 ^{0}C and injection temperature 250 ^{0}C with total flow 9 ml/min and column flow 1 ml/min. The EI mode for mass spectrometes has ion source temperature of 200 ^{0}C and interface temperature of 280 ^{0}C. Sample was injected in split mode. Total eluation time was 55 min. MS scanning was performed from m/z 40 to m/z 600.

GC/MS Identification of Components

Indentification of red ginger oil component was based on computer evaluation of mass spectra of sample from library (WILEY7.LIB). Identification was done by comparation of peaks and retention with standard compound by following the characteristic fragmentation of the mass spectra of compounds.

CFA-induced Inflammatory Pain

Mice in treatment group were anesthetized and injected by 40 μl CFA (Complete Freud's Adjuvant) in intraplantar site. Mice in sham group were injected by 40 μl normal saline. CFA was purchased from Sigma Chemical Co USA.

Hyperalgesia and Antinociceptive Respon Test

Twenty four mice were divided into 5 groups i.e sham, negative control, treatment (red ginger oil at three different doses: 100, 200, 400 and 600 mg/kgBW). Inflammatory condition was induced by intraplantar injection of CFA (*Completed Freud's Adjuvant*). Ginger oil (in treatment groups) were administrated by per oral once a day for seven consecutive days, at a week after CFA injection. Sham and negative control were administrated by tween 0,5%. Latency time toward thermal stimulus was measured on days 0, 1, 3, 5, 7, 8, 10, 12 and 14 after CFA injection. Hyperalgesia respon were hind paw lick, hind paw flick or jump (whichever came first). Paw thickness at the ipsilateral site was also measured on days 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 14 after CFA injection.

a. Result

b. Red Ginger Oil Specification

Red ginger for distillation is fresh red ginger with age 12 month. The yield from distillation is 0.43%. Red ginger oil from distillation was analyzed for density, the acid number and the ester number. The result can be shown at table 1.

The composition of essential oil of red ginger was analyzed by GC/MS. Twelve compound were identified by comparison with the library, as shown in picture 1 and table 2.

Table 3 showed that there were no significant different between latency time toward thermal stimulus of CFA and sham group on day 0 ($p>0,05$). This condition was significant different after CFA induction. Latency toward thermal stimulus were significantly decrease after CFA induction, lower than sham group on day 1,3,5 and 7 ($p<0,05$). Induction of CFA not only caused a damage of nerve system that caused increasing of latency toward thermal stimulus, but also caused increasing of paw thickness. Table 4 showed that CFA group have higher paw thickness than sham group on day 1, 3, 5 and 7 ($p<0,05$).

Red Ginger Oil Activity in CFA-induced Inflammatory Pain

Red ginger oil administration for 7 consecutive days changed the hyperalgesia response in mice. Table 5 and 6 showed that ginger oil increased latency time toward thermal stimulus and decreased paw thickness in mice. The increasing response after ginger oil administration were significantly different than sham group.

Discussion

Chronic pain can be caused by inflammation. This research use CFA (complete freud's adjuvants) that is composed of inactivated and dried mycobacteria (usually *M. tuberculosis*). CFA is effective in stimulating cell-mediated immunity and leads to potentiation of T helper cells that leads to production of certain immunoglobulin and effector T cells. CFA is responsible for inflammation in mice, because CFA caused stimulation phagocytosis system and cytokine secretion (Calder, 2006; Xie, 2011). CFA is a substance that caused inflammatory pain by increasing latency time toward thermal stimulus and paw thickness compared with baseline (before induction).

CFA as noxious stimulus caused imbalance between inhibitory and excitatory neurotransmitter in brain. CFA increased release of glutamate as excitatory

neurotransmitter that bind to NMDA receptor, caused depolarization and activation of central pathway. Longterm depolarization caused lossing of magnesium inhibitory in NMDA channel, affected calsium entry to intracelluler and activated NR2B receptor. This pathway was the key of chronic pain pathophysiology (Macintyre, *et al*., 2010; Zhuo *et al*., 2011).

