Original Article

Thai Journal of Pharmaceutical Sciences

The activity of red ginger oil in antioxidant study *in vitro* and antihyperalgesia effect in alloxan-induced painful diabetic neuropathy in mice

Fifteen Aprila Fajrin[1], Nidia Imandasari[1], Tsabit Barki[2], Gati Sulistyaningrum[1], Afifah[1], Nia Kristiningrum[2], Endah Puspitasari[3], Diana Holidah[1]

[1]Department of Community and Clinical Pharmacy, Faculty of Pharmacy, Universitas Jember, East Java, Indonesia, [2]Department of Chemistry Pharmacy, Faculty of Pharmacy, Universitas Jember, East Java, Indonesia, [3]Department of Biology Pharmacy, Faculty of Pharmacy, Universitas Jember, East Java, Indonesia

Corresponding Author:
Fifteen Aprila Fajrin, Department of Community and Clinical Pharmacy, Faculty of Pharmacy, Universitas Jember, East Java, Indonesia.
E-mail: fifteen.farmasi@unej.ac.id

Received: Dec 20, 2018
Accepted: Feb 28, 2019
Published: Jun 25, 2019

ABSTRACT

Background: Red ginger oil (RGO) contains the highest bioconstituent, especially its essential oil which is important for antioxidant, than the other varieties. Chronic diabetic triggers the increase of reactive oxygen species which responsible for a diabetic complication such as painful diabetic neuropathy (PDN). **Aim of the Study:** This study was aimed to prove the antioxidant activity of RGO and the activity of RGO as antihyperalgesia in PDN through reducing blood glucose level or direct action in nerves or both. **Materials and Methods:** Two varieties of ginger which were red ginger and ginger, destilated using water, then calculated the total phenolic content and the antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Ginger with the highest antioxidant effect was continued to *in vivo* study in diabetic mice. In PDN model, mice were induced PDN using alloxan 210 mg/kg body weight (BW) i.p. After 14 days, mice which were showed diabetic followed by hyperalgesia would randomized into 7 groups: Normal, diabetic, glibenclamide and RGO (with dose 100, 200, 400 and 600 mg/kg BW). Treatments were given once daily for 14 days. Hyperalgesia response was evaluated using a hot plate as latency time. The latency time and fasting blood glucose levels were observed at day – 0, 7, 14, 21, and 28. **Results**: RGO had higher total phenolic content (0.778 ± 0.175 mg GAE/g oil) and antioxidant activity using DPPH (inhibitory concentration 50 3.626 ± 0.357 µl/ml) than GO. *In vivo* evaluation using mice showed that RGO 600 mg/kg BW had the best activity in reducing hyperalgesia and delaying spinal cord damage after PDN compared to the diabetic group ($P < 0.05$). RGO also had slightly effect in reducing blood glucose level and postponing the islet pancreas cell breakage. **Conclusions:** RGO had stronger antioxidant effect compared to GO. RGO also reduced hyperalgesia in PDN mice due to the improvement of spinal cord morphology.

Keywords: Antioxidant, ginger, painful diabetic neuropathy, red ginger oil

INTRODUCTION

Today, the mortality of degenerative disease such as diabetes mellitus (DM) is still higher.[1] Based on the World Health Organization, Indonesia, was in the big four, together with the United States, China, and India as the country with the highest number of DM patient in the world.[2] Prolong hyperglycemia induces many serious problems, one of them is painful diabetic neuropathy (PDN) which characterized by hyperalgesia and allodynia due to the damage in the peripheral nervous system then spreads to the central nervous system.[3]

Pathophysiology of PDN is still unclear, although many previous studies explained there was a relationship between

reactive oxygen species (ROS) accumulation and peripheral nerve damage through activation of the transient receptor protein vanilloid (TRPV)-1 in PDN.[4] This damage will lead to signal transmission disturbances from peripheral to central and causes imbalance of neurotransmitter release in the central nervous system.[4,5] The highest number of N-methyl-D-aspartate receptor subunit 2B (NMDAR2B) expression in the spinal cord was found in PDN that associated with hyperalgesia and allodynia symptoms.[6]

The complex pathways that are involved cause PDN difficult to cure. Until now, the drugs that have been used for PDN did not have specific action as well as a lot of side effects.[4] Many patients reported their treatment was unuseful and some of them stopped their treatment. This condition affects another problem in the health-care system includes the increasing of health-care cost.[7] Lately, drugs for PDN are focused in reducing blood glucose level, inhibiting PDN pathophysiology such as antioxidant and direct inhibiting in nerves damage.[4]

As we know, recently, natural resources become a choice in the community to cure many symptoms or diseases because it is believed safer than chemical substances. One of them is red ginger that widely growth in Indonesia. Red ginger oil (RGO) was identified had a lot of important essential oil such as linelol, cineol, and camphene, which had a strong antioxidant effect to reduce ROS.[8] High content of essential oils also showed an antidiabetic activity, which also has a role to overcome the pain, thus prevent the damage of islet cells in the pancreas as a result of PDN conditions.[9] According to this, our research is aimed to prove the antioxidant activity of RGO and the activity of RGO as antihyperalgesia in PDN through reducing blood glucose level or direct action in nerves or both. This is the first report about the activity of RGO in PDN, so it is important to do further research about it.

