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# The antioxidant effect of bangle (*Zingiber cassumunar*) rhizome extract on superoxide dismutase (sod) activity in hyperlipidemic rats

**Novita Sari, Nurkhasanah* and Nanik Sulistyani**
Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, INDONESIA
*nurkhas@gmail.com

***Abstract***

*High-fat diet can cause increase in formation of reactive oxygen species (ROS). Bangle (Zingiber Cassumunar) rhizome is potential antioxidant and can restore oxidative balance. The purpose of this study was to evaluate the effects of extract Z. cassumunar rhizome on superoxide dismutase (SOD) enzyme activity in rat induced by high-fat diet (HFD). Twenty-five rats were divided randomly into five groups; group I: normal control (standard-fed); groups II: hyperlipidemia (HFD+propylthiouracil (PTU) 0.05%); group III, IV and V: administered HFD+PTU 0.05% and different dose of Z. cassumunar rhizome extracts ( 100, 200 and 400 mg/kg BB). The rats were fed with HFD+PTU 0.05% for 14 days and extract of Z. cassumunar treatment for 28 days simultaneously. Rats were fasted overnight and euthanized by neck dislocation.*

*Liver was separated for measurement of SOD activity. The statistical analysis that had been done used ANOVA with an LSD test. The intake of Rhizome Z. Cassumunar extract 400 mg/kg BW per day in rats induced HFD, significantly increased SOD activity compared to the control group and HFD group (P <0.05). Z. Cassumunar Rhizome has been shown to effectively increase antioxidant activity and minimize the adverse effects of HFD.*

**Keywords:** Superoxide Dismutase, *Zingiber cassumunar*, High-fat diet, antioxidant.

## Introduction

Hyperlipidemia is a metabolic disorder characterized by increased levels of total cholesterol, triglycerides, LDL and decreased HDL in the blood. Hyperlipidemia can be caused due to long-term use of a high-fat diet (HFD)[7]. The increase in ROS production causes oxidative stress which is characterized by an imbalance between oxidants and antioxidants[1]. Oxidative stress causes cardiovascular disease, degenerative diseases and metabolic diseases with various complications. Oxidative stress can damage the structure of DNA, cellular proteins and functional proteins present in cells[11].

Antioxidants are molecules that interact and neutralize free radicals, thus preventing cell damage in biological systems. The body makes endogenous antioxidants (antioxidant enzymes) used to neutralize free radicals[4]. Superoxide dismutase (SOD) is one of antioxidant enzymes that catalyses the dismutation of superoxide radical into hydrogen peroxide ($H_2O_2$) and molecular oxygen ($O_2$) and consequently an important defense[4]. In addition to enzymatic antioxidative factors, the protective system also comprises non-enzymatic antioxidants (exogen) such as vitamin E, vitamin C, phenols, flavonoid, or other compounds. One way of supplying these components is consuming fruits, vegetables and spices[16].

Bangle (*Zingiber cassumunar Roxb.*) is traditional plant commonly used as cooking spice in Indonesia[12]. The chemical content of bangle rhizome is phenolic groups, flavonoids, curcuminoids and essential oils which are potential antioxidant compunds[12,13]. The study has proven the antioxidant activity of *Z. cassumunar* rhizome *in vitro* using the DPPH test; extract of rhizome bangle has antioxidant activity in the 100 $\mu$g/ml with an inhibitory power of 41.23%[1].

The purpose of this study was to determine the antioxidant activity *in vivo* of rhizome bangle extract on the superoxide dismutase enzyme in rat induced by high-fat diet.

## Material and Methods

**Plant material and preparation of the extract:** Rhizome of *Z. Cassumunar* was collected from a local market at Sleman city, Yogyakarta and identified by Biology Laboratory, Universitas Ahmad Dahlan. ZC rhizome are rinsed with water, dried and cut into pieces and powdered with a grinder. This powder was extracted with 96% ethanol using the maceration method. Extract ZC is evaporated in water bath and the resulting brown residue is stored for further use.

**Determination of curcumin content:** Standard curcumin powder in ethanol is prepared with a concentration of 5 mg/mL, then prepare series solution with various concentration 0.2; 0.6; 0.8; 1.2; 1.4 and 1.6 mg/mL. For sample preparation, 50 mg of extract rhizome ZC was transferred to 10 ml volumetric flask. The sample was dissolved in ethanol. Five microliters of each standard solution and the sample solution are applied to the precoated plate of silica gel 60 $F_{254}$. Mobile phase used is a mixture of chloroform: ethanol: glacial acetic acid (94:5:1). Densitometric scanning was performed on Camag TLC Scanner 3 at 425 nm[18].

**Induction of hyperlipidemia:** Rats induced hyperlipidemia by feeding high-fat diet (standard pellet 300 g, chicken egg yolk 20 g, butter 100 g and beef fat 10 g moulded in the shape of pellets) and the addition of 0.05% propylthiouracil (PTU) in the drinking water[15].

