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 2 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 merah, merupakan tanaman yang banyak digunakan untuk mengatasi berbagai macam penyakit di masyarakat. Minyak jahe memperlihatkan aktivitas antiinflamasi, antinosiseptif dan imunomodulator. Kandungan minyak atsiri pada jahe merah terbanyak dibandingkan jenis yang lain. Minyak atsiri berperan sebagai antioksidan, salah satu mekanisme yang penting dalam penghambatan nyeri kronik. Nyeri kronik sendiri merupakan nyeri jangka panjang dengan durasi lebih dari 6 bulan yang umumnya menyertai berbagai macam penyakit, salah satunya inflamasi kronik dan neuropati. Tujuan penelitian ini adalah mengembangkan minyak jahe merah sebagai terapi nyeri kronik disebabkan inflamasi dan neuropati yang dibuktikan secara ilmiah. Parameter yang diamati pada tahun pertama perubahan respon nyeri dari mencit yang diinduksi nyeri kronik dengan inflamasi kronik oleh CFA dan neuropati dengan metode PSNL. Pada tahun kedua akan diamati histopatologi otak dan *spinal cord* mencit, mekanisme pada nyeri kronik secara imunohistokimia dan toksisitas akut dari minyak jahe merah.

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. Pemberian minyak jahe merah secara akut memberikan harga LD_{50} > 5000 mg/kg yang berarti dalam kategori aman. 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 pada keadaan inflamasi kronik dengan induksi CFA, neuropati dengan induksi PSNL dan diabetes neuropati. Aktivitas ini meningkat dengan peningkatan dosis. Kandungan minyak atsiri yang diduga bertanggung jawab terhadap aktivitas ini adalah champene. Pemberian minyak jahe merah memperbaiki histopatologi *spinal cord* mencit dengan nyeri kronik. Aktivitas minyak jahe merah ini berhubungan dengan hambatan pada COX-2 dan NMDA subunit NR2B pada otak dan *spinal cord* mencit dengan nyeri kronik.

Antihyperalgesia Activity of Red Ginger Oil (*Zingiber officinale* var. *rubrum*) in Inflammatory and Neuropathy-Induced Chronic Pain Condition in Mice

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Abstract

Chronic pain is a pain with duration more than six months that can be induced by chronic inflammation and neuropathy. This study was aimed to analyze the component of red ginger oil and proved its antihyperalgesia activity in chronic pain that was caused by chronic inflammation and neuropathy. Forty eight mice were divided into 2 groups ie inflammatory and neuropathy. Each groups were divided into 6 subgroups (sham, CFA or PSNL, red ginger oil doses 100, 200, 400 and 600 mg/kg). Inflammatory group was induced using CFA 40 µl intraplantar. Neuropathy group was induced by PSNL method. At day-7, all groups were given treatment once daily for seven days. Hyperalgesia and plantar thickness were measured at day 0,1,3,5,7,8,10,12 and 14. Quality of Red ginger oil was standarized by SNI 06-1312-1998. The red ginger oil compound was identified by GC/MS. The result showed that red ginger oil was qualified based on SNI 06-1312-1998. Camphene was the highest compound by GC/MS analysis. Red ginger oil administration in mice prolonged latency time toward thermal stimulus and significantly different with CFA and PSNL group. The conclusion of this study that red ginger oil have antihyperalgesia activity in mice with chronic pain.

Key word: Chronic pain, hyperalgesia, CFA, PSNL, Red ginger oil

Introduction

Pain is a multidimensional experience that affect to both sensory and emotional. Chronic pain is a pain with duration more than six months (1). Chronic pain usually follows chronic or degenerative diseases such as diabetes mellitus, cancer, chronic

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inflammation, infection and many else. Until now, treatments for chronic pain are still a challengge because of its effectiveness and side effects (2).

