Pakistan Journal of Pharmaceutical Sciences (PJPS)

Volume : 32, Issue : 4, Jul 2019
ISSN 1011-601X

Faculty of Pharmacy, University of Karachi
Pakistan

Free full-text Online Journal

Welcome

Pakistan Journal of Pharmaceutical Sciences (PJPS) is a peer reviewed multi-disciplinary pharmaceutical sciences journal. The PJPS had its origin in 1988 from the Faculty of Pharmacy, University of Karachi as a biannual journal, frequency converted as quarterly in 2005, and now PJPS is being published as bi-monthly from January 2013.

PJPS covers Biological, Pharmaceutical and Medicinal Research (Drug Delivery, Pharmacy Management, Molecular Biology, Biochemical, Pharmacology, Pharmacokinetics, Phytochemical, Bio-analytical, Therapeutics, Biotechnology and research on nano particles.

PJPS is abstracted and/or indexed in ASCI Database, AsiaNet, CAB Abstracts, CABI, Chemical Abstracts, EBSCO, EMBASE, EMRmedex, ESSCI, EVISA, GDPBM, Genamics, IMEMR, IndexCopernicus, IndexPharmacus, ISI, JournalSeek, MEDLINE/PubMed, NLM LocatorPlus, PakMediNet, PASTIC, PMRC, Pubget, ResearchGATE, SADCCT, SCIENCE Pakistan, SCImago, SCOPUS, Scribd, Serial Cited, SID, WebStatDomain and WorldCat.

Pakistan Journal of Pharmaceutical Sciences is Pakistani journal at the interface of chemistry, biology and medicine. It publishes original research and review articles from all fields of Pharmaceutical Sciences from authors all over the world. Its mission is to integrate the wide and flourishing field of medicinal and pharmaceutical sciences. PJPS is recognized by Higher Education Commission with “W” category and awarded Impact Factor 1.103 by Thomson Reuter in 2011.

Language

The journal accepts manuscripts in English only.

Submission of manuscripts

Manuscripts are preferred for submission electronically.

e-mails: pakjps@hotmail.com and pjps@uok.edu.pk

Instructions For Authors

- **Scope of the Pakistan Journal of Pharmaceutical Sciences**: Pakistan Journal of Pharmaceutical Sciences (PJPS) is a peer reviewed pharmaceutical and medicinal publication scheduled to appear bimonthly serving as a means for the exchange of scientific information at international level. All manuscripts are evaluated for their scientific content and significance by the Editor-in-Chief and at independent reviewers, which will be from technologically advanced country also. All submitted manuscripts should contain unpublished, original research not under consideration for publication anywhere else.
- In order to avoid unnecessary delay in publication, authors are requested to strictly comply the guidelines.
- Online Submission: Authors should ensure that papers conform to the scope of the journal. To expedite the publication process, the Journal encourages online submission through pjps@uok.edu.pk, pakjps@hotmail.com. The manuscript should be e-mailed or sent on a CD in MS Word 2000 or later format as a single file accompanied by a matched hard copy.
- Types of Manuscripts: Articles are comprehensive accounts of significant experimental or theoretical work, authors are asked to write their manuscripts in a clear and concise manner and to only include the data crucial for arriving at final conclusion.
- Communications are preliminary reports limited to approximately 1,500 words (including tables, figures and references). They must be of sufficient importance and general interest to justify accelerated publication.
- Reviews and mini-reviews are by invitation only. Authors interested in preparing a review article should provide a brief outline to the Editor-in-Chief requesting an invitation to submit a manuscript.
- The transmittal letter should designate one author corresponding indicated by a superscript *(e.g., Shah*). Authors are responsible for all statements made in their work and for the accuracy of the bibliographic information. The right is reserved to incorporate any change deemed necessary by the editorial board to make contributions harmonize with the editorial standards of the journal.
- **Suggested Reviewers**: Authors submitting manuscript are required to suggest at least two reviewers, one of them from technically advanced country. Detail contact information of the reviewer should be mentioned.
- **Structure of Article**: All manuscripts should be in English and written only on one side of A4 paper spacing on 8½ x 11”(21.5cm x 28.0 cm) white paper with one inch (2.5 cms) margin on all sides, pages should be numbered consecutively. It should not exceed 15 pages, excluding tables, references and figures. There should be no more than 45 refer-ences in an original article (no more than 100 in a review article). Brief reports contain less scientific data than the original articles and should not have more than one figure and/or table. The

original manuscript should include the original inked drawings or photographs of structural formulas for direct use. High-quality output from laser printers is acceptable. General information on the preparation of manuscripts may be found through pjps@uok.edu.pk, pakjps@hotmail.com