MATERIALS AND METHODS

Study Design

This research conducted at the Engineering Process of Agricultural Product Laboratory, Faculty of Agricultural Products Engineering and Biomedical Laboratory, Faculty of Pharmacy, Jember University, East Java, Indonesia. All *in vivo* procedures were approved by the Ethical Clearance Committee of Faculty of Medicine, Jember University (No: 774/H25.1.11/KE/2016).

Materials

In this study, rhizome of ginger (*Zingiber officinale* var. Roscoe) and red ginger (*Z. officinale* var. Rubrum) were provided from Kencong, Jember, East Java, Indonesia, that have been identified by Indonesian Institute of Sciences, UPT Balai Konservasi Tumbuhan Kebun Raya Purwodadi, Indonesia (No. 1458. IPH.6/HM/X/2015). All chemical reagents were purchased from Sigma (Singapore). Experimental animals in this study were adult male Balb/C mice (2–3 months and 20–30 g), obtained and certified from the Biomedical Laboratory, Faculty of Pharmacy, Universitas Jember, East Java, Indonesia.

Experimental Method

Preparation of ginger and RGO

The distillation process was using hydrodistillation method. The rhizome of ginger and red ginger was sliced thinly (±3 mm) then distilled with water in the ratio 1:2 for 5–6 h. Furthermore, the oil was separated using a separating funnel and filtered with filter paper, then stored in dark color glass bottles.[10]

Determination of total phenolic content

Each of 2.5 g sample (GO or RGO) was extracted using n-hexane: methanol/water (60:40 v/v) and centrifuged 3.500 rpm for 10 min. The methanol/water phase was separated. As much as, 1 mL of this solution was diluted with water ad 10.0 mL.[11]

Gallic acid was used as standard. 3 mg of gallic acid was diluted with water to get a concentration of 300 μg/mL. Standard solutions were prepared from dilution of 300 μg/mL into concentrations 3, 6, 12, 18, 24, 30, 36, and 48 μg/mL.[11] 0.5 mL from each methanol/water fraction of oil sample was added with 0.5 mL of Folin–Ciocalteu reagent. After incubation for 3 min, 0.5 mL of Na_2CO_3 10% and was incubated again in dark-room condition for 120 min. The absorbance was measured at 725 nm. The total phenolic content was determined using linier regression of gallic acid standart as equivalent with mg gallic acid of g oil (mg GAE/g).[11]

Determination of scavenging effect using 1,1-diphenyl-2-picrylhydrazyl (DPPH)

A certain amount of GO (25–70 μl/mL) and RGO (5–25 μl/mL) were prepared in methanol. Vitamin C as standard was prepared in 1000 μg/mL concentration, then diluted into several final concentration, i.e. 5, 10, 15, 20, 25, and 30 μg/mL.[12]

For DPPH solution, as much as 2 mg of DPPH was diluted with methanol ad 50.0 mL. This solution was stored at dark bottle until used.[12] For determination, 0.75 mL of sample was added with 0.75 mL of DPPH solution (0.1 mM). The solution was homogenized and incubated in room temperature for 60 min (GO and RGO) and 30 min (for ascorbic acid). The absorbance was measured at 516 nm. The inhibitory concentration 50 (IC_{50}) of samples was evaluated from the linear regression as the concentration that inhibited 50% of DPPH scavenger.[12]

In vivo study using PDN model in mice

Preparation of experimental animal

White-male mice strain Balb/C (20–40 g) was used in this study. The animals were adapted for 1 week. On day-0, the mice induced intraperitoneally with 210 mg/kg body weight (BW) of alloxan. Mouse was considered hyperglycemia when its blood glucose level is ≥250 mg/dL.[13]

Grouping of experimental animal

Twenty-eight mice were divided into six treatment groups. Each group was consisted of 4 mice. Group I as a normal control (without induction followed by tween 0.5% [1 ml/kg BW]). Group II as a negative control (mice induced by alloxan and tween 0.5% (1 ml/kg [BW]), Group III as a drug control (induced by alloxan and treated

with glibenclamide 1.3 mg/kg BW)), and Groups IV–VII as a treatment group mice induced by alloxan and treated with 100 mg/kg BW, 200 mg/kgBW, 400 mg/kg BW, and 600 mg/kg BW of RGO (emulsified with tween 0.5%) once daily for 14 days (from day 15 to day 28).[13] Our *in vivo* study is illustrated in Figure 1.