**Animals:** Six-to-eight-weeks old male Wistar rats weighing 150-200 gram, procured from faculty of pharmacy of Sanata Dharma University, were adapted for 7 days on animal house in conditions of temperature and humidity awake, with a 12 h light / 12 h dark. Standard water and pellets are provided *ad libitum*. This study was approved by the research ethics committee, Ahmad Dahlan University.

**Experimental design:** Twenty-five rats were divided randomly into five groups, group I: normal control (standard-fed); groups II: hyperlipidemia (HFD + propylthiouracil (PTU) 0.05%); group III, IV and V: administered HFD + PTU 0.05% and different dose of Z. cassumunar rhizome extracts ( 100, 200 and 400 mg/kg BB). The rats were fed with HFD + PTU 0.05% for 14 days and extract of Z. cassumunar treatment for 28 days simultaneously. Rats were fasted overnight and euthanized by neck dislocation. Livers was separated, washed with saline 0.9% and stored at -80 ℃ until analysis.

**Tissue preparation**: Mince the tissues to small pieces, then weigh and homogenize in PBS (0,01 M, pH 7.4) on ice, the volume of PBS (mL) : the weight of the tissue (g) = 9:1. The tissue homogenate is centrifuged at 3000 rpm for 15 min. collect the supernatant for detect[3].

**SOD enzyme activity assay:** SOD enzyme activity was determined using Elabscience kit (Elabscience Biotechnology Inc), which was based on the colorimetric method. Superoxide anion ($O_2^-$) produced by xanthine system can oxidize hydroxylamine to form nitrite which appears purplish red after chromogenic reaction. One unit of SOD activity is defined as 1 mg of tissue protein in 1 mL necessary to cause 50% inhibition. Measure the OD value with a spectrophotometer at 550 nm. SOD activity is calculated through the formula:

SOD activity ($U/mgprot$) =

$$\frac{ODcontrol - ODsampel}{ODcontrol} 50\% \, X \, \frac{volume\ total\ (mL)}{volume\ sampel\ (mL)}$$

where X = protein concentration of sample (mg/prot).

**Statistical Analysis:** Statistical analysis was processed using SPSS software Version 22. The data were analysed using one-way analysis of variance (ANOVA) and the differences between all groups were tested using the Least Significant Difference (LSD). A probability value less than 0.05 was statistically significant.

**Table 1**
**The content of curcumin in extract *Z. cassumunar***

| Sample replication | Area | Content of curcumin (mg/ml) | Content of curcumin (%) | Mean ± SD |
|---|---|---|---|---|
| I | 22837.67 | 0.33 | 6.6 | 7.3 ± 0.58 |
| II | 23990.91 | 0.38 | 7.7 | |
| III | 23842.28 | 0.37 | 7.5 | |

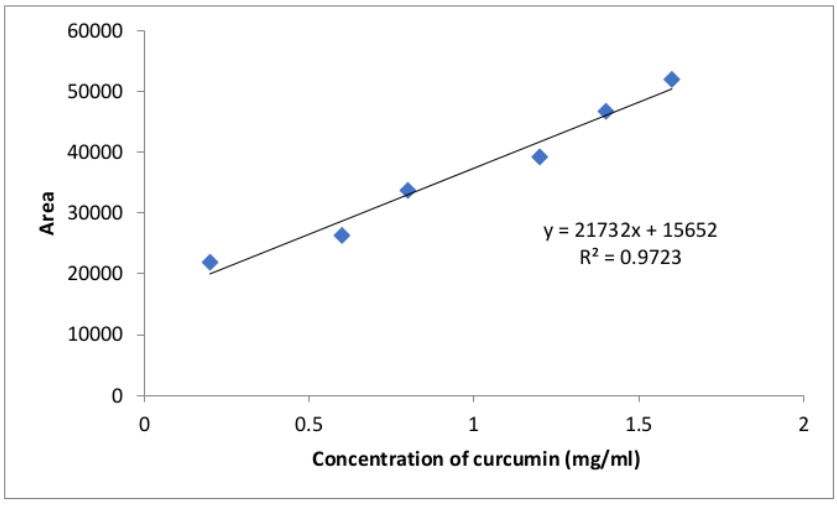


**Fig. 1: Standard curve of curcumin**

## Results and Discussion

The extraction process was done 1 kg of Simplicia of *Z. Cassumunar* Rhizome and produced a viscous extract of 48.2 g (4.82%). The viscous extract is brown with a distinctive odor and tastes slightly rough. The solvent used in this process is 96% ethanol, it will break down the cell wall so the solvent can penetrate the cell and attract the secondary metabolites contained in it. 96% ethanol is a universal solvent because it has a significant solvent partition coefficient that attracts polar and non-polar compounds[13].

