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Effect of *Zingiber zerumbet* Essential Oils and Zerumbone Inhalation on Body Weight of Sprague Dawley Rat

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Abstract: *Zingiber zerumbet* contained the typically essential oils. The research aims to evaluate the effect *Z. zerumbet* essential oil and zerumbone inhalation on rats body weight, food consumption, parasympathetic nerve activity and brown adipose tissue temperature. The essential oils of *Z. zerumbet* was isolated from the rhizome of *Z. zerumbet*. The component in the oil and zerumbone structure was determined by gas chromatography-mass spectroscopy. The structure of zerumbone crystal was determined by nuclear magnetic resonance spectroscopy. The *Sprague dawley* male adult rats were divided into 4 groups namely Normal Diet (ND) group, High Fat Diet (HFD) group, HFD inhaled *Z. zerumbet* essential oils group and HFD inhaled zerumbone group. The results showed that inhalation of *Z. zerumbet* essential oils and zerumbone increased the food consumption as well as increased the body weight. The increasing body weight of rats which inhaled *Z. zerumbet* essential oils and zerumbone is by decreasing the sympathetic nerve activity. In conclusion, inhaling *Z. zerumbet* essential oils and zerumbone as the major component of the oils increased the weight gain.

Key words: *Zingiber zerumbet*, zerumbone, inhalation

INTRODUCTION

Zingiber zerumbet is one of Indonesia medicinal plant and among the South-East Asian countries (Fansworth and Bunyapraphatsara, 1992). In Indonesia, it is known as *lemputang gajah* and used as one of ingredient in *jammu* (Burkill, 1966). The rhizome of *Z. zerumbet* had been used for anti-inflammation, anti-ulceration, antioxidant, anti-pyretic, analgesic and anti-microbe (Somchit *et al.*, 2005). This rhizome reported contain of alkaloid, saponin, flavonoid and polyphenol as well as the volatile oil.

The volatile oil of *Z. zerumbet* contains zerumbone as the major component (Bhuiyan *et al.*, 2009). Zerumbone revealed many activities such as antinociceptive (Sulaiman *et al.*, 2010) through the suppression of free radical generation, iNOS expression and TNF- α release, also acts as an anti-intestinal inflammatory agent

(Murakami *et al.*, 2002, 2003). Zerumbone also potent as anticancer by suppressing skin tumor initiation (Murakami *et al.*, 2004), inducing apoptosis in a variety of human colonic adenocarcinoma cell line (Murakami *et al.*, 2002), as well as active to Human Cervical HeLa cells (Abdul *et al.*, 2008a, b; Wahab *et al.*, 2008) and CHO Cell lines (Al-Zubairi, 2012). Beside that, it has antimicrobial activity against *Salmonella choleraesuis* (Abdul *et al.*, 2008a) and has benefit for osteoarthritis and hepatotoxicity (Ganabadi *et al.*, 2009; Al-Saffar *et al.*, 2010; Fakurazi *et al.*, 2008, 2009).

Related to volatile oil, Shen *et al.* (2005a, b) reported that inhalation of grapefruit oil reduced the bodyweight of rats but inhalation of lavender oil increased the bodyweight of rats. The effect of inhalation of *Z. zerumbet* oil on bodyweight has not been reported yet. Information on the inhalation effect could add information about the utilization of *Z. zerumbet*.

The decreasing or increasing body weight of rats by inhalation was resulted from the effect of aroma on the autonomic nerves. Olfactory stimulation of aroma could affect autonomic nerves which related to lipolysis, heat production, appetite and finally affects the body weight. In the view of the uses of *Z. zerumbet* in Southeast Asia, the objective of this current investigation was to evaluate the effect of inhalation of *Z. zerumbet* essential oil and zerumbone on body weight, food consumption, fecal excretion, parasympathetic nerve activity and brown adipose tissue temperature.

MATERIALS AND METHODS

Isolation of *Zingiber zerumbet* essential oils: The materials used were *Zingiber zerumbet* rhizome collected from Indonesian Spices and Medicinal Crops Research Institute, Bogor. About 25 kg *Z. zerumbet* rhizome distilled by water distillation for 12 h yielded 0.12%. Distillate was kept on 4°C and pale yellow crystal formed. The pale yellow crystal then separated from the oils. Both oils and crystal were analyzed by gas chromatography-mass spectrometry (GC-MS). The structure of pale yellow crystal was determined by JEOL-NMR 500 MHz.