Blood glucose measurement

Fasting blood glucose levels were determined at day-0 (as a baseline, before alloxan-induced), day – 7, 14, 21, and 28. Blood samples were taken from retro-orbital plexus then centrifuged at 2000 rpm for 10 min to separate the serum. Which based on glucose oxidase-peroxidase method with procedure as written in the kit. The percentage of decreasing blood glucose level was calculated using.[13]

$$\text{\% decreasing of blood glucose level} = \frac{(\text{blood glucose levels at day14} - \text{blood glucose levels at day28})}{\text{blood glucose levels at day 14}} \times 100\%$$

Hyperalgesia measurement using hot plate test

All mice were adapted for 1 h before the experiment. Each mouse was placed individually on the hot plate that was set at 50°C. The time that was needed for each mouse to show pain response was considered as latency time. Pain responses were showed as licking, jumping, and rearing.[14] Cut of time for this experiment was 12 s to prevent nerve damage. The maximum possible effect (MPE) was calculated according to the following formula: [15] % MPE = (test pressure threshold – pretest pressure threshold/(cut off pressure threshold – pretest pressure threshold) ×100.

Histopathology of the pancreas and spinal cord of mice

At the end of the study (day-24), mice were sacrificed by cervical dislocation. Pancreas and spinal cord were isolated and fixated in neutral buffer formalin 10% before staining. The pancreas was stained using aldehyde-fuchsin and spinal cord was stained using hematoxylin-eosin.

Data Analysis

Results were expressed as mean ± standard error mean. All the data included blood glucose levels, BW, latency time toward the thermal stimulus, and the percentage of MPE were statistically analyzed using one-way analysis of variance followed by Tukey analysis to compare various groups with each other. The significantly different was considered when $P < 0.05$. The histopathology of the pancreas and spinal cord of mice was illustrated descriptively and compared between groups.

RESULTS AND DISCUSSION

Distillation

Our result showed the percentage of yield of RGO slightly lower than ginger. Based on Table 1, the yield for RGO was 0.36%, compared to GO was 0.44%. The percentage of yield was determined using a comparison of weight between oil and rhizome in 100%.

Table 1: The percentage of yields from red ginger and ginger oil

Sample	Rhizome weight (g)	Oil weight (g)	Yield (%)
Red ginger	6.00	21.80	0.36
Ginger	8.10	35.95	0.44

Table 2: Determination of total phenolic compounds in red ginger and ginger oil

Sample	Total phenolic compound (mg GAE/g oil, *n*=3)
Red ginger oil	0.778±0.175[a]
Ginger oil	0.348±0.022[b]

Data were presented as mean±SD and different letter means significantly different between groups ($P<0.05$) using the independent *t*-test.
SD: Standard deviation

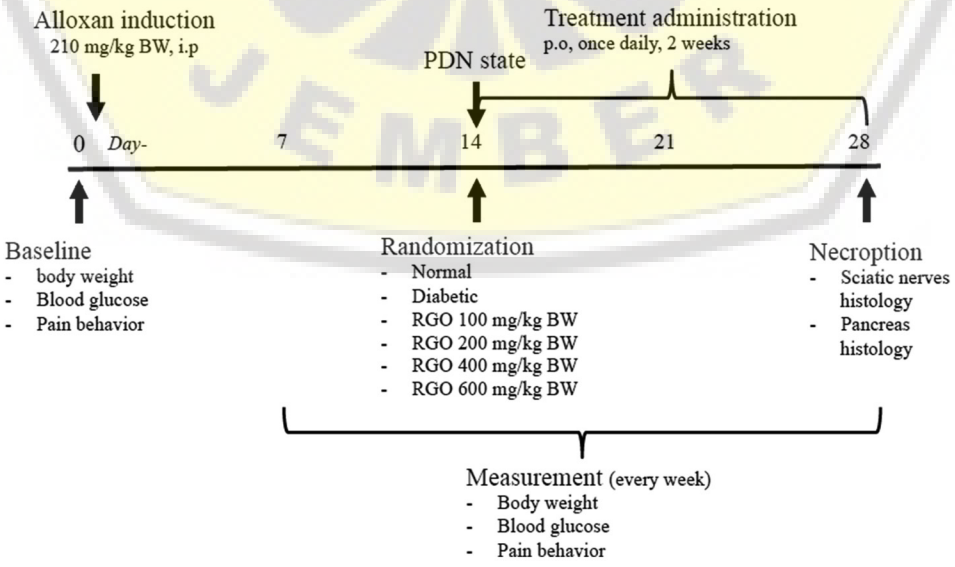

Figure 1: The scheme of work of alloxan-induced PDN *in-vivo*.

Determination of Total Phenolic Content

Gallic acid standardization curve [Figure 2] showed a linear equation between concentration and absorbance. The equation was y = 0.0827x + 0.162 with linearity (R^2) 0.99269. Determination total phenolic compound based on gallic acid standardization curve was obtained that RGO had total phenolic compound as much as twice higher than ginger oil [Table 2].