- **Text**:Submit your text in DOC format.
- **Pictures**:To ensure the highest print quality, must be submitted in TIF/JPEG format with high resolutions.
- Abstract: An abstract should be concise (80-200 words) self-contained summary, should include the background/ objective, purpose of the study (including its statistical significance), methods, results, and conclusion.
- Keywords: For indexing purposes each submitted article should include three to five key words chosen from the medical subject heading (MeSH). In addition to facilitating indexing of articles, our keyword system assists in the assignment of qualified reviewers for your manuscript.
- **Abbreviations**: Standard abbreviations should be used without periods throughout the manuscript. All nonstandard abbreviations should be kept to a minimum and must be defined in the text following their first use.
- **Experimental Sectio**n: The experimental procedures should be described in sufficient detail to enable others to repeat the experiments. Names of products and manufacturers should be included only if alternate sources are deemed unsatisfactory. Novel experimental procedures should be described in detail, but published procedures should merely be referred to by literature citation of both the original and any published modifications. The purity of key compounds and descriptions(s) of the method(s) used to determine purity should be included in this section.
- Manuscripts containing data generated from animal and/or human studies must specify the committee and the institution that approved the experimental protocols used to generate these data.
- **Results**:Concise tables and figures should be designed to maximize the presentation and comprehension of the experimental data. The same data should not be presented in more than one figure or in both a figure and a table. As a rule, interpretation of the results should be reserved for the discussion section of an article.
- **Discussion**:The purpose of the discussion is to interpret the results and to relate them to existing knowledge in the field in as clear and brief a fashion as possible. Information given elsewhere in the manuscript should not be repeated in the discussion. Extensive reviews of the literature should be avoided.
- **References**:References should be assembled al-phabetically on a separate sheet. In the text, they should be referred by name and year (Harvard system). When referring to more than one paper of a same author and same year, the alphabets a, b, c, etc. should be placed next to the year of publication. In the text, when referring to a work by sole author, the name of author should be given like (Robinson, 1998) and (Robinson, 1999; Jeong, 2000). When referring to a work by two authors, the name of authors should be given like (Robinson and Jeong, 2001). When more than two authors, the name of the first author should be given followed by *et al.* such as (Robinson *et al.,* 2002). Literature references must

consist of names and initials of all authors, title of the paper referred to, abbreviated title of the journal, the volume, and page numbers of the paper. List submitted manuscripts as “in press” only if formally accepted for publication; otherwise, use “unpublished results” after the names of authors. The style and punctuation of the references should confirm with the following examples:

- *For journals:* Yoneto K, Li SK, Higuchi WI, Jiskoot W and Herron JN (1996). Fluorescence probe studies of the interactions of 1-alkyl-2-pyrrolidones with stratum corneum lipid liposomes. *J. Pharm. Sci.,* 85: 511-517. *For edited books*: Rall TW and Schleifer LS (1985). Drugs effective in the therapy of the epilepsies. In: Gilman AG, Goodman LS, Rall TW, Murad F editors. The pharmacological basis of therapeutics, 7th ed., Macmillan Publishing Co.,New York, pp.446-472.
- **How to order**:To purchase a copy you can deliver order at Office of the Editor-in-Chief, PJPS, Faculty of Pharmacy,University ofKarachi, Karachi-75270,Pakistan or pjps@uok.edu.pk, pakjps@hotmail.com
- **Supporting Information**:The Supporting Information format of this journal can accommodate and make readily available almost any type of supplementary figures or data (e.g., reproductions of spectra, experimental procedures, tabulated data, expanded discussion of peripheral findings, etc.). The author should include a Supporting Information Available statement that describes the material at the end of the printed manuscript text. Consult a current issue of the Journal for the proper wording of this statement. Supporting Information should be clear and of high contrast (suitable for direct photoreproduction) and submitted in quadruplicate on 8.5- × 11-in. paper. All pages of supporting information must be consecutively numbered. Captions or legends for figures, spectra, etc., must appear directly on the figure.
- **Acknowledgments**:This section should acknowledge financial support, technical assistance, advice from colleagues, gifts, etc. Permission must be received from persons whose contribution to the work is acknowledged in the manuscript.
- **Spectral Data**:It may be desirable to include such data for representative compounds in a series, for novel classes of compounds, and in structural determinations. Usually, it is not desirable to include routine spectral data for every compound in the manuscript. Papers where interpretations of spectra are critical to structural elucidation and those in which band shape or fine structure needs to be illustrated may be published with spectra included. When such presentations are deemed essential, only pertinent sections should be reproduced.
- **Biological Data**. Biological test methods must be referenced or described in sufficient detail to permit the experiments to be repeated by others, detailed descriptions of methods should be placed in the experimental procedures section. Data may be presented as numerical expressions or in graphical form, statistical limits (statistical significance) are usually required, if these cannot be provided, the number of determinations and some indication of the variability and reliability of the results should be given and references of calculation should be included. Doses and concentrations should be expressed as molar quantities (e.g., mmol/kg, mM) when comparisons of potencies are made on compounds having large differences in molecular weights. The routes of administration of test

compounds and vehicles used should be indicated.

