

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 an 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 antioxidants¹. 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 cells¹¹.

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 radicals⁴. 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 defense⁴. 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 spices¹⁶.

Bangle (*Zingiber cassumunar Roxb.*) is traditional plant commonly used as cooking spice in Indonesia¹². The chemical content of bangle rhizome is phenolic groups, flavonoids, curcuminoids and essential oils which are potential antioxidant compounds^{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 $\mu\text{g/ml}$ 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 F₂₅₄. 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 nm¹⁸.

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 water¹⁵.

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 °C 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 detect³.

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 ($U/mgprot$) =

$$\frac{OD_{control} - OD_{sampel}}{OD_{control}} 50\% \times \frac{volume\ total\ (mL)}{volume\ sampel\ (mL)}$$

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 \pm SD
I	22837.67	0.33	6.6	7.3 \pm 0.58
II	23990.91	0.38	7.7	
III	23842.28	0.37	7.5	

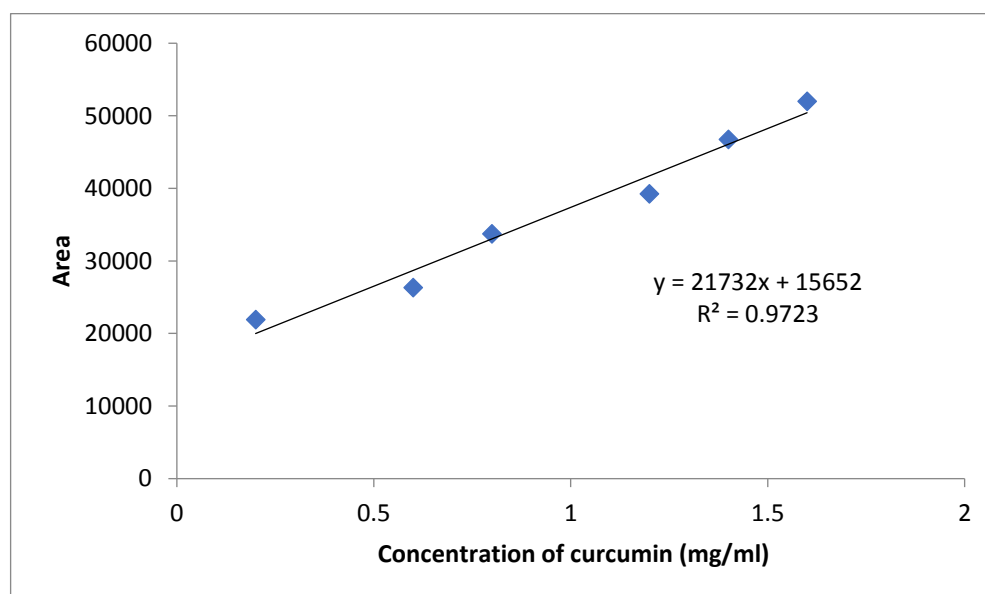


Fig. 1: Standard curve of curcumin

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 compounds¹³.

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 + <i>Z. cassumunar</i> 100 mg/kg b.w	207,4 ± 13,33	268,8 ± 6,97	14,75 ± 2,99
High-Fat Diet + <i>Z. cassumunar</i> 200 mg/kg b.w	217,8 ± 18,14	266,2 ± 34,68	12,1 ± 5,22
High-Fat Diet + <i>Z. cassumunar</i> 400 mg/kg b.w	203,2 ± 21,24	250 ± 26,61	11,7 ± 4,38

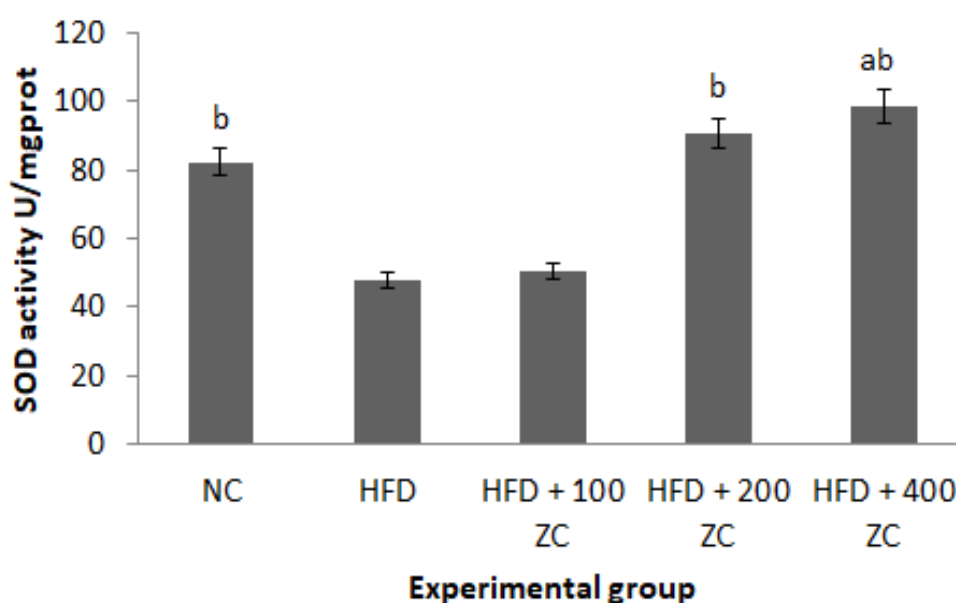


Fig. 2: Antioxidant effect of *Z. cassumunar* rhizome extract on SOD activity in rat liver; data are expressed as mean ± SD; n = 5 in each group; NC = normal control; HFD + 100 ZC = high-fat diet + *Z. cassumunar* 100 mg/kg bw per day; HFD + 200 ZC = high-fat diet + *Z. cassumunar* 200 mg/kg bw per day; 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 oxide³. 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

The authors like to thank the Ministry of Research, Technology and Higher Education of Indonesia for postgraduate research funding.