The Effect of Red Ginger and GO on Free Radical Scavenging using DPPH Method

Antioxidant activity was measured using the DPPH method. The IC_{50} value was compared to Vitamin C, a compound that had been proved with a high potency of antioxidant effect. Our studies showed the IC_{50} of RGO lower than GO [Table 3]. It means that RGO was more potent as antioxidant compared to GO. This result was in concordance with the total phenolic compound determination result. Based on our result, the antioxidant activity of RGO was not significantly different compared to Vitamin C, as control.

The Effect of Red Ginger on BW and Blood Glucose Level of PDN Mice

Our result showed that GO treatment did not impact the BW [Figure 3a]. Otherwise, blood glucose level decreased after GO treatment, compared to the diabetic group ($P < 0.05$; Figure 3b). The highest dose of GO (600 mg/kg BW) significantly decreased blood glucose level (mean = 273.16 ± 23.36 mg/dL, $P < 0.05$) from diabetic condition (mean = 467.08 ± 25.80 mg/dL). Even though it was significant, the blood glucose level after the highest dose treatment of RGO was still more than 250 mg/dL with the percentage of decreasing was 37.23%.

The Effect of RGO on Thermal-induced Hyperalgesia of PDN Mice

RGO administration for 2 weeks successfully improved pain behavior in PDN mice. This effect was determined hyperalgesia using the hot plate test. RGO showed the increasing of the latency time toward thermal stimulus [Figure 4]. The latency time toward thermal stimulus after RGO was significantly different compared to the diabetic group ($P < 0.05$). However, there were significant differences of latency time between the lower doses of red ginger with the highest dose (600 mg/kg BW) ($P < 0.05$; Table 4). The percentage of MPE after the highest dose of RGO treatment wTIas 62.35%, and this result was significantly higher than glibenclamide as control drug ($P < 0.05$; Table 4). It was reasonable because glibenclamide as antihyperglycemic drug did not have activity in peripheral or central nerves.

We can also determine the effective dose (ED_{50}) from this experiment. Using probit analysis, the ED_{50} of RGO for treating hyperalgesia in PDN was 154.17 mg/kg BW (the data did not show). Our data explained that the activity of RGO in reducing hyperalgesia in PDN did not due to its main activity in maintaining blood glucose level but probably due to its direct activity in nerves system.

The Effect of RGO on Histopathology of Pancreas and Spinal Cord of PDN Mice

Aldehyde-fuchsin staining was aimed to determined α and β cell of the pancreas, which were shown as blue (β cell) and red (α cell) color. The normal group did not show any damage in β cell [Figure 5a] compared to diabetic group [Figure 5b] that showed many damages in pancreas cell and also decreasing of β cell number. Treatment of RGO showed a delayed of the progression of islet cells damage. RGO dose 200 mg/kg BW [Figure 5d] increased the number of β cell pancreas compared to the diabetic group and similar with the control group (treated with glibenclamide, Figure 5c). Otherwise, the highest dose of RGO (600 mg/kg BW, Figure 5f) showed the best morphology of pancreas after treatment and closed to normal group (Figure 5a).

RGO treatment [Figure 6d-f] also delayed of spinal cord damage in PDN mice. After treatment, there was seen a lot of glia cell surrounding the nerve compared to the diabetic group [Figure 6b]. Besides, there was also no vasodilatation and neutrophil cell after treatment, especially with the highest dose of RGO (600 mg/kg BW; Figure 6f).

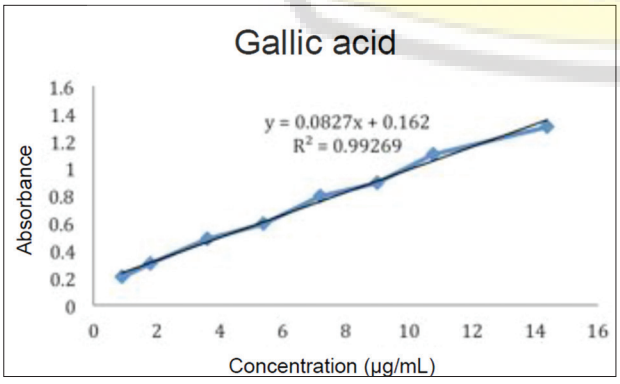

Figure 2: Standart curve of gallic acid.