- **Tables**:Tabulation of experimental results is encouraged when this leads to more effective presentation or to more economical use of space, tables should be numbered consecutively with Arabic numerals with a brief title and a brief heading for each column. Clearly indicate the units of measure (preferably SI) and preferred in *.doc format.
- **Illustrations**:Electronic submission of illustrations is encouraged, preferred formats for graphics and artwork are TIFF (tagged image file format) and EPS (Encapsulated PostScript). If not submitted electronically, the chemical structures, graphs, photographs, or other illustrations you send with your paper will be scanned into the journal page using a digital scanner. The scanner is very sensitive; it will faithfully copy all flaws such as smudges, uneven lines, incomplete erasures, etc.
- **Chemical structures**: Structures should be produced with the use of a drawing program such as ChemDraw. Authors using the current versions of ChemDraw, ChemIntosh, and ChemWindows will find the necessary parameters incorporated into these programs ("JOC Document" under the Windows menus for ChemDraw and "Reduce 60% for JOC Style" under the Options menu for ChemIntosh/ChemWindows). In ChemDraw version 4.5, files should be saved in TIFF format to allow use of electronic files in production (see journal home page "Information for Authors" for further guidelines).
- The chemical names for drugs should be used. If the terminology is unwieldy, nonproprietary names of drugs may be used throughout the manuscript after the first mention and identification. Formally adopted nonproprietary names listed in *USAN, INN* or approved by the World Health Organization, should be used. Trade names and laboratory codes should not be used except as additional information.
- **Proofs**:Proofs are sent to the corresponding author of the manuscripts. Proofs should be verified against the manuscript and appropriate corrections made. Substantial changes in a manuscript after composing has been done require editorial approval and in some cases may be the cause for re-reviewing.
- **Confirmation of manuscript content must accompany initial submission**:Manuscripts submitted to the Journal of Pharmaceutical Sciences should contain significant, unpublished and original data not being considered simultaneously for publication elsewhere. All authors should be aware of and in agreement with the submission of this manuscript and share responsibility for its content. The manuscript should provide full and appropriate credit to those who contributed to the underlying hypothesis and the generation and interpretation of the experimental data. Related research in the field should be acknowledged in the manuscript through appropriate literature citations. Manuscripts should be devoid of any forms of plagiarism with respect to ideas, words, graphic materials or other forms of communication. Protocols used to generate animal and/or human experimental data were approved by an authorized Institutional Animal Care Committee and/or Human Experimentation Committee. Authors who violate any of these basic rules of scientific ethics may be barred from publishing in the Journal of Pharmaceutical Sciences at the sole discretion of the Editors.

- **Anti-Plagiarism Policy**: Manuscripts should not be plagiarized, if plagiarized material is found more than 20% in the manuscripts, the concerned authors are required to modify the submitted manuscript.
- **Submission of undertaking**:All concerned authors are bound to submit an undertaking duly original signed by them, which shows that this paper has not been submitted elsewhere and is also not plagiarized according to the policy of Higher Education Commission, Islamabad, Pakistan, contributors of all authors are also required to mention against their names.
- **Supplementary issue**: To demand of quick publication of research articles, PJPS has arranged publication of supplementary issues which can be published with each regular issue, comprising of twenty research articles at-least. Contributors who need urgent publication of their research articles can get this opportunity. Rs.10,000/- or US$125 will be charged on each rejected/withdrawn paper in account of processing charges in Fast Track.
- **Publication charges**:
- *Regular issue* Rs.2000/- per page (within Pakistan)
- US$ 50.00 per page (Foreign)
- *Special/ Supplementary/Additional issue*
- Rs.10,000/- per page (within Pakistan)
- US$ 150.00 per page (Foreign)
- The charges can be deposited through on-line banking within a month for regular issue and a week for fast track. Details of bank account: Pakistan Journal of Pharmaceutical Sciences, United Bank Limited, University Campus Branch, University of Karachi, A/c No.1146-010-1731-3, Swift Code: UNILPKKA, IBAN – PK10 UNIL 0112 1146 01073 13
- **How to order:** To purchase a copy you can deliver order at Office of the Editor-in-Chief, PJPS, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan
- e-mails: pjps@uok.edu.pk, pakjps@hotmail.com

Volume : 32, Issue : 4, Jul 2019

Antihyperalgesia potency of *Zingiber officinale* var. *Rubrum* in inflammatory and neuropathy-induced chronic pain condition in mice

Fifteen Aprila Fajrin[1], Azham Purwandhono[2], Fransisca Maria Christianty[1], Gati Dwi Sulistyaningrum[1], Afifah[1], Nidia Imandasari[1] and Tsabit Barki[1]
[1]Faculty of Pharmacy, University of Jember, East Java, Indonesia
[2]Faculty of Medicine, University of Jember, East Java, Indonesia