References

1. Agustin N., Penelusuran Fraksi Aktif Anti Oksidan Ekstrak Etanol Rimpang Bangle (*Zingiber Cassumunar Roxb*) Dengan Metode DPPH, Universitas Ahmad Dahlan (2016)
2. Amiya E., Interaction of hyperlipidemia and reactive oxygen species: Insights from the lipid-raft platform, *World J Cardiol*, **8**(12), 689-694 (2016)
3. Assady M., Farahnak A. and Golestani A., Superoxide dismutase (SOD) enzyme activity assay in *Fasciola* spp, *Iranian Journal of Parasitology*, **6**(4), 17-22 (2011)
4. Bouayed J. and Bohn T., Exogenous Antioxidants—Double-Edged Swords in Cellular Redox State: Health Beneficial Effects at Physiologic Doses versus Deleterious Effects at High Doses, *Oxid Med Cell Longev*, **3**(4), 228-237 (2010)
5. Cai H. and Harrison D., Endothelial Dysfunction in Cardiovascular Diseases: The Role of Oxidant Stress, *Circ Res*, **87**(10), 840-4 (2000)
6. Diplock A., Charleux L. and Crozier W., Functional food science and defence against reactive oxidative, *British Journal of Nutrition*, **80**(1), 77-112 (1998)
7. Karam I., Yang Y.J. and Li J.Y., Hyperlipidemia Background and Progress, *SM Atherosclerosis Journal*, **1**(1), 1-8 (2017)
8. Marliani L., Juanda D. and Rubianto A., Isolation of Antioxidant Compounds from Ethanol Extract of Temu Kunci (*Boesenbergia pandurata* Roxb.) Rhizomes, *Acta Pharmaceutica Indonesia*, **XXXVIII**(2), 48-51 (2013)
9. McCord J.M. and Fridovich I., Superoxide dismutase, An enzymic function for erythrocyte (hemocuprein), *J Biol Chem*, **244**(22), 6049-55 (2006)
10. Melekh B., Ilkiv I., Lozynskyi A. and Sklyarov A., Antioxidant enzyme activity and lipid peroxidation in rat liver exposed to celecoxib and lansoprazole under epinephrine-induced stress, *J Appl Pharm Sci*, **7**(10), 94-99 (2017)
11. Nita M. and Grzybowski A., The Role of the Reactive Oxygen Species and Oxidative Stress in the Pathomechanism of the Age-Related Ocular Diseases and Other Pathologies of the Anterior and Posterior Eye Segments in Adults, *Oxid Med Cell Longev*, doi: 10.1155/2016/3164734, 1-23 (2016)
12. Nurkhasanah, Santoso R.D. and Fauziah R., The immunomodulatory effect of *Zingiber cassumunar* ethanolic extract on phagocytic activity, nitric oxide and reactive oxygen intermediate secretions of macrophage in mice, *IOP Conf Ser Mater Sci Eng* (2017)
13. Padmasri P.D., Astuti K.W. and Warditiani N.K., Skrinning Fitokimia Ekstrak Etanol 70% Rimpang Bangle (*Zingiber purpureum* Roxb.), *Journal Farmasi Udayana*, **2**(4), 1-7 (2013)
14. Paramita S., Aminyoto M., Ismail S. and Arung E.T., Anti-hypercholesterolemic effect of *Zingiber montanum* extract, *F1000Research*, **7**, 1798 (2018)
15. Rini S., Pengaruh Pemberian Diet Lemak Tinggi Terhadap Kadar Trigliserida Pada Tikus, Univ Muhammadiyah Surakarta (2012)
16. Rissanti I., Fachriyah E. and Kusri D., Isolasi dan Identifikasi Senyawa Aktif dari Ekstrak Aseton Rimpang Bangle (*Zingiber cassumunar* Roxb.) sebagai Antioksidan, *J Kimia Sains Dan Apl*, **17**(3), 75-79 (2019)
17. Valko M., Leibfritz D., Moncol J., Cronin M.T.D., Mazur M. and Telser J., Free radicals and antioxidants in normal physiological functions and human disease, *Int J Biochem Cell Biol*, **39**(1), 44-84 (2007)
18. Yusuf F.M. and Nurkhasanah N., Evaluasi Kadar Kurkumin dalam Jamu Tradisional Kunir Asam yang Dijual di Pasar Kota Gede Bulan Februari 2015, *Pharm Sci Res*, **2**(3), 115-123 (2017).

(Received 10th June 2019, accepted 15th August 2019)