GC-MS analysis: *Z. zerumbet* essential oils and pale yellow crystal were separated with column DB-5 MS (0.25 mm×30 m) with helium gas (flow rate 42 mL min⁻¹). The injector and detector temperature were 80°C and 250°C, respectively. The separation condition started at 80°C for 5 min and increasing the temperature with rate 5°C min⁻¹ until 250°C and constant for 45 min. The EI MS analyzes were performed in 70 eV. MS data was collected and the spectrum compared with the library data.

NMR analysis: Zerumbone (Fig. 1), pale yellow crystal from *Z. zerumbet* essential oil, C₁₅H₂₂O ¹H-NMR (CD₃OD, 500MHz): δ(ppm) 1.05 (3, s, H-14), 1.21 (3H, s, H-15), 1.54

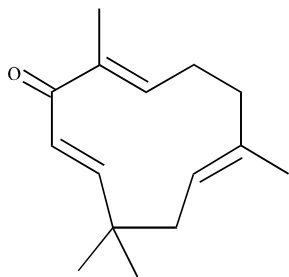


Fig. 1: Structure of Zerumbone

(3H, s, H-12), 1.75 (3H, s, H-13), 1.86-1.89 (1H, d, 14.8 Hz, H-2), 2.21-2.39 (6H, m, H-1, H-4, H-5), 5.94 (1H, s, H-10), 6.05 (1H, d, 16.4 Hz, H-9), 6.07 (1H, d, 16.4 Hz, H-6), ¹³C-NMR (CD₃OD, 125MHz): δ(ppm) 11.86 (C-13), 15.36 (C-12), 24.48 (C-14), 25.48 (C-5), 29.77 (C-15), 38.33 (C-11), 40.33 (C-4), 43.24 (C-1), 126.02 (C-2), 128.06 (C-9), 137.61 (C-3), 138.87 (C-7), 151.22 (C-6), 163.19 (C-10), 206.79 (C-8). All of the NMR data were compared with data reported by Abdul *et al.* (2008b).

In vivo analysis: Male *Sprague Dawley* rats were kept in cages with wood shaving as bedding of 3 rats per cages. The animals were adapted to laboratory conditions for 7 days prior to the experiments. They were given water *ad libitum*. Animals were grouped into 4 groups randomly based on their body weight with 6 animals in each group. The first group consumed Normal Diet (ND) without inhalation treatment as normal control; the second group consumed High Fat Diet (HFD) without inhalation as control group. The third and fourth groups consumed high fat diet with inhalation of *Z. zerumbet* essential oil and zerumbone 100 times dilution in water, respectively. During treatment period for 5 weeks, all animals were measured every week for body weight, daily weight of food consumption and fecal-urination excretion (based on bedding weight) every three days. After treatment, animals were sedated with combination of ketamine and xylazine with dosage 80 and 10 mg kg⁻¹ b.wt., respectively. Blood was collected intracardially to measure the lipid profile (cholesterol and triglyceride) using standard routine kit. The animals were euthanized with sodium phentobarbital (20 mg kg⁻¹ b.wt.) to collect and weight adipose tissue from scrotal and mesenteric area.

Electrophysiological recording: Male Wistar rats (10-11 weeks) weighing 270-310 g were used after adapted to the environment for at least 1 week prior to the experiment. Animals were anaesthetized with urethane (1 g urethane/1 kg b.wt.) prior to surgery. Small incision was made above the scapula to separate Brown Adipose Tissue (BAT) from muscle. One of the nerve branches was dissected and isolated. The isolated nerve was placed on a pair silver wire electrodes. The original signal of efferent discharges from the electrodes was amplified and filtered using a Bioelectric Amplifier ER-1. The amplified signal was converted to digital signals by Power Lab (AD Instruments, Colorado, USA). Baseline BAT sympathetic nerve activities were measured 40 min prior to olfactory stimulation with

samples and water. Samples used diluted 100 times with water. The rat's nose was placed inside the paper cup consisted of samples for 10 min. Spike above a threshold voltage level set just above background were counted by spike histograms.