Abstract: Chronic inflammation and neuropathic pain are classified into chronic pain. Until now there are so many drugs that have been used for chronic pain but the effectiveness still lower. One of the plants that are commonly used for medicine in Indonesia is red ginger (*Zingiber officinale* var. *rubrum*). This study was aimed to analyze the component of red ginger oil and proved its antihyperalgesia potency in chronic pain using two models, inflammatory pain and neuropathy pain. Forty-eight mice were divided into 2 groups i.e. inflammatory and neuropathy. Each group was divided into 6 subgroups (@4 mice) i.e. for inflammatory model (sham, negative control, red ginger oil doses 100, 200, 400 and 600 mg/kg) and for neuropathy model (sham, negative control, red ginger oil doses 100, 200, 400 and 600 mg/kg). Inflammatory model was induced using Completed Freud's Adjuvant (CFA) 40 µl intraplantar. Neuropathy model was induced using Partial Sciatic Nerve Ligation (PSNL). At day-7, all groups were given orally treatment, once daily for seven days. The latency time toward thermal stimulus and plantar thickness were measured at day 0,1,3,5,7,8,10,12 and 14 after induction. Quality of red ginger oil was standardized by Indonesia standard (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. Red ginger oil 200 mg/kgBW and 400mg/kgBW administration in mice gave the best result in prolong the latency time toward thermal stimulus using hot plate and significantly different with inflammatory and neuropathy group. From GC/MS analysis, camphene was known as the highest compound of red ginger oil that might be important for its antihyperalgesia effect. The conclusion of this study that red ginger oil have antihyperalgesia activity in mice with chronic pain and could be developed further to be antihyperalgesia.

Keyword: Chronic pain, hyperalgesia, CFA, PSNL, red ginger oil.

INTRODUCTION

Pain is a multidimensional experience that affect to both sensory and emotional aspect of human being. Chronic pain, a pain with duration more than 6 months, usually follows chronic or degenerative diseases such as diabetes mellitus, cancer, chronic inflammation, infection and many else. Approximately one of five populations is diagnosed by chronic pain (van Hecke *et al*., 2013). The prevalence rate was different in every country. The estimation of chronic pain incidence were 20.2% in Denmark, 22.1% in Australia, 31.4% in Sweden, 35.1% in Finland, 35.5% in the USA and 46% in Israel (Igumbor *et al*., 2011).

Until now, treatments for chronic pain are still a challenge because of its lower effectiveness and higher side effects (Baron *et al*., 2010; Cole, 2002). Without an appropriate treatment, it will be difficult for patients to improve their quality of life. Many patients with chronic pain get inability to do their daily activities and some of them loss their job. This condition may lead to stress and depression condition that enhance the pain threshold as well. Because of all of this, the health financial cost also increases. In Europe the cost for chronic pain is 200 billion Euro for a year and 635 billion US dollar for a year in United States (van Hecke *et al*., 2013).

Regarding on pathophysiology, chronic pain are triggered by inflammation and neuropathy condition. Complex pathophysiology of chronic pain involves the increasing of oxidative stress. Peripheral nerves injuries by chronic inflammation and neuropathy cause increasing of super oxides (SO) and activate peroxynitrite (PN) (Singh *et al*., 2014). The higher accumulation of PN triggers 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 then stimulates central sensitization in chronic pain (Farmer *et al*., 2012).

Red ginger is one of the species from ginger that have been used by Indonesian people as treatment in many diseases (Shimoda *et al*., 2010). Red ginger had an antioxidant effect higher than ginger (Oboh *et al*., 2012a). Based on previous results, red ginger had higher ability to inhibit lipid peroxidation in rat's brain than ginger, represent of the previous statement (Oboh *et al*, 2012b).

**Corresponding author:* e-mail: fiezz_15@yahoo.co.id

The active compounds of ginger include volatile oil and pungent phenol compounds such as gingerol and shogaol (Hernani, 2011). Red ginger oils composition are consisted of essential oils, such as cineol and zingiberen. Essential oils from red ginger have been known as compound that responsible for antioxidant activity (Quintnas-Junior *et al*., 2013). Based on relationship between pathophysiology of chronic pain with oxidative stress, it was possible to block central sensitization by antioxidant activity (Anfenan, 2014). This study was aimed to analyze the component of red ginger oil and proved its antihyperalgesia potency in chronic pain using two models, inflammatory pain and neuropathy pain.

MATERIALS AND METHODS

Forty-eight of 8-week-old mice (male) were used for the experiment. They were purchased and kept in the Animal House, Faculty 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 (10 month) was purchased from farmer group "PeciFarm" in Kencong, Jember, East Java, Indonesia. CFA (Completed Freud's Adjuvant) was purchased from Sigma Chemical Co USA. For neuropathy pain, we used suture 8/0 (Ethicon) and silk suture (One Med).

This research was done in Faculty of Pharmacy University of Jember and got the ethical approval from Committee 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 was prepared in destilator with aquadest (1:2). Distillation process was done in 100-121 ^{0}C, for 5-6 hours. Red ginger oil was emulated 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 physic-chemical characteristics, included density, the acid number and the ester number. The analytical method used the national (Indonesia) standard in accordance with the quality standards referred, 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 (C146-E087F, Shimadzu, Tokyo, Japan). The sample was detected on a column 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 had ion source temperature of 200 ^{0}C and interface temperature of 280 ^{0}C. Samples were injected in split mode. Total elution time was 55 min. MS scanning was performed from m/z 40 to m/z 600.

GC/MS Identification of Components

Identification 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 Model

Twenty-four mice were divided into 6 groups (4 mice in each group) i.e. sham, negative control, treatment (red ginger oil at four different doses: 100, 200, 400 and 600 mg/kgBW). Inflammatory condition was induced by intraplantar injection of CFA. Mice were anesthetized by ether 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) was administrated orally once a day for seven consecutive days, a week after CFA injection. Sham and inflammation control were administrated by tween 0.5%.