Table 3: DPPH scavenging activity (IC_{50})

Compound	IC_{50} (µg/mL±S.E.M)
Vitamin C (control)	3.544±0.106[a]
Red ginger oil	3.626±0.357[a]
Ginger oil	12.445±0.111[b]

Data were presented as mean±SD. Letter (a) showed significantly different of IC_{50} value compared to letter (b) using one-way ANOVA ($P<0.05$).
SD: Standard deviation, DPPH: 1,1-diphenyl-2-picrylhydrazyl, S.E.M: Standard error mean, IC_{50}: Inhibitory concentration 50, ANOVA: Analysis of variance

Table 4: The percentage of maximum possible effect after RGO treatment in PDN mice

Group	% MPE±S.E.M
RGO 100 mg/kg BW	46.57±4.50[a]
RGO 200 mg/kg BW	52.67±3.07[a]
RGO 400 mg/kg BW	55.81±3.74[a]
RGO 600 mg/kg BW	62.35±5.67[b]
Glibenclamide 1.3 mg/kg BW	42.02±4.82[a]

Different letter indicated significantly different between groups ($P<0.05$) using one-way ANOVA. ANOVA: Analysis of variance, PDN: Painful diabetic neuropathy, BW: Body weight, RGO: Red ginger oil, MPE: Maximum possible effect

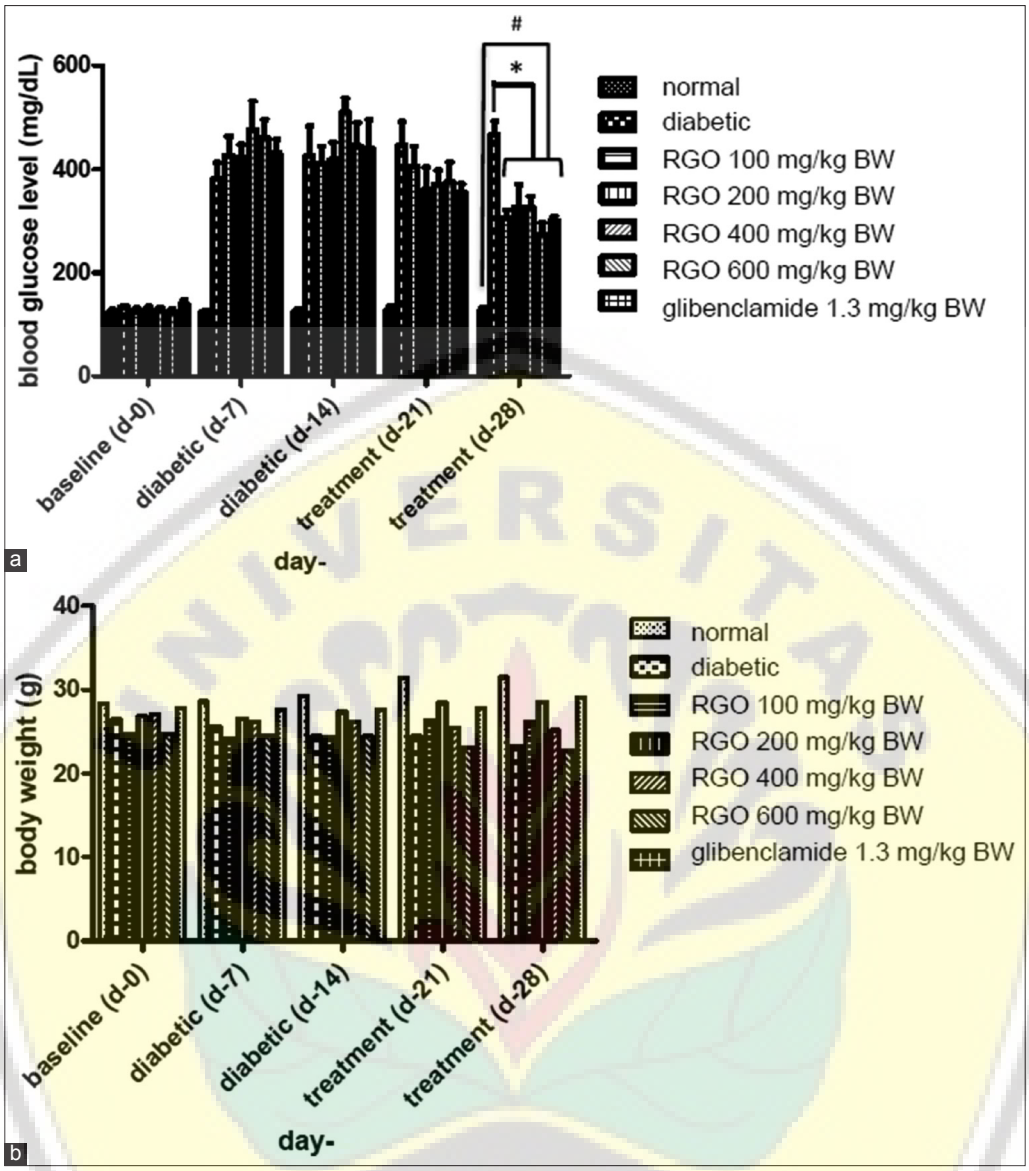

Figure 3: The differences of body weight (a) and blood glucose level (b) between groups. * indicated significantly different of blood glucose levels compared to diabetic ($P < 0.05$) and # indicated significantly different of blood glucose levels compared to normal ($P < 0.05$) using one way ANOVA. There was no significantly different of body weight between groups.