Regarding on pathophysiology, chronic pain are caused by inflammation and neuropathy. Pathophysiology of chronic pain involves increasing of stress oxidative. Peripheral nerves injury by chronic inflammation causes increasing of superoxides (SO) and activates peroxynitrite (PN) (3). 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), then stimulates central sensitization in chronic pain (4).

Red ginger is one of the species from ginger that have been used by Indonesian people as treatment in many diseases (5). Red ginger has an antioxidant effect higher than ginger (6). Essential oils from red ginger have been known as compound that responsible for this effect.

Red ginger oils composition, have been known consisted by essensial oils, such as cineol and zingiberen. This compound had antioxidant activity. Based on relationship between pathophysiology of chronic pain with stress oxidative, it was possible to block central sensitization by antioxidant effect (7). This research was aimed to analyze the component of red ginger oil and prove its activity in reducing hyperalgesia in mice with chronic pain induced by inflammation and neuropathy condition.

Materials and Methods

Forty eight 8-week-old mice (male) 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 ^{0}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. CFA (Completed Freud's Adjuvant) was purchased from Sigma Chemical Co USA.

This research was done in Faculty of Pharmacy University of Jember and got the ethical approval from Committe of Ethics from Faculty of Medicine, University of Jember (No: 774/H25.1.11/KE/2016)

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, 400 and 600 mg/kgBW.

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

Distillates were analyzed for physico-chemical characteristics, included density, the acid number and the ester number. The analytical method used 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 spectrometers has ion source temperature of 200 ^{0}C and interface temperature of 280 ^{0}C. Samples were

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 analyzed by comparation of peaks and retention with standard compound by following the characteristic fragmentation of the mass spectra of compounds.

CFA (Completed Freud's Adjuvant)-induced Inflammatory Pain

Twenty four mice were divided into 6 groups i.e sham, negative control, treatment (red ginger oil at four different doses: 100, 200, 400 and 600 mg/kg). Inflammatory condition was induced by intraplantar injection of CFA. Mice were anesthetized by aether and injected by 40 µl of CFA in intraplantar site. Mice in sham group were injected by 40 µl normal saline. Red ginger oil (in treatment groups) were administrated by per oral once a day for seven consecutive days, a week after CFA injection. Sham and negative control were administrated by tween 0.5%.

PSNL (Partial Sciatic Nerve Ligation)-induced Neuropathy Pain

Twenty four mice were divided into 6 groups i.e sham, negative control, treatment (red ginger oil at four different doses: 100, 200, 400 and 600 mg/kg). Mice were induced neuropathy with PSNL method. Mice were anesthetized with aether and tying 1/3-1/2 of dorsal portion of sciatic nerve on the left lumbar nerve of mice with 8-0 silk. sham group, the sciatic nerve was exposed without ligation. Red ginger oil (in treatment groups) were administrated by per oral once a day for seven consecutive days, a week after CFA injection. Sham and negative control were administrated by tween 0.5%.

Hyperalgesia and Antinociceptive Respon Test

Latency time toward thermal stimulus were measured using hot plate at temperature 50 °C on days 0 (baseline), 1, 3, 5, 7, 8, 10, 12 and 14. Hyperalgesia responses were mice showed hind paw lick, hind paw flick or jump (whichever came first). Paw thickness of inflammatory group at the ipsilateral site was also measured on days 0 (baseline), 1, 3, 5, 7, 8, 10, 12 and 14.

Statistical analysis

Latency time towards thermal stimulus and paw thickness were expressed as mean±SEM. The significant differences between injury (inflammatory and neuropathy) and sham group on day 0,1,3,5 and 7 were tested by independent t-test ($p<0.05$). The significant differences between groups on day 8,10,12 and 14 were tested by one-way ANOVA followed by Tukey's HSD ($p<0.05$).

Results

Red Ginger Oil Specification

Red ginger for distillation was fresh red ginger with 10 month aged. The yield from distillation was 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.