PSNL (Partial Sciatic Nerve Ligation)-induced Neuropathy Pain Model

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 ether and tied 1/3-1/2 of dorsal portion of sciatic nerve on the left lumbar nerve of mice with 8-0 silk. In sham group, the sciatic nerve was exposed without ligation. Red ginger oil (in treatment groups) was administrated orally once a day for seven consecutive days, a week after PSNL induction. Sham and neuropathic control were administrated by tween 0.5%.

Hyperalgesia and Antinociceptive Response Test

Latency time toward thermal stimulus were measured using hot plate at temperature 50±0.5 °C on days 0 (baseline), 1, 3, 5, 7, 8, 10, 12 and 14. Hyperalgesia responses of mice were showed with licked, flicked or jumped (whichever came first). Paw thickness at the ipsilateral site of mice in inflammatory model 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 was 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

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.

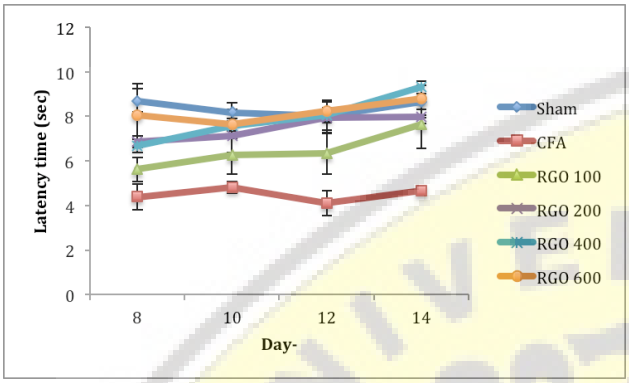

Fig. 1: Average of Latency Toward Thermal Stimulus After Ginger Oil Administration on day 8, 10, 12 and 14. RGO: Red ginger oil

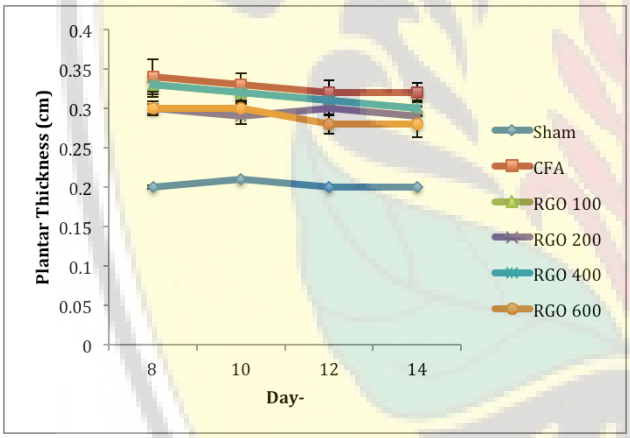

Fig. 2: Average of Paw Thickness of Mice After Red Ginger Oil Administration on day 8, 10, 12 and 14. RGO: red ginger oil

CFA-induced Inflammatory Pain

Table 2 and 3 showed that at baseline (day-0), there was no significant different between the paw thickness and latency time toward thermal stimulus between sham and CFA group ($p>0.05$). It described that all mice in the same condition at the beginning of the study. One day after CFA induction, the paw thickness of CFA group (0.36±0.01 cm) was significantly bigger than sham group (0.21±0.01 cm). It means that CFA induced inflammatory process that characterized by edema in plantar site. This condition was occurred until day-7 and we concluded that it was a chronic inflammation.

The increasing of paw thickness was followed by the decreasing of the latency time toward thermal stimulus. One day after CFA induction (table 3), the latency toward thermal stimulus of CFA group (7.13±0.23) was significantly lower ($p<0.05$) than sham group (8.96±0.76). The latency toward thermal stimulus in CFA group was continued to decrease until day-7. It showed that mice in CFA group in hyperalgesia state.

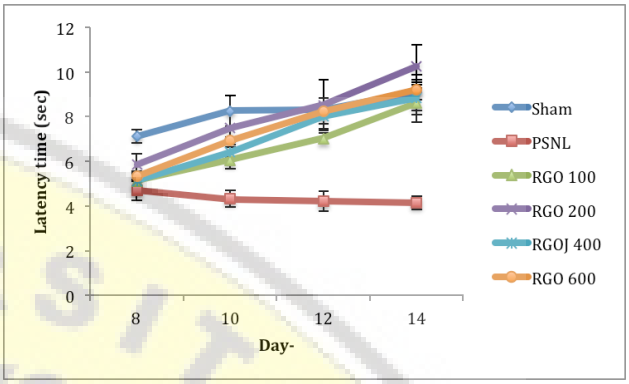

Fig. 3: Average of Latency Toward Thermal Stimulus After Ginger Oil Administration on day 8, 10, 12 and 14. RGO: Red ginger oil