BAT temperature recording: Between the interscapular BAT in the subcutaneous space, the temperature sensor was implanted. The temperature was recorded before, during and after inhalation of *Z. zerumbet* essential oils and zerumbone. Concentration of samples used was 0.1% or 100x diluted with water.

Statistical analysis: The data obtained were analyzed with Completely Randomized Design (CRD) and ANOVA (analysis of variance) at the confidence level of 90 or 95% (α level of 0.01 or 0.05). The p-values <0.05 indicates that there is a real difference to the measured response. Further test used was Duncan test. All data were analyzed with SAS program.

RESULTS

The essential oil yield of *Z. zerumbet* was 0.12%. The oil was colorless and had specific smell of *Z. zerumbet*. Based on GC-MS analysis, *Z. zerumbet* essential oils consisted of monoterpene, monoterpene alcohol, sesquiterpene and sesquiterpene alcohol. The oil was rich in sabinene (32.96%), β -myrcene (13.27) and zerumbone (11.05%) (Table 1). The yellow pale crystal yield was 0.04% and determined as zerumbone based on the GC-MS analysis (Table 1) and NMR data analysis. The structure of zerumbone is shown in Fig. 1.

The inhalation effect on percentage of increase body weight, food consumption and fecal and urination are shown in Table 2. The animal which consumed only normal diet had no change food consumption on adaptation periods (20.00 g/animal) compared with in treatment periods. The same condition also found in HFD group which inhaled *Z. zerumbet* essential oils and group inhaled zerumbone. The food consumption on HFD group is the lowest compared to other groups as well as the fecal and urination excretion. Treated animals with zerumbone of 1% had the highest percentage of weight gain compared to the other 4 groups.

Animal consumed high fat diet had a trend of higher adipose tissue compared to others even there was no significance different between all groups (Table 3). The liver tissue also had no significance different between all groups (p<0.05). The concentration of blood triglyceride

and cholesterol (Table 3) was no significance different although the total cholesterol concentration of HFD groups was the highest.

The inhalation effects on BAT Sympathetic Nerve Activity (BSNA) of *Z. zerumbet* essential oil and zerumbone were shown in Fig. 2. Inhalation of *Z. zerumbet* oil had no direct effect on BAT sympathetic nerve activity, but inhalation of zerumbone could decrease the BAT sympathetic nerve activity.

Effect of sniffing of *Z. zerumbet* and zerumbone on BAT temperature described on Fig. 3. The temperature of BAT decreased during sniffing the *Z. zerumbet* oil and the

Table 1: Constituents of *Zingiber zerumbet* rhizome essential oil and pale yellow crystal

Group name	Compound name	Percentage	
		Essential oil	Pale yellow crystal
Monoterpene	α -tujene	1.70	
	α -pinene	1.16	
	Camphene	0.98	
	Sabinene	32.96	
	δ -myrcene	13.27	
	α -terpinene	0.71	
	Limonene	0.86	
	γ -terpinene	1.24	
	Camphor	2.91	
Monoterpene alcohol	Linalool	0.77	
	1,8-sineol	4.23	
Sesquiterpene	δ -gurjunene	1.50	
	Trans-caryophyllene	3.73	
	α -humulene	2.72	1.36
	Germacrene	3.04	
Sesquiterpene alcohol	zerumbone	11.05	97.52

Table 2: Percentage of increase body weight, average food consumption and fecal and urination excretion in all groups

Treatment groups	% increased body weight compared to baseline	Food consumption (g/animal)	Fecal and urination excretion (g/animal)
ND (normal diet)	44.34±14.62 ^a	19.99±0.02 ^b	14.23±0.15 ^b
HFD (high fat diet)	49.32±7.21 ^{ab}	18.99±0.16 ^a	13.31±0.42 ^a
HFD+ <i>Z. zerumbet</i> essential oil	57.80±18.71 ^{ab}	19.99±0.01 ^b	14.39±0.20 ^b
HFD+zerumbone	61.80±10.17 ^a	19.93±0.09 ^b	14.44±0.03 ^b

ND: Normal diet, HFD: high fat diet, Different letter indicate significance at p>95%

Table 3: Percentage of adipose tissue to body weight, percentage of liver to body weight, total triglyceride, total triglyceride and total cholesterol in all animals in each groups