Red ginger oil action was related to induction of GABA agonist. The availability of GABA restored the balancing between exitatory and inhibitory neurotransmitter in brain (Chandra, *et al*., 2005). GABA suppressed the release of glutamate and blocked intake calsium to intracelluler. This condition decreased NR2B activity and pain sensitization. The activity of red ginger oil is based on the composition of essential oil. Sivasothy *et al* (2011) found that red ginger oil was consisted by monoterpene, with camphene (14.5%), geranial (14.3%) and geranyl acetate (13.7%). This essensial oil showed an antioxidant effect by reducing oxidative stress in pathophysiology of chronic pain (Janes *et al*., 2011). One of the mechanism of how oxidative stress induced sensitization in chronic pain was by causing loss of GABA neuron. Antioxidant activity showed increasing of GABA activity then reimbalanced neurotransmitter in brain and reduced chronic pain (Yowtak, *et al*., 2013).

Coclusion

Red ginger oil administration ameliorated hyperlagesia condition in mice with increased latency time toward thermal stimulus and decreased paw thickness after administration for seven consecutive days.

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Conflict of Interest

The autors declare no conflict of interest.

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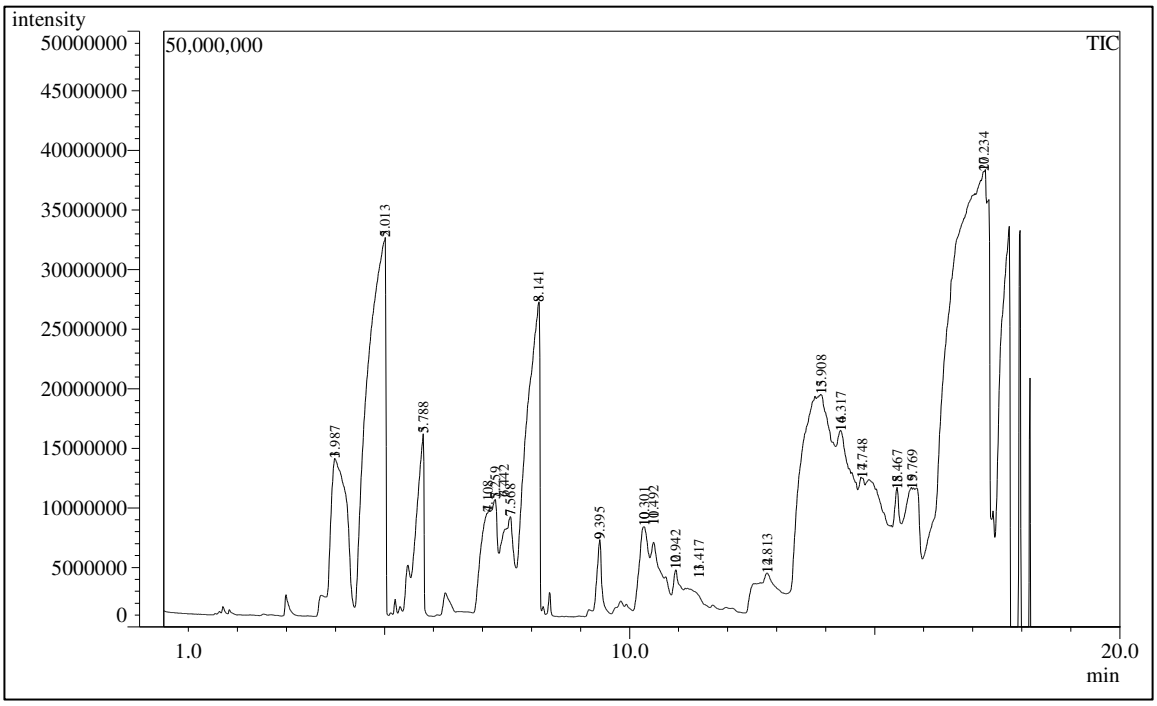
Table and Figures