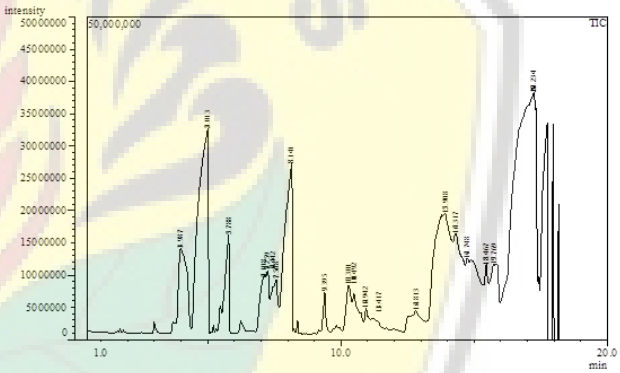

Fig. 4: The Chromatogram Profile of Essensial Oil from Red Ginger by GC/MS

PSNL-induced Neuropathy Pain

Table 4 described that previously, there was 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. After PSNL induction, the latency toward thermal stimulus in PSNL group significantly decreased ($p<0.05$), lower than sham group on day-1, 3, 5 and 7 from 8.76±0.29 sec to respectively, 5.11±0.33 sec, 3.94±0.18 sec, 3.32±0.15 sec and 3.13±0.20 sec.

Red Ginger Oil Activity in CFA-induced Inflammatory Pain

Red ginger oil administration for 7 days changed the hyperalgesia response in mice. fig. 1 and table 5 showed that red ginger oil increased latency time toward thermal stimulus by increasing the percentage of latency time toward thermal stimulus compared to CFA group. One day after treatment, latency time toward thermal stimulus

Table 1: Red ginger oil specification

No	Criteria	SNI*) (06-1312-1998)	Result	Description
1	Color	-	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

*) SNI (Standar Nasional Indonesia)

Table 2: The average of paw thickness of mice between cfa and sham groups on day 0, 1, 2, 3, 4, 5, 6 and 7

Group	Average of Paw Thickness of Mice on day- (cm ± SEM)					% Increasing of Paw Thickness On day-7
	0	1	3	5	7	
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
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

Table 3: The average of latency time toward thermal stimulus between CFA and sham groups on Day 0, 1, 3, 5 and 7

Group	Average of Latency Time Toward Thermal Stimulus on day- (cm±SEM)					% Decreasing of Latency Time on day-7
	0	1	3	5	7	
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
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

Table 4: The average of latency time toward thermal stimulus between psnl and sham groups on day 0, 1, 3, 5 and 7

Group	Average of Latency Time Toward Thermal Stimulus on day- (sec ± SEM)					% Decreasing of Latency Time on day-7
	0	1	3	5	7	
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
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.

Table 5: 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)	Saline	-2.75	2.97
CFA (n=20)	Saline	-42.76	-44.54
	RGO 100 mg/kg	-15.20	-5.22
	RGO 200 mg/kg	-25.53	-9.14
	RGO 400 mg/kg	-10.21	10.37
	RGO 600 mg/kg	-9.14	4.63

RGO: Red ginger oil

of mice significantly increased (p<0.05) compared to CFA group, but still significantly lower than sham group (p<0.05). At the end of treatment (day-7), red ginger oil dose 100, 200, 400 and 600 mg/kgBW had the latency time toward thermal stimulus as same as sham group. On the other hand, the paw thickness of treatment group after seven days were significantly decreased (p<0.05) compared to CFA group, but still significanly bigger (p<0.05) than sham group as shown as fig. 2.

Red Ginger Oil Activity in PSNL-induced Neuropathy Pain

Red ginger oil administration dose 100 mg/kgBW, 200 mg/kgBW, 400 mg/kgBW and 600 mg/kgBW for 7 days changed the hyperalgesia response in mice. The latency time toward thermal stimulus continuously improved with longer duration of treatment. The increasing of the dose also gave significant improvement of the latency time toward thermal stimulus, as well. At day-12 and 14 (5 and 7 days after treatment), red ginger oil administration in all

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)	Saline	18.19	30.08
PSNL (n=20)	Saline	-38.10	-40.54
	RGO 100 mg/kg	-13.32	23.21
	RGO 200 mg/kg	7.45	46.85
	RGO 400 mg/kg	-8.31	26.36
	RGO 600 mg/kg	-0.43	32.09

RGO: Red ginger oil

Table 7: The Essential 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

doses had latency time toward thermal stimulus as same as sham group. Even if red ginger oil dose 200 mg/kgBW had latency time toward thermal stimulus, but it did not significantly different compared to sham group as seen as table 6.

GC/MS analysis for red ginger oil

The composition of essential oil of red ginger was analyzed by GC/MS. There were 19 compound identified with the library, as shown in fig. 4 and table 7. From the result, champene was the highest compound in red ginger oil, followed by 1,8-cineole.

DISCUSSION

Chronic pain can be caused by chronic inflammation and neuropathy. In this research, we used two models to induce chronic pain. For inflammatory model, CFA that is consisted of inactivated and dried mycobacteria, used as inducer for chronic inflammation. CFA is effective in stimulating cell-mediated immunity and leads to potentiation of T-helper cells that stimulates production of certain immunoglobulin and effect or T-cells (Anfenan, 2014; Calder, 2006). This process initiates inflammatory pain and responsible for the increasing of latency time toward thermal stimulus and paw thickness, compared with baseline (before induction). On the other side, for neuropathy model, we used PSNL method. The advantages of PSNL are because this method is analog to neuropathy in human that caused 60% of hyperalgesia and allodynia (Bridges *et al*., 2001; Xie, 2011). That statement was consistent with our result, that PSNL induced hyperalgesia by 64.27%.

