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Antihyperlipidemic and Hepatoprotective Effect of *Zingiber cassumunar* Rhizome Extract in High-Fat Diet-Induced Hyperlipidemic Rats: The Role of Antioxidant Activity

(Kesan Antihiperlipidemik dan Hepatopelindung Ekstrak Rizom *Zingiber cassumunar* pada Tikus Hiperlipidemik Disebabkan Diet Tinggi Lemak: Peranan Aktiviti Antioksidan)

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ABSTRACT

Hyperlipidemia and oxidative stress are major risk factors for the onset of cardiovascular diseases, and the oxidative stress caused by high level of lipids can cause liver damage. *Zingiber cassumunar* has been reported to contain a high antioxidant content that may provide therapeutic advantages. The present study was to evaluate the antihyperlipidemic and hepatoprotective effects of *Z. cassumunar* rhizome extract (ZCRE) in high-fat diet (HFD)-induced hyperlipidemic rats model and investigate the mechanism through its effect on the endogenous antioxidant enzymes. In this study, the rhizomes of *Z. cassumunar* was extracted using ethanol 96% (v/v) and evaporated to get the concentrated *Z. cassumunar* rhizome extract (ZCRE). Thin layer chromatography (TLC)-densitometry was performed to determine the curcumin content in the extract. High fat diet-induced hyperlipidemia model was used to evaluate the anti-hyperlipidemic and hepatoprotective activities of ZCRE in rats. Male Wistar rats were randomly divided into five groups: normal control; High fat diet-induced hyperlipidemic rats (HFD); High Fat Diet and 100 mg/kgBW of ZCRE (HFD + 100 mg/kgBW); High Fat Diet and 200 mg/kgBW of ZCRE (HFD + 200 mg/kgBW); and High Fat Diet and 400 mg/kgBW of ZCRE (HFD + 400 mg/kgBW). The antihyperlipidemic and hepatoprotective potential of ZCRE were assessed through a series of analyses of body weight, blood biochemical parameters, which include total cholesterol (TC), triglycerides (TG), the serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT). The antioxidant activity of catalase (CAT) and glutation peroxidase (GSH-Px) were assessed on the liver homogenate. Data of the study were presented as mean ± SD and analyzed by using one way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test for multiple comparisons. The TLC analysis showed that ZCRE contained a significant amount of Curcumin. In addition, the study has also shown that ZRCE was able to significantly lower the levels of total cholesterol, triglyceride, SGPT, and SGOT as compared to hyperlipidemic rats ($p < 0.05$). Concomitantly, the activity of CAT and GSH-Px was found significantly increased ($p < 0.05$) when compared to hyperlipidemic control, with the dose of 400 mg/kg BW being the most effective. This study showed the significant antihyperlipidemic and hepatoprotective effects of ZCRE in HFD-induced hyperlipidemic rats, which mechanism might possibly connect to the increased antioxidant enzyme activities.

Keywords: Antihyperlipidemic; antioxidant enzymes; catalase (CAT); glutathione peroxidase (GSH-Px); hepatoprotective; *Zingiber cassumunar*

ABSTRAK

Hiperlipidemia dan tekanan oksidatif adalah faktor risiko utama untuk permulaan penyakit kardiovaskular dan tekanan oksidatif yang disebabkan oleh tahap lipid yang tinggi boleh menyebabkan kerosakan hati. *Zingiber cassumunar* telah dilaporkan mengandungi kandungan antioksidan yang tinggi yang mungkin memberikan kelebihan terapeutik. Kajian ini adalah untuk menilai kesan antihiperlipidemik dan hepatopelindung ekstrak rizom *Z. cassumunar* (ZCRE) pada model tikus hiperlipidemik akibat diet tinggi lemak (HFD) dan mengkaji mekanisme melalui kesannya ke atas enzim antioksidan endogen. Dalam kajian ini, rizom *Z. cassumunar* diekstrak menggunakan etanol 96% (v/v) dan disejat untuk mendapatkan ekstrak rizom *Z. cassumunar* pekat (ZCRE). Kromatografi lapisan nipis (TLC)-densitometri