Curcumin of *Z. cassumunar* extract is a compound that has the potential for antioxidant activity. The content of curcumin extract *Z. cassumunar* is obtained by entering the sample area into the linear regression equation from the standard curve y = 21732x + 15652 as in figure 1. The results of the determination of the curcumin content of extract *Z. cassumunar* determined by TLC-densitometry are shown in the table 1. According to Farmakope Herbal Indonesia, the content of curcumin in the rhizome of bangle should not be less than 5%. Curcumin levels in this study met the requirements.

The body weight determinations are shown in table 2. All rats gained weight in the experimental period. However, hyperlipidemia group rats experienced a significantly high body weight per day as compared with the other groups (*P<0.05). The study showed consumption. *Z. cassumunar* reduced weight growth compared to eating HFD and 0.05% PTU in drinking water after four weeks of treatment and the difference was not statistically significant. The function of PTU is increasing cholesterol levels by inhibiting the synthesis of thyroid hormones. HFD-fed together with PTU is unable to control cholesterol and fat levels.[15]

**Table 2**
**The effect of *Z. cassumunar* extract treatment on high-fat diets.**

| Group | Initial body weight (g) | Final body weight (g) | Weight gain (g/day) |
|---|---|---|---|
| Normal Control | 229,6 ± 30,84 | 255,8 ± 17,12 | 9,8 ± 4,77 |
| High-Fat Diet | 196,8 ± 28,77 | 272,2 ± 20,11 | 16,2 ± 3,12* |
| High-Fat Diet + *Z cassumunar* 100 mg/kg b.w | 207,4 ± 13,33 | 268,8 ± 6,97 | 14,75 ± 2,99 |
| High-Fat Diet + *Z cassumunar* 200 mg/kg b.w | 217,8 ± 18,14 | 266,2 ± 34,68 | 12,1 ± 5,22 |
| High-Fat Diet + *Z cassumunar* 400 mg/kg b.w | 203,2 ± 21,24 | 250 ± 26,61 | 11,7 ± 4,38 |

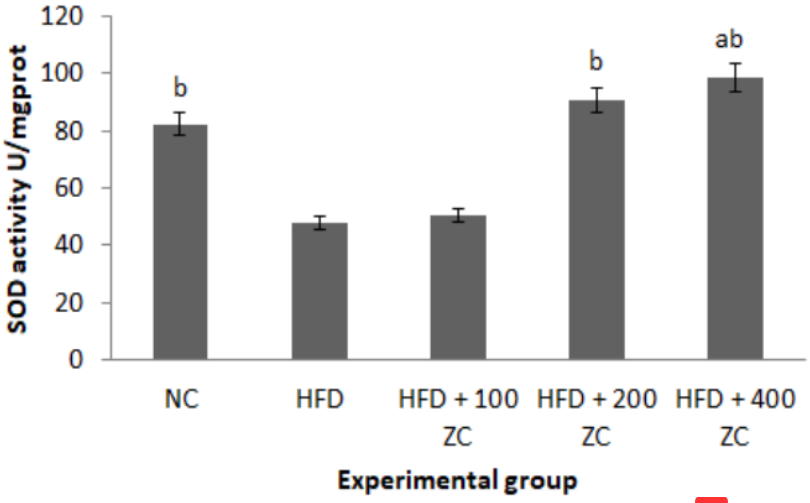


**Fig. 2: Antioxidant effect of *Z. cassumunar* rhizome extract on SOD activity in rat liver; data are expressed as mean ± SD; n = 5 in each group; NC = normal control; HFD + 100 ZC = high-fat diet + *Z. cassumunar* 100 mg/kg bw per day; HFD + 200 ZC = high-fat diet + *Z. cassumunar* 200 mg/kg bw per day; HFD + 400 ZC = high-fat diet + *Z. cassumunar* 100 mg/kg bw per day; a = P<0.05 compared NC group; b = P<0.05 compared HFD group**

The study showed a decreasing effect of SOD enzyme activity on HFD-fed rats. The decrease in SOD enzyme activity in this study was caused by HFD consumption implicated in the excess production of ROS. However, enzyme SOD activity is increased when consuming *Z. cassumunar*. The activity of the enzyme SOD in each group is shown in figure 2. The intake of extract Rhizome ZC 400 mg/kg BW per day in rats induced HFD, significant increase in SOD activity compared to the control group and HFD group (ab=P <0.05). The SOD enzyme catalyses superoxide to hydrogen peroxide and oxygen, thereby reducing the likelihood of superoxide anion reacting with nitric oxide[3]. This study suggests that the extract *Z. cassumunar* rhizome has an antioxidant activity and is capable of ameliorating the effect ROS of HDF.

## Conclusion

*Z. Cassumunar* rhizome has effectively increased antioxidant activity and minimized the adverse effects of HFD. It increases SOD enzyme activity after four weeks, especially at a dose of 400 mg/kg BW.

## Acknowledgement

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