Chronic inflammation and neuropathy caused imbalance between the composition of inhibitory and excitatory neurotransmitter in central nervous system (CNS). Influx of oxidative stress in chronic pain increases the release of glutamate as excitatory neurotransmitter that bonds to N-Methyl-D-Aspartate (NMDA) receptor, caused depolarization and activation of central pathway. Long-term depolarization causes losing of magnesium

inhibitory in NMDA channel, affects calcium entry to intracellular and activated NMDA receptor NR2B subunit. This pathway is the key of chronic pain pathophysiology (Macintyre *et al*., 2010; Zhu *et al*., 2011).

After treatment using red ginger oil, there was an improvement in pain behavior of mice with chronic inflammation and PSNL-induced chronic pain. But, the effective dose that was needed to reduce pain was slightly different. In CFA-induced inflammatory pain, red ginger oil dose 400mg/kgBW was the most effective dose to minimize the hyperalgesia by increasing of latency time toward thermal stimulus 10.37%. Meanwhile, in PSNL-induced neuropathy pain, red ginger oil dose 200 mg/kgBW had the best antihyperalgesia activity by increasing of latency time toward thermal stimulus 46.85%, higher than in inflammatory pain. Even tough the administration of red ginger oil could reduce hyperalgesia in CFA-induced inflammatory pain condition, but the paw thickness of mice still not returned yet to its baseline. It might be because of the lower ability to reduce hyperalgesia, just 10.37%.

The activity of red ginger oil was 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%). From our GC/MS analysis, the biggest component of red ginger oil was camphene, that one of the monoterpene compound.

The latest studies showed that monoterpenoid had antinociceptive and anti-inflammatory activity (Hernani, 2011; Kumari *et al*., 2014). Other research also found that this compound had antioxidant effect through reducing of oxidative stress in pathophysiology of chronic pain (Janes *et al*., 2011; Quintans-Junior *et al*., 2013). One of the mechanisms of how oxidative stress-induced sensitization in chronic pain is by causing loss of gamma-amino butyric acid (GABA) neuron. Antioxidant treatment showed increasing of GABA activity then rebalanced neurotransmitter in CNS and reduced chronic pain (Yowtak *et al*., 2013). Red ginger oil action was related to induction of GABA's action (Choi *et al*., 2017). The availability of GABA restored the balancing between excitatory and inhibitory neurotransmitter in CNS (Chandra *et al*., 2005). GABA suppresses the release of glutamate and blocks calcium intake into intracellular. This condition decreases NR2B activity and pain sensitization.

The other mechanism of red ginger oil is probably related to decreasing of pro-inflammatory mediator such as prostaglandin that is produced via biosynthetic of cyclooxygenase. When this way is blocked, the production of prostaglandin will decrease and reduces the inflammation (Nogueira *et al*., 2011; Vendruscolo *et al*., 2006). Even if red ginger oil could reduce the paw thickness by this pathway, but the thickness paw of mice with CFA-induced inflammation not returned yet to baseline. It might be because there were complex mechanisms in chronic inflammation that involved the immunity system.

CONCLUSION

Red ginger oil has antihyperalgesia activity in mice with chronic pain that are induced by inflammation and neuropathy by prolong the latency time toward thermal stimulus. Camphene, the highest compound in red ginger oil, is believed responsible for this action. Based on this research, red ginger oil have an opportunity to be developed further to be antihyperalgesia.

ACKNOWLEDGEMENT

Ministry of Research, Technology and Higher Education-Directorate General of Resources of Science, Technology, and Higher Education by Hibah Bersaing Programme 2015-2016 financially supported the research for this paper.

REFERENCES

Anfenan MLK (2014). Evaluation of Nutritional and Antidiabetic Activity of Different Forms of Ginger in Rats. *Middle-East Journal of Scientific Research*, **21**(1): 56-62.

Baron R, Binder A, Wasner (2010). Neuropathic Pain: Diagnosis, Pathophysiological Mechanisms, and Treatment. *The Lancet Neurology*, **9**(8): 807-819.

Bridges D, Thompson SWN and Rice ASC (2001). Mechanisms of Neuropathic Pain. *British Journal of Anaesthesia*, **87**(1): 12-26.

Calder PC (2006). N 3 Polyunsaturated Fatty Acids, Inflammation, and Inflammatory Diseases. *The American Journal of Clinical Nutrition*, **83**(suppl): 1505S-1519S.

Chandra D, Korpi ER, Miralles CP, de Blas AL and Homanics GE (2005). $GABA_A$ Receptor γ2 Subunit Knockdown Mice Have Enhanced Anxiety-like Behaviour but Unaltered Hypnotic Response to Benzodiazepines. *BMC Neuroscience*, **6**(30): 1-13.