Table 1 Red Ginger Oil Spesification

No	Criteria	SNI (06-1312-1998)	Result	Description
1	Colour	-	yellow-red	-
2	Density	0.872-0.889	0.881 g/ml	qualify
3	The acid number	max 2	0.202	qualify
4	The ester number	max 15	2.0635	qualify

GC/MS Analysis for Red Ginger Oil

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 figure 1 and table 2.

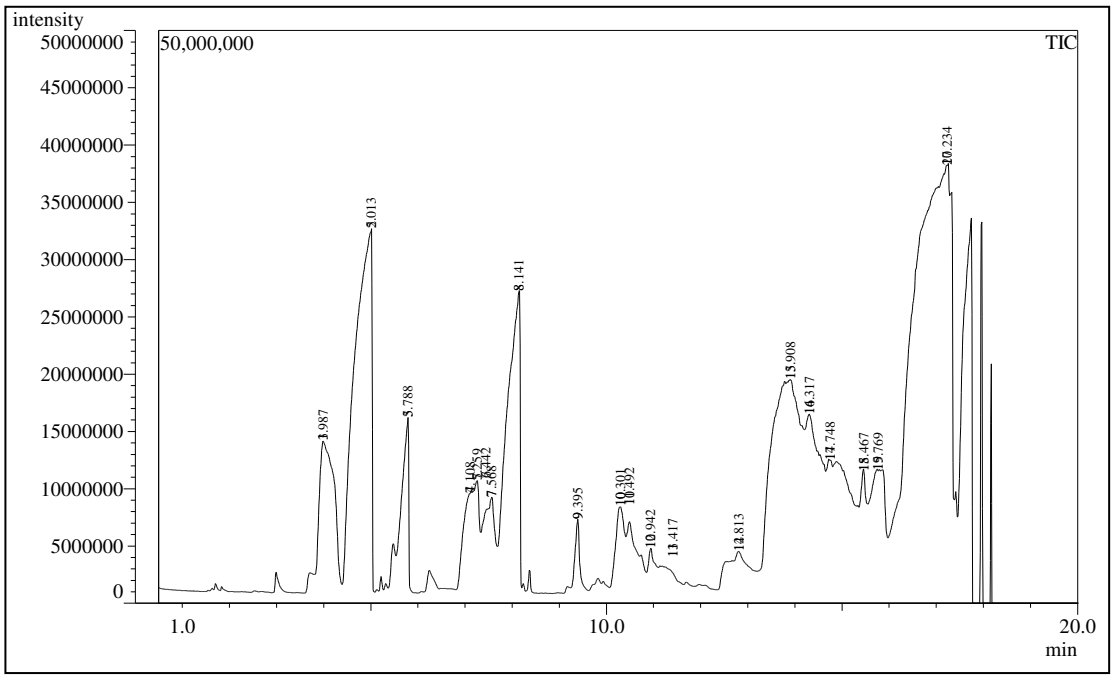

Fig. 1 The Chromatogram Profile of Essensial Oil from Red Ginger by GC/MS

Table 2 The Essensial Oil Composition of Red Ginger Oil

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

CFA-induced Inflammatory Pain

Table 3 showed that previously, there were no significantly different between latency time toward thermal stimulus of CFA and sham group on day 0 (*p>0.05*) and this condition was significantly 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 increased latency time toward thermal stimulus, but also caused increasing of paw thickness. Table 4 showed that CFA group have higher paw thickness than sham group before red ginger oil administration on day 1, 3, 5 and 7 (*p<0,05*).