Table 1 Red Ginger Oil Spesification

Criteria	SNI (06-1312-1998)	Result	%CV	Description
Colour	-	yellow-red	-	-
Density	0,872-0,889	0,881 g/ml	0,845%	qualify
The acid number	max 2	0,202	9,06%	qualify
The ester number	max 15	2,0635	30,23	qualify



Picture 1 Chromatogram of Essensial Oil from Red Ginger by GC/MS

Table 2 Essensial Oil Composition of Red Ginger

Peak	RT	% Area	% Height	Name
1	3.987	5.24	5.85	Alpha-pinene (-)-
2	5.013	14.55	14.09	Camphene
3	5.788	2.81	6.78	beta-Myrcene
4	7.108	2.54	3.75	Cyclohexene,1-methyl-4-(1-methylethenyl),-
5	7.259	1.10	4.26	Cyclohexene,1-methyl-4-(1-methylethenyl),-
6	7.442	1.33	3.11	beta-Phellandrene
7	7.568	1.20	3.65	Sabinene
8	8.141	8.99	11.48	1,8-Cineole
9	9.395	0.71	2.57	alpha-Terpinolene
10	10.301	0.68	3.16	Linalool
11	10.492	1.68	2.54	3-Methyl-2-(2-methyl-2-butenyl)-furan
12	10.942	0.62	1.44	trans-3(10)-Caren-2-ol
13	11.417	0.59	0.48	Bicyclo(2.2.1)heptan-2-ol, 1,5,5-trimethyl-(C)
14	12.831	1.52	1.23	Citronella
15	13.908	13.04	7.27	endo-Borneol
16	14.317	5.08	5.70	3-Cyclohexene-1-methanol, alpha.,alpha.,tr
17	14.748	4.93	3.70	beta-Citronellol
18	15.467	0.94	2.90	Z-Citral
19	15.769	2.21	2.74	beta-Citronellol
20	17.234	29.24	13.28	2,6-Octadien-1-ol, 3,3-dimethyl-(CAS) 3,7D

Table 3 Average of Latency Time Toward Thermal Stimulus on Day 0, 1, 3, 5 dan 7

No	Group	Average of Latency Time Toward Thermal Stimulus on day (secon)				
		0	1	3	5	7
1	CFA (n = 20)	9,52±0,43	7,13±0,23	6,98±0,20	6,65±0,25	5,60±0,26
2	*Sham* (n = 5)	9,03±0,65	8,96±0,76*	8,36±0,18*	8,98±0,55*	8,42±0,34*

*Data are presented as mean (SEM), *)p<0.05 means significant different versus* sham group.

Table 4 Average of Paw Thickness of Mice on day 0, 1, 2, 3, 4, 5, 6, and 7

No	Group	Average of Paw Thickness of Mice on day (cm)				
		0	1	3	5	7
1	*Sham* (n = 5)	0,19±0,01	0,21±0,01	0,19±0,01	0,20±0,01	0,20±0,01
2	CFA (n = 20)	0,20±0,01	0,36±0,01*	0,34±0,01*	0,34±0,01*	0,34±0,01*

*Data are presented as mean (SEM), *)p<0.05 means significant different versus* sham group

Table 5 Average of Latency Toward Thermal Stimulus After Ginger Oil Administration on day 8, 10, 12 and 14

Group	Treatment	Average of Latency Toward Thermal Stimulus (secon) on day-			
		8	10	12	14
Sham (n=5)	normal salin	8,57±0,53[a]	8,19±0,41[a]	8,02±0,66[a]	8,67±0,73[a]
CFA (n=20)	normal salin	4,39±0,57[b]	4,82±0,27[b]	4,11±0,57[b]	4,67±0,14[b]
	MJ 100 mg/kgBB	6,85±0,54[c]	7,14±0,85[a]	7,96±0,91[a]	7,98±1,09[a]
	MJ 200 mg/kgBB	5,63±0,26[c]	6,27±0,76[b]	6,33±0,70[a]	7,65±0,32[a]
	MJ 400 mg/kgBB	6,66±0,30[c]	7,56±0,22[a]	8,04±0,34[a]	9,31±0,29[a]