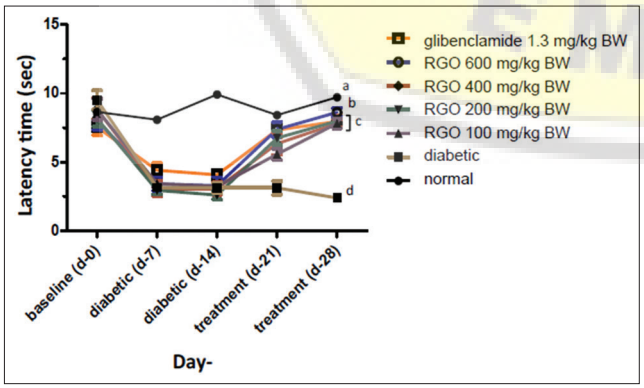

Figure 4: Latency time toward thermal stimulus after red ginger oil treatment in PDN mice. Different letter indicated significantly different between groups ($P < 0.05$) using one way ANOVA.

Pathogenic mechanisms underlying the progressive of nerve fiber loss are multifactorial, including the polyol pathway, advanced glycation, and ROS.[16] Chronic hyperglycemia affects cellular proteins, gene expression, and cell surface receptor expression, resulting in progressive pathologic changes and diabetic complications. Our study showed that intraperitoneal injection of alloxan significantly induced higher blood glucose levels through the inhibition of oxidative phosphorylation, caused the formation of ROS and disturbance pancreas function to secrete insulin.[17] Higher ROS concentration induced nerves damage and related to the increasing of the nociceptive threshold using thermal stimuli than normal mice. This condition indicated that diabetic mice exhibit thermal hyperalgesia. Our result was in concordance with another previous research that alloxan-induced thermal hyperalgesia and allodynia in mice.[13]

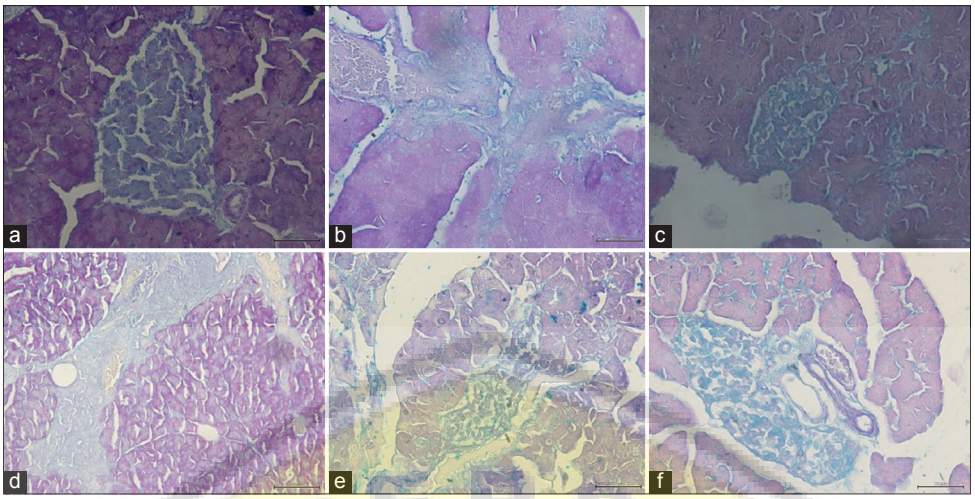

Figure 5: Islets pancreas morphology between groups in PDN mice i.e. normal (a), diabetic (b), glibenclamide (c), red ginger oil 100 mg/kg BW (d), red ginger oil 200 mg/kg BW (e) and red ginger oil 600 mg/kg BW (f). Pancreas was stained using aldehide-fuchsin with 400 magnification. Blue color: β cell and red color: α cell of pancreas.

