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Zingiber officinale var. Rubrum Reduces the Rate of Prostaglandin Production

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Abstract

In Indonesia, red ginger (*Zingiber officinale* var. Rubrum) is usually used as topical pain reducer by directly applying the fresh rhizome. The aim of this research was to provide information regarding the pharmacological activity of *Z. officinale* var. Rubrum rhizome infusion on the rate of prostaglandin production. The *Z. officinale* var. Rubrum rhizome used in this research was purchased from Research Institute for Spices and Medicinal Plants (Balittro) Manoko Lembang, West Java, Indonesia. This research was conducted by applying TMPD (N,N,N',N'-tetramethyl-p-phenylendiamine) as the reagent. COX-1 and COX-2 enzyme inhibitory activity can be seen from TMPD chromogenic changes that occur during PGG2 reduction to PGH2. Phytochemical screening showed that flavonoid, quinone, and monoterpenoid/sesquiterpenoid were detected in both dried rhizomes and the water extract. Three spots were detected on thin-layer chromatography system which employing chloroform-methanol (5:5) as the eluent. The rate of prostaglandin formations either by *Z. officinale* var. Rubrum rhizome infusion or acetylsalicylic acid on COX-1 is slower (at 25th minutes) rather than COX-2 (5th minutes). We concluded that the rhizome of *Z. officinale* var. Rubrum reduces the rate of prostaglandin production. The rhizome of red ginger reduces the rate of prostaglandin production, which is slower in COX-1 than in COX-2. This plant could be further developed as anti-inflammatory drug candidate.

Keywords: acetosal, antiinflammation, cyclooxygenase, NSAIDs, red ginger

Introduction

Inflammation is a normal protective response to tissue injury caused by physical trauma, microbial toxin, or harmful chemicals. Inflammation is caused by the release of chemical mediators (histamine, prostaglandins) from damaged tissue.[1] Symptoms of inflammatory processes include heat (calor), redness (rubor), swelling (tumor), and impaired function (function laesa).[2] Statistical data shows that the

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prevalence of inflammation is quite high, i.e. gastrointestinal inflammation reaches 396 out of 100,000 cases.[3] The cases of gout arthritis occurred as much as 45.9% of the 100,000 cases with the most sufferers were men.[4]

The most common target of inflammatory treatment is by inhibiting prostaglandin formation as a major inflammatory mediator. Prostaglandin, which is produced from arachidonic acid, is catalyzed cyclooxygenase (COX) enzymes.[5] COX serves as a catalyst in the first stage of the biosynthesis process of prostaglandins, thromboxane, and prostacyclin. There are two COX isoforms, namely COX-1 and COX-2. COX-1 is found in many tissues and is responsible for maintaining normal body function including gastric mucosal integrity. Meanwhile, COX-2 was not found in tissues under normal condition, its expression is induced by various stimuli associated with prostaglandin production during inflammatory, pain, and pyretic.[6,7] The prostaglandin formation can be inhibited by nonsteroidal antiinflammatory drugs (NSAIDs). NSAIDs therapy may cause gastrointestinal side effects due to its nonselective inhibition on both COX isoforms.[8-10]

Many plants, particularly those that contains secondary metabolite compounds i.e. terpenoid groups, flavonoids and phenolates, show antiinflammatory activity through inhibition of COX enzymes.[11,12] The development of drugs from natural resource is expected to be a source of novel anti-inflammatory drugs with lower side effects. In Indonesia, red ginger (*Zingiber officinale* var. Rubrum) is usually used as topical pain reducer by directly applying the fresh rhizome. *Z. officinale* var. Rubrum contains essential oil (1-3%), oleoresin, and protease. The oleoresin contains many active ingredients and most of them give a spicy flavor effect, including gingerol, shogaol, eugenol, myristic acid, paradol, zingiberen and zingeron.[13] Its essential oil consists of monoterpenes such as geranial (citral a) and neral (citral b) and sesquiterpenes such as bisabolon, zingiberen, and sesquiterpene.*13* Zingiberen compounds, bisabolon, gingerol, and shogaol are known to have anti-inflammatory activity and antiulcer.[14]

Phenolic compounds in *Z. officinale* var. Rubrum shows anti-inflammatory activity (IC_{50} 5.5 µM) on prostaglandin biosynthesis.[15] The terpenoid group compounds are known to inhibit significant gastric lesions.[16] The use of oral ginger water extract can reduce inflammation effectively but is weaker than indomethacin.[17]

The aim of this research was to provide information regarding the pharmacological activity of Z. officinale var. Rubrum rhizome infusion on the rate of prostaglandin production. COX-1 and COX-2 enzyme inhibitory activity can be seen from TMPD chromogenic changes that occur during PGG2 reduction to PGH2.18

Methods

Instruments

The instruments used in this study were microplate reader (MRX TC revelation), 96-wells plate (Cayman Chemical), freeze dryer (Telstar), UV 254 and 366 nm (Camag UV Betrachter) lamps, water bath, eppendorf tubes, thermometers, analytical scales (Sartorius), micropipette tips, vortex mixer (VM-300), and glasswares commonly used in analytical laboratories.