Table 3 The Average of Latency Time Toward Thermal Stimulus between CFA and sham groups on Day 0, 1, 3, 5 dan 7

No	Group	Average of Latency Time Toward Thermal Stimulus on day (secon)					% Decreasing of Latency Time
		0	1	3	5	7	
1	Sham (n = 4)	9.03±0.65	8.96±0.76*	8.36±0.18*	8.98±0.55*	8.42±0.34*	6.76
2	CFA (n = 20)	9.52±0.43	7.13±0.23	6.98±0.20	6.65±0.25	5.60±0.26	41.18

*Data were presented as mean (SEM), *)p<0.05 means significant different compared to sham group using independent t-test.*

Table 4 The Average of Paw Thickness of Mice between CFA and Sham Groups on day 0, 1, 2, 3, 4, 5, 6, and 7

No	Group	Average of Paw Thickness of Mice on day (cm)					% Increasing of Paw Thickness
		0	1	3	5	7	
1	Sham (n = 4)	0.19±0.01	0.21±0.01	0.19±0.01	0.20±0.01	0.20±0.01	5.26
2	CFA (n = 20)	0.20±0.01	0.36±0.01*	0.34±0.01*	0.34±0.01*	0.34±0.01*	70.00

*Data were presented as mean (SEM), *)p<0.05 means significant different compared to sham group using independent t-test.*

PSNL-induced Neuropathy Pain

Table 5 showed that previously, there were no significantly different between latency time toward thermal stimulus of PSNL and sham group on day 0 ($p>0.05$) and this condition was significantly different after PSNL induction. Latency toward thermal stimulus were significantly decrease after PSNL induction, lower than sham group on day 1, 3, 5 and 7 ($p<0.05$).

Table 5 The Average of Latency Time Toward Thermal Stimulus between PSNL and Sham Groups on Day 0, 1, 3, 5 dan 7

No	Group	Average of Latency Time Toward Thermal Stimulus on day (secon)					% Decreasing of Latency Time
		0	1	3	5	7	
1	Sham (n = 4)	7.62±0.60	6.22±0.66	4.92±0.26	6.18±0.26	6.98±0.22	8.40
2	PSNL (n = 20)	8.76±0.29	5.11±0.33*	3.94±0.18*	3.32±0.15*	3.13±0.20*	64.27

*Data were presented as mean (SEM), *)p<0.05 means significant different compared to sham group using independent t-test.*

Red Ginger Oil Activity in CFA-induced Inflammatory Pain

Red ginger oil administration for 7 days changed the hyperalgesia response in mice. Table 6 and figure 2 showed that red ginger oil increased latency time toward thermal stimulus by increasing the percentage of latency time toward thermal stimulus compared to day-7. The latency time on day 8, 10, 12 and 14 were better than CFA group. Figure 3 showed that there were no significant differences of paw thickness between before and after administration of red ginger oil.

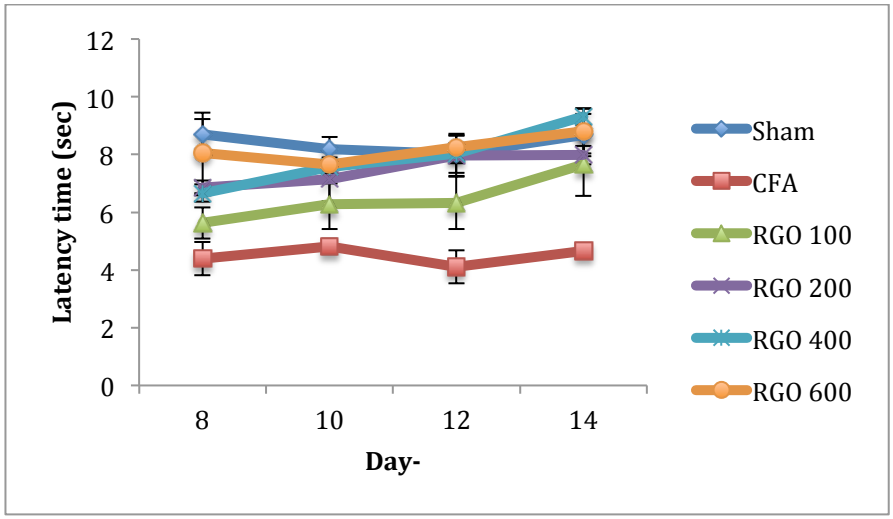

Figure 2 Average of Latency Toward Thermal Stimulus After Ginger Oil Administration on day 8, 10, 12 and 14. RGO: Red ginger oil