Choi JY, Kim SY, Jeong M and Oh MS (2017). Pharmacotheraupetic Potential of Ginger and Its Compounds in Age-related Neurological Disorders, *Pharmacology and Therapeutics*, article in press.

Cole BE (2002). Pain Management: Classifying, Understanding and Treating Pain. *Hospital Physician*, 23-30.

Farmer KL, Li C and Dobrowsky RT (2012). Diabetic Peripheral Neuropathy: Should a Chaperone

Accompany Our Therapeutic Approach?. *Pharmacol. Rev*., **64**(4): 880–900.

Hernani, Winarti C (2011). Kandungan Bahan Aktif Jahe Dan Pemanfaatannya Dalam Bidang Kesehatan. Bogor: Balai Besar Penelitian dan pengembangan Pasca Panen, pp.125-142.

Igumbor EU, Puoane TR, Gansky SA, Plesh O (2011). Chronic Pain in The Community: A survey in a township in Mthatha, Eastern Cape, South Africa, South Afr. *J. Anaesth. Analg*., **17**(5): 329-337.

Janes K, Neumann WL, Salvemini D (2011). Anti-superoxide and Anti-peroxynitrite Strategies in Pain Supression. *Biochimica et Biophysica Acta*, **1822**: 815-821.

Kumari, Venkateshwarlu, Choukse and Anandan (2014). Effect of Essential Oil and Aqueous Extract of Ginger (Zingiber Officinale) on Oxidative Stability of Fish oil-in-Water Emulsion. *Food Processing & Technology*, **6**(1): 1-5.

Macintyre PE, Scott DA, Schug SA, Visser EJ and Walker SM (2010). *Acute Pain Management: Scientific Evidence*, 3rd Ed, Melbourne: ANZCA & FPM, pp.1-98.

Nogueira de Melo G, Grespan R, Fonseca J, Farinha T, da Silva E, Romero A, Bersani-Amado C and Cuman R (2011). Inhibitory Effects of Ginger Essential Oil on Leukocyte Migration *in vivo* and *in vitro*. *Journal of Natural Medicines*, **65**(1): 241-246.

Oboh G, Akinyemi AJ and Ademiluyi AO (2012). Antioxidant and Inhibitory Effect of Red Ginger (Zingiber officinale var. Rubra) and White Ginger (Zingiber officinale Roscoe) on Fe2+ induced Lipid Peroxidation in Rat Brain *In vitro*. *Experimental and Toxicologic Pathology*, **64**(2012): 31-36.

Oboh G, Ademiluyi AO and Akinyemi AJ (2012). Inhibition of Acetlycholinesterase Activities and Some Pro-oxidant Induced Lipid Peroxidation in Rat Brain by Two Varieties of Ginger (Zingiber officinale). *Experimental and Toxicologic Pathology*, **64**(2012): 315-319.

Quintans-Junior L, Moreira JCF, Pasquali MAB, Rabie SMS, Pires AS, Schroder R, Rabelo TK, Santos JPA, Lima PSS, Cavalcanti SCH, Araujo AAS, Quintans JSS and Gelain DP (2013). Antinociceptive Activity and Redox Profile of the Monoterpenes (+)-Camphene, p-Cymene and Geranyl Acetate in Experimental Models. *ISRN Toxicology*, pp.1-11.

Singh VP, Bali A, Singh N and Jaggi AS (2014). Advanced Glycation End Products and Diabetic Complications. *Korean J. Physiol. Pharmacol*., **18**(1): 1-14.

Shimoda Shan, Tanaka Seki, Seo Kasajima, Tamura Ke and Murakami (2010). Anti-Inflammatory Propertiies of Red Ginger (*Zingiber officinale* var. Rubra Extract and Suppression of Nitric Oxide Production by Its Consistuents. *Journal of Medicinal Food*, **13**(1): 156-162.

Sivasothy Y, Chong WK, Hamid A, Eldeen IM, Sulaiman SF and Awang K (2011). Essential Oils of Zingiber officinale var rubrum Theilade and Their Antibacterial Activities. *Food Chemistry*, **124**(2): 514-517.

van Hecke O, Torrance N and Smith BH (2013). Chronic Pain Epidemiology-where do lifestyle factors fit in?, *British Journal of Pain*, 0(0): 1-9.

Vendruscolo Takaki, Bersani-Amado Dantas and Bersani-Amado Cuman (2006). Antiinflammatory and Antinociceptive Activities of *Zingiber officinale* Roscoe Essential Oil in Experimental Animal Model. *Indian Journal of Pharmacology*, **38**(1): 58-59.

Xie W (2011). Assessment of Pain in Animals In Ma, C., and Zhang JM (Eds). *Animal Models of Pain*, New York: Humana Press, pp.23-76.

Yowtak J, Wang J, Kim HY, Lu Y, Chung K and Chung JM (2013). Effect of Antioxidant Treatment on Spinal GABA Neurons in a Neuropathic Pain Model in the Mouse. *Pain*, **154**(11): 1-16.

Zhuo M, Wu G and Wu LJ (2011). Neuronal and Microglial Mechanism of Neurophatic Pain (Review). *Molecular Brain*, **31**(4): 1-12.