Materials

Materials used were double distilled water (Ipha Laboratories), amyl alcohol (Great eternal tower), ammonia (Great eternal tower), 2N hydrochloric acid (Great

eternal tower), iron (III) chloride (Merck), 96% ethanol (Merck) , ether (Merck), ethyl acetate (Merck), gelatin (Merck), potassium hydroxide 5%, Colorimetric COX inhibitor screening assay kit No. 701050 (Cayman Chemical), chloroform (Merck), Dragendorff reagent (bismuth (III) nitrate in 10 M hydrochloric acid), Liebermann-Burchard reagent (acetic acid anhydrous in concentrated sulfuric acid), Mayer reagents (mercury (II) chloride and potassium iodide in distilled water), vanillin reagents in sulfuric acid, magnesium powder, and GF254 silica (Merck).

Plants determination

The *Z. officinale* var. Rubrum rhizome used in this research was purchased from Research Institute for Spices and Medicinal Plants (Balittro) Manoko Lembang, West Java, Indonesia (http://balittro.litbang.pertanian.go.id/?p=993&lang=en). The plant was determined in the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran.

Infundation

A total of 100 g of dried Z. officinale var. Rubrum rhizome was boiled in 1 L of water for 15 minutes. The extract was freeze-dried and stored in tightly sealed container.

Phytochemical screening

Phytochemical screening was carried out as per the standard methods described by Tiwari and colleagues at the Faculty of Pharmacy, Universitas Padjadjaran, West Java, Indonesia.

Thin layer chromatography (TLC)

A total of 20 µL of extract dissolved in 96% ethanol was put on GF254 silica plate and left for a while until the solvent evaporates. The TLC plates were eluted using a mixture of chloroform-methanol (5:5). The resulting spots were then observed under visible and UV lights.

Sample preparation

Stock solution was prepared by dissolving 50 mg of the extract in 10 mL ethanol 96%. This solution was diluted until various concentrations: 2.5 mg/mL, 1.25 mg/

Table 1. TLC extract optimization result

Mobile phase	Spots Number	Rf	Colour detection			Colour detection Vanilin Sulphate
			UV 254nm	UV 366nm	Vanilin Sulphate	
Methanol : Water (5:5)	1	0.325	-	-	Purple	Yellow
	2	0.8125	-	-	Purple	Yellow
Chloroform : Methanol (2:8)	1	0.7125	-	Green	Purple	-
Chloroform : Methanol (5:5)	1	0.125	Black	-	Purple	Yellow
	2	0.275	Black	-	Purple	Yellow
	3	0.65	Black	Green	Purple	-
Chloroform : Methanol (7:3)	1	0.1000	Black	Green	Purple	-
	2	0.8750	-	-	Purple	Yellow
Chloroform : Methanol (8:2)	1	0.1250	Black	-	Purple	Yellow
	2	0.7500	-	-	Purple	-
Ethyl Acetate	1	0.9125	Black	-	Purple	-

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mL, 0.625 mg /mL and 0.3125 mg/mL, respectively, were obtained. Cayman COX Inhibitor assay kit no. 701050

Cayman COX Inhibitor Assay Kit No. 701050 reagents were prepared as per standard instruction.

COX inhibitory activity assay

COX inhibitory activity assay was performed as per standard instruction written in the Cayman COX Inhibitor assay kit no. 701050 data sheet.

Data analysis

The average value of absorbance for all samples were calculated and converted into a graph between absorbance of time variation and absorbance histogram to concentration.

Results and Discussion

The yield of the *Z. officinale* var. Rubrum rhizome extract was 8.78%. Phytochemical screening showed the presence of secondary metabolites of flavonoid, quinone, monoterpenes and sesquiterpenes.

The TLC results is provided in Table 1. The TLC system resulted three distinct spots (Rs > 1). There were 2 values of Rs (between spots 1-2 and spots 2-3) of 1.78 and 4.91. The purple spots resulted by vanilin-sulphate reagent indicated the presence of