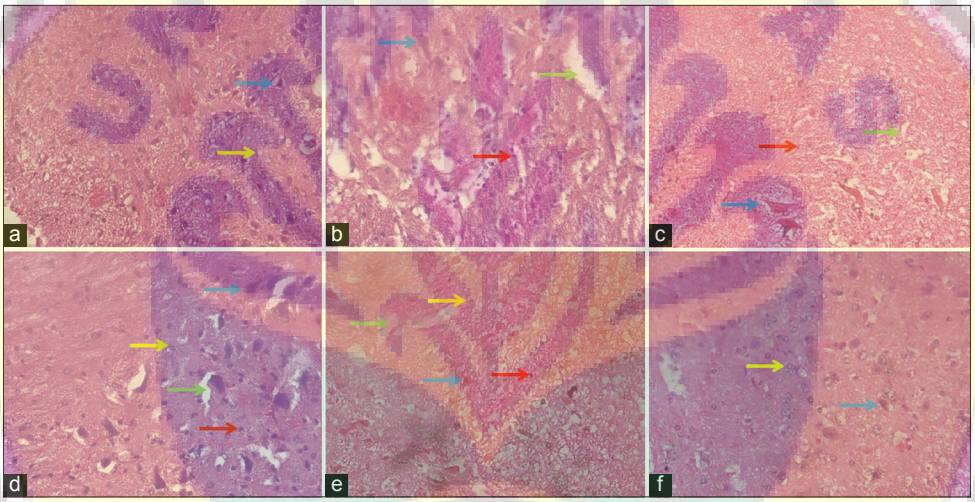

Figure 6: Histology of the spinal cord of mice (a) normal, (b) diabetic, (c) glibenclamide, (d) red ginger oil 100 mg/kg BW, (e) red ginger oil 200 mg/kg BW and (f) red ginger oil 400 mg/kg BW. green arrow: vasodilatation; yellow arrow: glial cell; red arrow: inflammatory cell; blue arrow neuron. Spinal cord was stained using hematoxylin-eosin with 400 magnification.

The uncontrolled hyperalgesia due to early diabetic neuropathy occurred after 7 days of alloxan induction and sustained until day-14, before treatment. Our result was also similar to the other study in rats, which found that hyperalgesia was first happened from day-7 after alloxan injection.[18] The decreasing of mice latency time toward heat stimuli was caused by the damage of peripheral nerves due to glucose accumulation. This condition will activate pain pathways begin from TRPV1 activation and lead to depolarization. In depolarization, the terminal button of nerves released neurotransmitter such as glutamate, which bound NMDA receptor NR2B subunit then caused pain sensitization.[4,5] The higher amount of glucose also increasing of ROS concentration, disturbed nutrients, and oxygen supply into the peripheral nerve fibers, and then damaged the nerves.[4]

Red ginger is one of the ginger species in Indonesia. RGO was previously reported had antidiabetic and antioxidant activity.[9] The administration of RGO 600 mg/kg BW for 2 weeks in our study, successfully decreasing the hypersensitivity threshold in thermal hyperalgesia better than the lower doses. RGO treatment also decreased blood glucose levels as same as glibenclamide, even though it did not come back into normal. This action was correlated with histopathology result in the pancreas and spinal cord staining. Even the activity of RGO was seen better than control drug, glibenclamide, the potency of RGO was lower than glibenclamide to reach the same effect. It is reasonable because RGO was composed of many active substances and further is needed regarding the content of active compounds that play a role in antidiabetic and antioxidants activity in RGO.

One of the ginger's mechanism in decreasing blood glucose levels is working as a serotonin antagonist-receptor. The mechanism may further lead pancreatic beta cells to increase the secretion of insulin.[19] Other mechanism of GO in delaying pancreas damage as well as spinal cord damage is antioxidant activity.[20] Antioxidant compound acts as a donor a hydrogen

atom, therefore, forms radical to stable and stops the oxidation chain reaction of free radical formation.[21,22] Although in our result RGO showed the highest antioxidant effect *in vitro*, it is still needed further exploration about the detailed relationship between antioxidant and antihyperalgesia effect in PDN.

The most content in the RGO is terpenoid group (such as monoterpenoid and sesquiterpenoids) and phenolic compounds, as shown in our result, which was associated with antidiabetic and antioxidant activity.[8] Camphene and cineole as a compound that identified in RGO were suspected as antioxidant and antidiabetic activity of GO. Both of them significantly reduced the production of ROS and protected cells including the pancreas and spinal cord from the damage after ROS attacks.[23] Inhibition of ROS accumulation caused a direct effect in decreasing of TRPV1 activation. This action initiated deactivation of NMDAR2B in the dorsal horn of spinal cord and reducing hyperalgesia in PDN.[6]

CONCLUSIONS

According to our result, RGO showed better antioxidant activity *in vitro* compared to GO. In *in vivo* study, RGO treatment reduced hyperalgesia in PDN through ameliorating of spinal cord histology of mice. Further research is needed to explain the relationship between antioxidant effect and antihyperalgesia effect in PDN.

ACKNOWLEDGMENT

This research was supported by Grant Research from the Ministry of Research, Technology and Higher Education Republic of Indonesia 2016.