Treatment groups	% adipose tissue to body weight	% liver to body weight	Triglyceride (mg dL ⁻¹)	Cholesterol (mg dL ⁻¹)
ND (normal diet)	1.44±0.38 ^a	3.79±0.34 ^a	73.87±10.18 ^a	86.13±16.10 ^a
HFD (high fat diet)	1.66±0.49 ^a	3.87±0.28 ^a	70.35±9.97 ^a	90.00±16.13 ^a
HFD+ <i>Z. zerumbet</i> essential oil	1.28±0.22 ^a	3.76±0.29 ^a	71.25±11.09 ^a	80.75±11.51 ^a
HFD+zerumbone	1.38±0.35 ^a	3.81±0.48 ^a	76.75±10.97 ^a	86.25±11.61 ^a

ND: Normal diet, HFD: High fat diet, Different letter indicate significance at p>95%

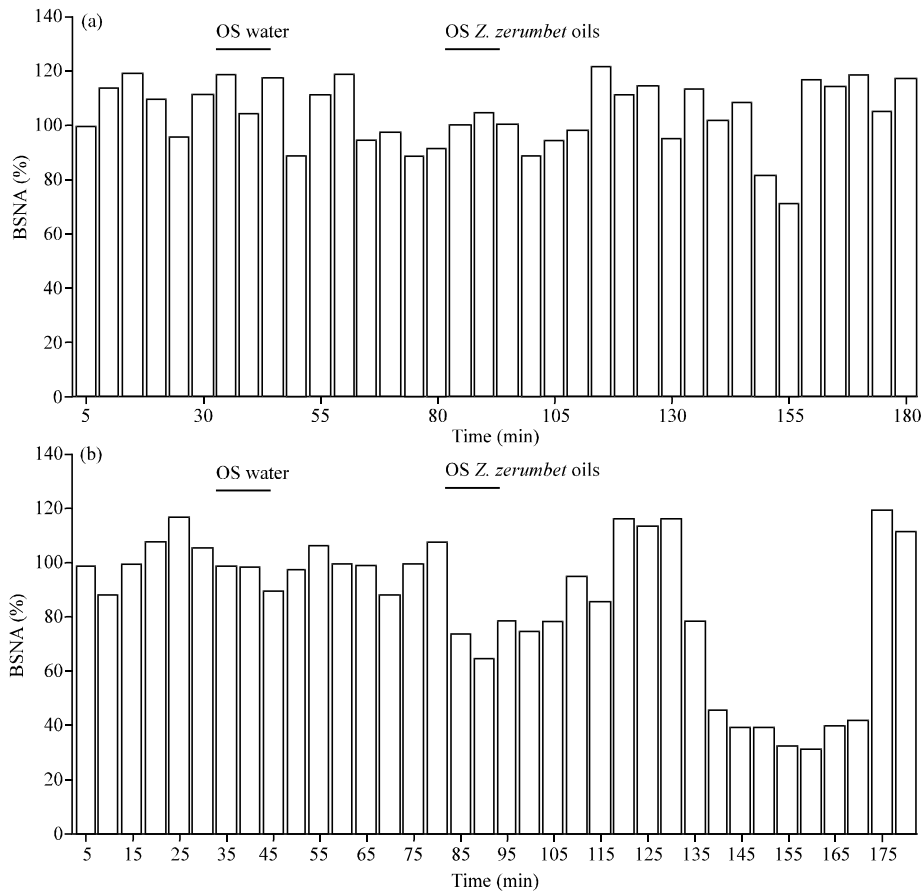


Fig. 2: BSNA (%) effect of sniffing the *Z. zerumbet* (a) Essential oils and (b) Zerumbone