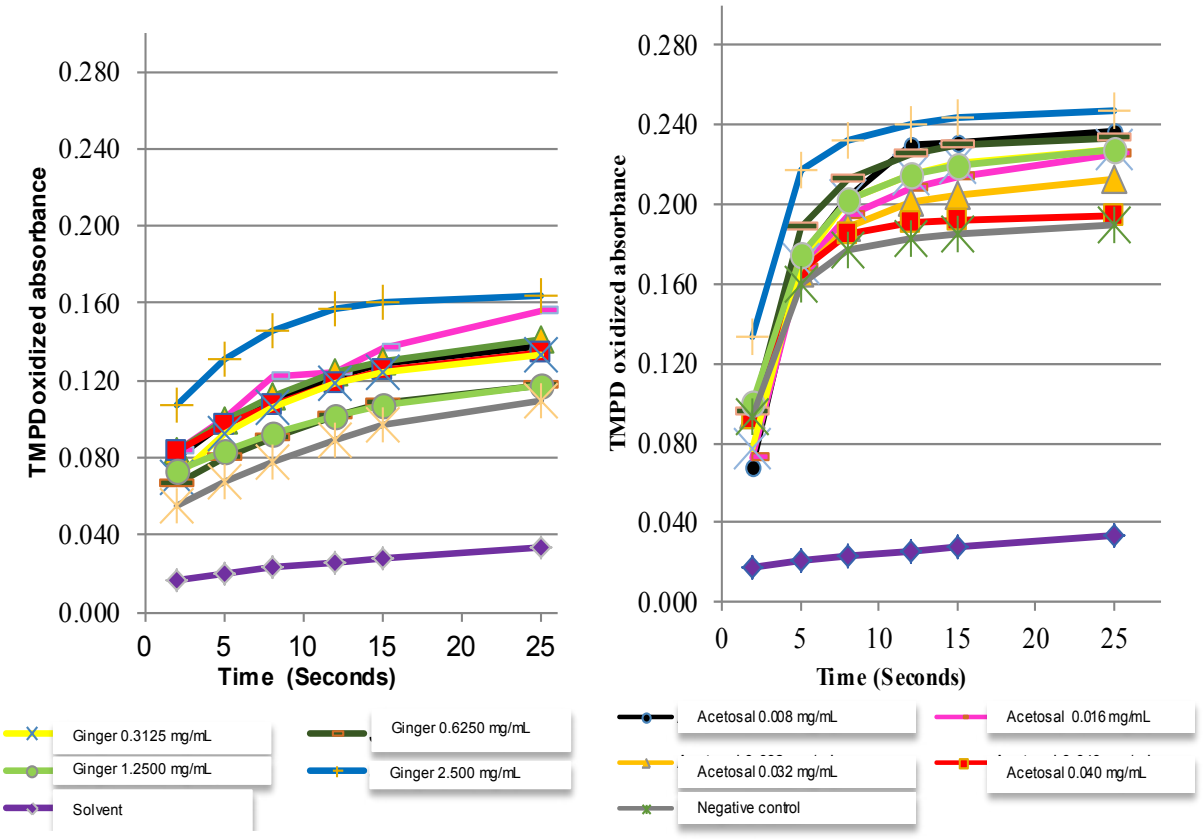

Figure 1. The TMPD kinetics curve

Information :

1. SD (a) = ± 0.0219, SD (b) = ± 0.03707; n = 3
2. The solvent contained test buffer, heme, ethanol, arachidonic acid and TMPD
3. Negative control contained test buffer, heme, ethanol, COX enzyme, arachidonic acid and TMPD

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monoterpenoid/sesquiterpenoid compounds.

COX inhibitory activity assay

There are three classes of secondary metabolites that could inhibit COX enzymes, *i.e.,* terpenoids, flavonoids and phenolates.[19] The presence of terpenoids and flavonoids in *Z. officinale* var. Rubrum rhizome is predicted belongs to these compounds.

COX inhibitory activity assay was performed in vitro using a Colorimetric COX Inhibitor Screening Assay kit. 701050 from Cayman Chemical Company. In this kit Tris-HCl buffer retained enzyme stability, heme as enzyme cofactor, COX-1 and COX-2 enzymes, arachidonic acid as enzyme substrate, KOH, and TMPD (N, N, N', N'- tetramethyl-p-phenyliamediamine) as a colorimetric substrate. TMPD served as an indicator or compound marker of COX enzyme activity.[20] The principle of this enzyme kit is the TMPD oxidation reaction due to heme peroxidase activity, so TMPD releases one electron to form a colored compound which absorbs at λ 590 nm.21 The TMPD oxidation reaction is equivalent to the PGG2 reduction reaction to PGH2 by the activity of the COX enzyme. The higher the activity of the COX enzyme against the arachidonic acid substrate, the more TMPD is oxidized so that the higher the absorbance value. If the inhibitory activity of extracts on large COX enzymes, then the reduction of PGG2 to PGH2 will decrease, resulting in fewer oxidized TMPDs or lower absorbance values. In enzyme-catalyzed reactions, the arachidonic acid substrate occupies the active side of the COX enzyme forming a complex of temporary enzymes where this complex will loose again resulting in free COX enzymes and prostaglandin products. The reaction of the complex formation of these enzymes is reversible. As the reaction progresses, over time, the enzyme will be occupied entirely by the arachidonic acid substrate so that the resulting prostaglandin product will remain relatively constant over a period of time.