Table 6. Increasing of Latency Time Toward Thermal Stimulus After Red Ginger Oil Administration on day 10 and 14 after CFA-induced Chronic Pain

Group	Treatment	Increasing of Latency Time (%) Day-	
		10	14
Sham (n=4)	normal salin	-2.73	2.97
CFA (n=20)	normal salin	-13.93	-16.61
	RGO 100 mg/kg	11.96	36.61
	RGO 200 mg/kg	27.50	42.50
	RGO 400 mg/kg	35.00	66.25
	RGO 600 mg/kg	36.61	57.32

RGO: Red ginger oil

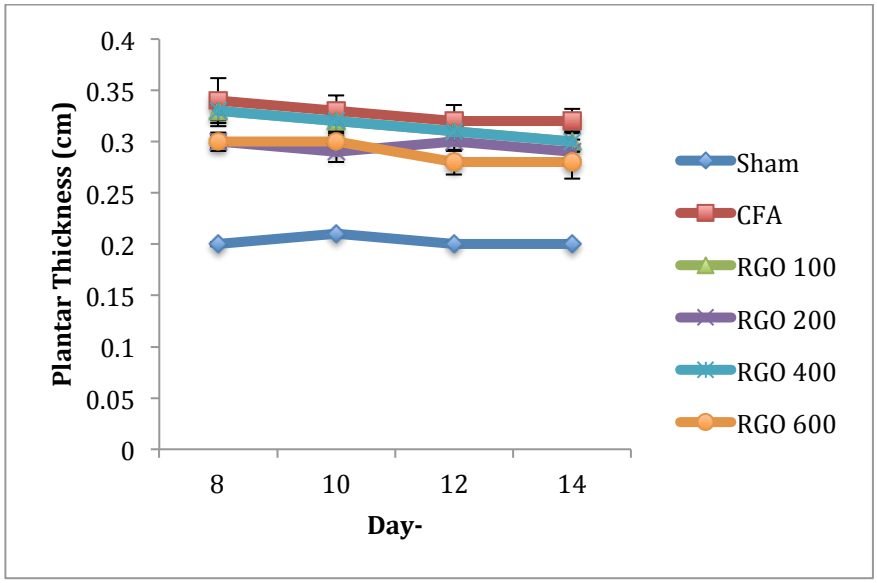

Figure 3 Average of Paw Thickness of Mice After Red Ginger Oil Administration on day 8, 10, 12 and 14. RGO: red ginger oil

Red Ginger Oil Activity in PSNL-induced Neuropathy Pain

Red ginger oil administration for 7 days changed the hyperalgesia response in mice. Table 7 showed that red ginger oil increased latency time toward thermal stimulus by increasing the percentage of latency time toward thermal stimulus compared to day-7. The latency time on day 8, 10, 12 and 14 were better than PSNL group.