Data are presented as mean (SEM), different letter means significant different between groups

Table 6 Average of Paw Thickness of Mice on day 8, 10, 12 and 14

Group	Treatment	Average of Plantar Thickness (cm) on day			
		8	10	12	14
Sham (n=4)	normal salin	0,20±0,00[a]	0,21±0,00[a]	0,20±0,00[a]	0,20±0,00[a]
CFA (n=20)	normal salin	0,34±0,02[b]	0,33±0,03[b]	0,32±0,02[b]	0,32±0,01[b]
	MJ 100 mg/kgBB	0,33±0,02[c]	0,32±0,03[a]	0,31±0,01[a]	0,30±0,01[a]
	MJ 200 mg/kgBB	0,30±0,01[c]	0,29±0,01[b]	0,30±0,01[a]	0,29±0,01[a]
	MJ 400 mg/kgBB	0,33±0,01[c]	0,32±0,01[a]	0,31±0,01[a]	0,30±0,01[a]

Data are presented as mean (SEM), different letter means significant different between group

Luaran 2

PENURUNAN HIPERALGESIA MINYAK JAHE EMPRIT DIBANDINGKAN MINYAK JAHE MERAH TERHADAP TIKUS DENGAN INFLAMASI KRONIK AKIBAT INDUKSI COMPLETED FREUD'S ADJUVANT (CFA)

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Latar Belakang : Nyeri kronik dapat disebabkan karena inflamasi, salah satunya oleh bakteri. Jahe merupakan tanaman yang digunakan secara luas sebagai antioksidan, diantaranya adalah jahe emprit (Zingiber officinale var amarum) dan jahe merah (Zingiber officinale var rubrum). Berbagai macam studi menyebutkan bahwa kandungan 6-gingerol pada jahe dapat menghambat produksi ROS dan sitokin seperti TNF-α dan IL-1β. Berdasarkan pustaka, kandungan 6-gingerol dari jahe merah lebih tinggi dibandingkan jahe emprit.

Tujuan : Penelitian ini bertujuan untuk membandingkan aktivitas nyeri kronik karena inflamasi setelah induksi CFA pada minyak jahe emprit dan jahe merah.

Metode : Jahe emprit dan jahe merah dibuat menjadi minyak melalui *steam-water destillation* selama 5-6 jam. Dua puluh ekor mencit terbagi menjadi 4 kelompok, yaitu sham, kontrol negatif, minyak jahe emprit dosis 100 mg/kgBB dan minyak jahe merah dosis 100 mg/kgBB. Nyeri kronik karena inflamasi diinduksi oleh pemberian CFA intraplantar sebesar 40 μl. Pada hari ke-7 setelah induksi CFA, mencit diberi perlakuan dengan minyak jahe dalam tween 1% satu hari sekali secara per oral, selama tujuh hari berturut-turut. Kelompok sham dan kontrol negatif diberikan tween 1% secara per oral. Waktu ketahanan terhadap stimulus panas diukur pada hari ke 0,1,3,5,7,8,10,12 dan 14 setelah induksi CFA. Tebal plantar diukur pada hari ke 1,2,3,4,5,6,7,8,10,12, dan 14 setelah induksi CFA.

Parameter yang diukur : waktu ketahanan terhadap stimulus panas dan tebal plantar dalam bentuk rata-rata±SEM. Analisis data menggunakan *independent t-test* dan *one way anova* dengan kepercayaan 95%.

Results : Minyak jahe dan jahe merah dapat menurunkan waktu ketahanan terhadap stimulus panas dan tebal plantar dibandingkan kontrol negatif.

Conclusion : Minyak jahe dan jahe merah dapat menurunkan hiperalgesia pada keadaan nyeri kronik akibat inflamasi dengan induksi CFA.

Keywords : Nyeri inflamasi, CFA, minyak, jahe