The results of COX inhibitory activity assay

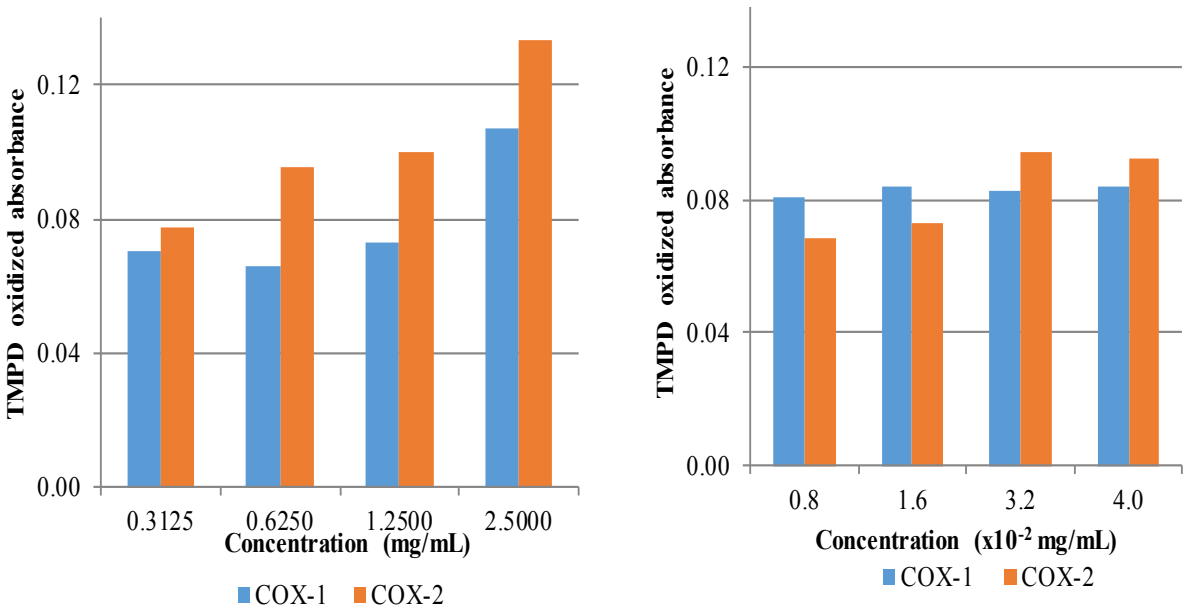

Figure 2: Histogram inhibition of COX enzyme by red ginger rhizome infusion (a) and acetosal (b)

is provided in Figure 1. Based on the results of testing of both COX enzymes, the measured TMPD absorbance increases with time. This indicates that the amount of oxidized TMPD was increasing as the product of prostaglandins produced from COX enzymes and arachidonic acid substrate increases. In Figure 1 (a), increased absorbance of oxidized TMPD or prostaglandin formation in COX-1 was relatively slower than the formation of prostaglandins in COX-2. This means that in COX-1, the *Z. officinale* var. Rubrum rhizome had a higher affinity with the enzyme, so the arachidonic acid slowly reacts with the enzyme (in the test procedure, arachidonic acid and TMPD was added last after incubation of the enzyme with infusa for 5 min). Figure 1 (b) in the time span of 2 to 12 minutes the absorbance value of oxidized TMPD or measurable prostaglandin formation increases rapidly, while in the 12-25 minutes period the absorbance value is relatively constant. This is because the arachidonic acid substrate has reacted entirely to the COX-2 enzymes so that the product of the oxidized prostaglandin or absorbance TMPD is relatively constant. This also applies to acetosal where the rate of prostaglandin formation in COX-1 is slower than COX-2.

In Figure 2 it can be seen that the inhibitory power of *Z. officinale* var. Rubrum rhizome in COX-1 and COX-2 is weaker than acetosal. This is evident from the large concentrations of *Z. officinale* var. Rubrum rhizome required to provide an almost equivalent inhibition with acetosal. In COX-1, an acetosal concentration of 3.2×10-2 mg/ml gave an absorbance of 0.083 while *Z. officinale* var. Rubrum rhizome requires a concentration of 1.25 mg/ml to give an absorbance of 0.073. In COX-2, acetosal with concentration 0.8x10-2 mg/ml gave absorbance 0.068 wherein red ginger rhizome infusion requires concentration of 0.3125 mg/ml to give absorbance of 0.0777.

Conclusions

The rhizome of red ginger reduces the rate of prostaglandin production, which is slower in COX-1 than in COX-2. This plant could be further developed as anti-inflammatory drug candidate.

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None.

Conflict of Interest

None.

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