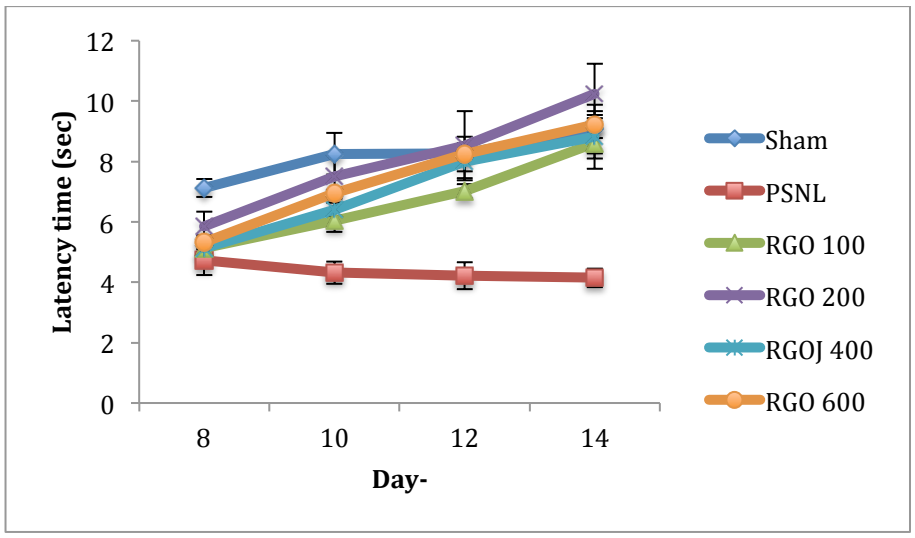

Figure 4 Average of Latency Toward Thermal Stimulus After Ginger Oil Administration on day 8, 10, 12 and 14. RGO: Red ginger oil

Table 6. Increasing of Latency Time Toward Thermal Stimulus After Red Ginger Oil Administration on day 10 and 14 after PSNL-induced Chronic Pain

Group	Treatment	Increasing of Latency Time (%) Day-	
		10	14
Sham (n=4)	normal salin	7.63	19.16
PSNL (n=20)	normal salin	-50.68	-52.62
	RGO 100 mg/kg	-30.82	-1.83
	RGO 200 mg/kg	-14.38	17.01
	RGO 400 mg/kg	-26.94	0.68
	RGO 600 mg/kg	-20.66	5.25

RGO: Red ginger oil

Discussion

Chronic pain can be caused by chronic inflammation and neuropathy. This research used CFA that was consisted of inactivated and dried mycobacteria (usually *M. tuberculosis*) as inducer for chronic inflammation. CFA was effective in stimulating

cell-mediated immunity and leaded to potentiation of T helper cells that stimulated production of certain immunoglobulin and effector T cells. CFA was responsible for inflammation in mice, because CFA caused stimulation phagocytosis system and cytokine secretion (8,9). CFA was a substance that caused inflammatory pain by increasing latency time toward thermal stimulus and paw thickness compared with baseline (before induction). Neuropathy was related with neuronal damage then reduced the time to response thermal stimulus as noxious stimulus. Model for neuropathy that was used in this research was PSNL. The advantages of PSNL was analog to neuropathy in human with caused 60% of hyperalgesia and allodynia(9,10).

Noxious stimulus that was happened by chronic inflammation and neuropathy, caused imbalance between inhibitory and excitatory neurotransmitter in central nervous system (CNS). CFA increased the release of glutamate as excitatory neurotransmitter that binded to N-Methyl-D-Aspartate (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 NMDA receptor NR2B subunit. This pathway was the key of chronic pain pathophysiology (11,12).

From GC/MS analysis, the biggest component of red ginger oil was terpenoid. Red ginger oil consisted of monoterpenoid such as champene, cineol, linalool and alpha-pinena. The latest studies showed that monoterpenoid had antinociceptive and antiinflammatory activity (7,13).

The activity of red ginger oil was based on the composition of essential oil. Sivasothy *et al* (14) 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 (15). 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 CNS and reduced chronic pain (16). Red ginger oil action was related to induction of GABA agonist. The availability of GABA restored the balancing between exitatory and inhibitory neurotransmitter in CNS (17). GABA suppressed the release of glutamate and blocked calsium intake to intracelluler. This condition decreased NR2B activity and pain sensitization.

The other mechanism of red ginger oil was probably related to decreasing of pro-inflammatory mediator such as prostaglandin that was producted by biosyntethis of cyclooxygenase. When this way was blocked, the production of prostaglandin would be decreased and reduced the inflammation (18,19).

Conclusion

Camphene is the highest compound in red ginger oil. Red ginger oil administration ameliorated hyperalgesia condition in mice with chronic inflammation and neuropathy condition that characterized by prolonged the latency time toward thermal stimulus.

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Conflict of Interest

The autors declare no conflict of interest.

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