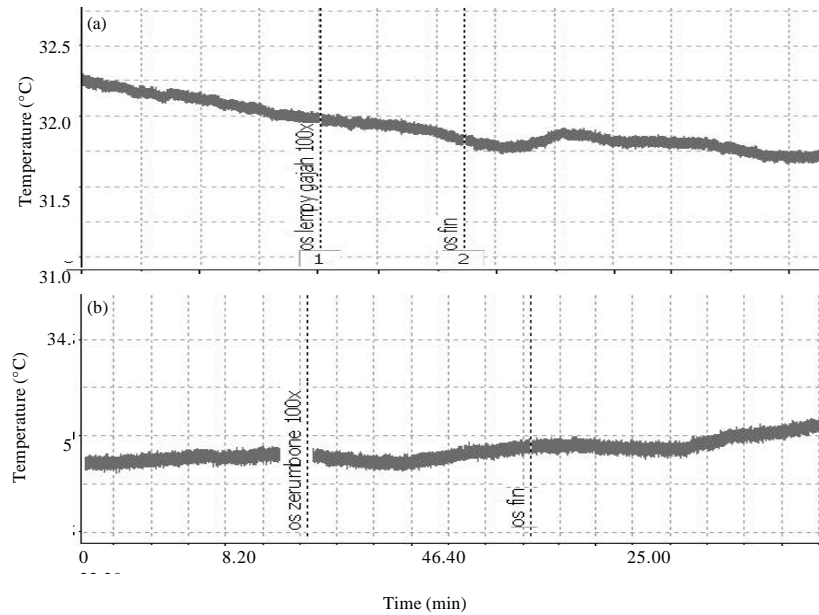


Fig. 3: BAT temperature before, on and after sniffing *Z. zerumbet* (a) Essential oil and (b) Zerumbone

temperature increased again after the sniffing time finished. On sniffing zerumbone, the BAT temperature decreased and increased again after 5 min sniffing. This effect also found after sniffing time finished.

DISCUSSION

Zingiber zerumbet rhizome oils from Bogor, Indonesia had the same major component with *Z. zerumbet* oil from Chittagong, Bangladesh (Bhuiyan *et al.*, 2009), Reunion Island (Chane-Ming *et al.*, 2003) and from Vietnam (Dung *et al.*, 1993). The different is found on the concentration of each component. Oil used in this study is only consisted of 11.05% of zerumbone (Table 1) while oil from Bangladesh consisted of 36.98% (Bhuiyan *et al.*, 2009), from reunion Island consisted of 37% (Chane-Ming *et al.*, 2003) and from Vietnam about 72.3% (Dung *et al.*, 1993). The low concentration of zerumbone from our oils might be due to zerumbone cyrstalization during storage in low temperature. The crystal and oils were separated before used in this study.

The data of inhalation effect of perfumes decreased the body weight in Wistar rats (Akunna *et al.*, 2011; Shen *et al.* (2005a). The recent data showed that inhalation of *Z. zerumbet* essential oils as well as its major component, zerumbone, could increase the body weight (Table 2). This correlates with effect lavender oil inhalation (Shen *et al.*, 2005b).

The increase in body weight also correlates with increased in food consumption. The group treated with HFD and *Z. zerumbet* oil and zerumbone had the same food consumption with ND group. The effect of HFD feeding on HFD group is decreasing the appetite, but with inhaling the oil, the appetite comes to normal. This result confirmed that *Z. zerumbet* is traditionally used to reduce appetite (Somchit and Nur-Shakirah, 2003).

Body weight increased the highest in animals treated with zerumbone compared with other groups ($p > 0.05$). However, the increase in body weight is not followed by the increase of adipose tissue. The adipose tissue of group which inhaled the essential oils was the lowest and lower than the normal diet group (Table 3). The lipid profile of all groups was not significantly different. Interestingly the plasma cholesterol concentration in *Z. zerumbet* essential oil groups was the lowest compared to other groups and also lower than the normal diet group.

The liver ratio to the body weight also not significantly different between each groups (Table 3) while the inhaled of perfumed from Nigerian decreased the

ratio of liver to body weight (Akunna *et al.*, 2011). This can be assumed that the inhalation of *Z. zerumbet* essential oil and zerumbone did not affect the liver.

Increase in appetite can be produce when the BAT sympathetic nerve activity decreased (Bray *et al.*, 1989). It is also reported that reduction of the sympathetic nervous activity is a potential mechanism in affecting the weight gain (Spraul *et al.*, 1993). The decrease of BAT sympathetic nerve activity was found in animals sniffing the *Z. zerumbet* essential oil and sniffing zerumbone (Fig. 2). The decreasing activity of sympathetic nerve also is followed by decreasing the BAT temperature (Fig. 3). Thermogenesis will appeared when the sympathetic nerve activity increased and BAT will convert fatty acids as fuel (Ma and Foster, 1986).

In conclusion, inhalation of *Z. zerumbet* essential oils and its major component, zerumbone, increased the food consumption by decreasing the BAT sympathetic nerve activity. As the results, it increased the body weight.

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