REFERENCES

1. World Health Organization (WHO). Noncommunicable Diseases Country Profiles. Geneva: WHO Publishing; 2011.
2. World Health Organization (WHO). Definition, Diagnosis and Classification of Diabetes Melitus and it's Complications. Geneva: WHO Publishing; 2010.
3. Kawano T. A current overview of diabetic neuropathy-mechanisms, symptoms, diagnosis, and treatment. In: Peripheral Neuropathy. Vol. 10. London: InTech; 2014. p. 89-105.
4. Farmer KL, Li C, Dobrowsky RT. Diabetic peripheral neuropathy: Should a chaperone accompany our therapeutic approach? Pharmacol Rev 2012;64:880-900.
5. Luongo L, Costa B, D'Agostino B, Guida F, Comelli F, Gatta L, *et al.* Palvanil, a non-pungent capsaicin analogue, inhibits inflammatory and neuropathic pain with little effects on bronchopulmonary function and body temperature. Pharmacol Res 2012;66:243-50.
6. Zhuo M. Long-term potentiation in the anterior cingulate cortex and chronic pain. Philos Trans R Soc Lond B Biol Sci 2014;369:20130146.
7. Hartemann A, Attal N, Bouhassira D, Dumont I, Gin H, Jeanne S, *et al.* Painful diabetic neuropathy: Diagnosis and management. Diabetes Metab 2011;37:377-88.
8. Murhananto H, Endah JH, Listyarini T, Pribadi ST. Khasiat Manfaat Jahe Merah si Rimpang Ajaib. Jakarta: Agro Media Pustaka; 2002.
9. Anfenan ML. Evaluation of nutritional and antidiabetic activity of different forms of ginger in rats. Middle East J Sci Res 2014;21:56-62.
10. Rialita T, Rahayu WP, Nuraida L, Nurtama B. Aktivitas antimikroba minyak esensial jahe merah *(Zingiber officinale* Var. Rubrum) dan lengkuas merah (*Alpinia purpurata* K. Schum) terhadap bakteri patogen dan perusak pangan. Agritech 2015;35:1-8.
11. Fuentes E, Báez ME, Bravo M, Cid C, Labra F. Determination of total phenolic content in olive oil samples by uv–visible spectrometry and multivariate calibration. Food Anal Methods 2012;5:1311-9.
12. Singh G, Kapoor IP, Singh P, de Heluani CS, de Lampasona MP, Catalan CA, *et al.* Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale*. Food Chem Toxicol 2008;46:3295-302.
13. Deshpande TC, Une HD, Lahoti SR, Dehghan MH. Protective effect of ethyl acetate soluble fraction of ethanolic extract of *Terminalia chebula* retz, fruits on diabetic neuropathy in mice. Eur J Exp Biol 2011;1:139-14.
14. Fong SW, Lin HC, Wu MF, Chen CC, Huang YS. CPEB3 deficiency elevates TRPV1 expression in dorsal root ganglia neurons to potentiate thermosensation. PLoS One 2016;11:e0148491.
15. Nash MS, McIntyre P, Groarke A, Lilley E, Culshaw A, Hallett A, *et al.* 7-tert-butyl-6-(4-chloro-phenyl)-2-thioxo-2,3-dihydro-1H-pyrido[2,3-d]pyrimidin-4-one, a classic polymodal inhibitor of transient receptor potential vanilloid Type 1 with a reduced liability for hyperthermia, is analgesic and ameliorates visceral hypersensitivity. J Pharmacol Exp Ther 2012;342:389-98.
16. Mohini A, Phansea SB, Padhyeb MJ, Patila AR, Bafnaa AR, Takawalec VV. *Thespesia populnea* extract attenuates thermal hyperalgesia in diabetic mouse model of neuropathic pain. Iran J Pharm Sci 2010;6:269-76.
17. Dobretsov M, Romanovsky D, Stimers JR. Early diabetic neuropathy: Triggers and mechanisms. World J Gastroenterol 2007;13:175-91.
18. Bhatti R, Rawal S, Singh J, Ishar MP. Effect of *Aegle marmelos* leaf extract treatment on diabetic neuropathy in rats: A possible involvement of α2 adrenoceptors. Int J Pharm Pharm Sci 2012;4:632-7.
19. Heimes K, Feistel B, Verspohl EJ. Impact of the 5-HT3 receptor channel system for insulin secretion and interaction of ginger extracts. Eur J Pharmacol 2009;624:58-65.
20. Baroty GE, Baky HH, Farag RS, Saleh MA. Characterization of antioxidant and antimicrobial compounds of cinnamon and ginger essential oils. Afr J Biochem Res 2010;4:167-74.
21. Basak SS, Candan F. Chemical composition and *in vitro* antioxidant and antidiabetic activities of *Eucalyptus camaldulensis* dehnh essential oil. J Iran Chem Soc 2010;7:216-26.
22. Kumari J, Venkateshwarlu G, Choukse MK, Anandan R. Effect of essential oil and aqueous extract of ginger (*Zingiber officinale*) on oxidative stability of fish oil-in-water emulsion. J Food Process Technol 2014;6:1-5.
23. Tiwari M, Kakkar P. Plant derived antioxidants-geraniol and camphene protect rat alveolar macrophages against t-BHP induced oxidative stress. Toxicol *In Vitro* 2009;23:295-301.