telah dijalankan untuk menentukan kandungan kurkumin dalam ekstrak. Model hiperlipidemia yang disebabkan oleh diet lemak tinggi digunakan untuk menilai aktiviti anti-hiperlipidemik dan hepatopelindung ZCRE pada tikus. Tikus Wistar jantan dibahagikan secara rawak kepada lima kumpulan: kawalan normal; Tikus hiperlipidemik (HFD) akibat diet tinggi lemak; Diet Tinggi Lemak dan 100 mg/kgBW ZCRE (HFD + 100 mg/kgBW); Diet Tinggi Lemak dan 200 mg/kgBW ZCRE (HFD + 200 mg/kgBW); dan Diet Tinggi Lemak dan 400 mg/kgBW ZCRE (HFD + 400 mg/kgBW). Potensi antihiperlipidemik dan hepatopelindung ZCRE dinilai melalui satu siri analisis berat badan, parameter biokimia darah yang termasuk jumlah kolesterol (TC), trigliserida (TG), serum glutamik-oksaloasetik transaminase (SGOT) dan serum glutamik-piruvik transaminase (SGPT). Aktiviti antioksidan katalase (CAT) dan glutation peroksidase (GSH-Px) dinilai pada homogenat hati. Data kajian dibentangkan sebagai min ± SD dan dianalisis dengan menggunakan analisis varians sehala (ANOVA) diikuti dengan ujian Perbezaan Ketara (LSD) untuk pelbagai perbandingan. Analisis TLC menunjukkan bahawa ZCRE mengandungi sejumlah besar Curcumin. Di samping itu, kajian juga telah mendedahkan bahawa ZRCE mampu menurunkan paras jumlah kolesterol, trigliserida, SGPT dan SGOT dengan ketara berbanding tikus hiperlipidemik ($p < 0.05$). Pada masa yang sama, aktiviti CAT dan GSH-Px didapati meningkat dengan ketara ($p < 0.05$) jika dibandingkan dengan kawalan hiperlipidemik dan dos 400 mg/kg BW adalah yang paling berkesan. Kajian ini menunjukkan kesan antihiperlipidemik dan hepatopelindung ZCRE yang ketara pada tikus hiperlipidemik yang disebabkan oleh HFD, yang mekanismenya mungkin bersambung dengan peningkatan aktiviti enzim antioksidan.

Kata kunci: Antihiperlipidemik; enzim antioksidan; katalase (CAT); glutation peroksidase (GSH-Px); hepatopelindung; *Zingiber cassumunar*

Introduction

Hyperlipidemia is one of the risk factors of cardiovascular disease (Harikumar et al. 2013). Hyperlipidemia was characterized by an increase in total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), and a decrease in high-density lipoprotein (HDL) in the blood. The main risk factor for hyperlipidemia was excessive consumption of foods high in cholesterol and saturated fats. Long-term consumption of a high-fat diet can result in hyperlipidemia (Karam, Yang & Li 2017). According to earlier research, subendothelial LDL oxidation and hyperlipidemia both contribute to atherosclerosis. Hyperlipidemia also reported an important role in inducing oxidative stress. Increasing the production of free radicals and decreasing enzymatic and non-enzymatic antioxidants are the main features of oxidative stress (Borza et al. 2013). Contrarily, numerous findings point to the advantages of antioxidant supplementation in avoiding cardiovascular disease and dyslipidemia (Amiya 2016; Nita & Grzybowski 2016).

Antioxidants are important mechanism in preventing cell damage caused by excessive free radicals. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) were involved in the protection against free radicals (Gusti et al. 2021). Superoxide dismutase is a metalloenzyme that catalyzed the radical reduction reaction of anion superoxide (O_2-) to hydrogen peroxide (H_2O_2) and oxygen (O_2). Catalase (CAT) then catalyzed the reduction reaction of the hydrogen peroxide (H_2O_2) molecule to oxygen (O_2) and water (H_2O). Glutathione peroxidase (GSH-Px) will catalyze the reduction reaction of H_2O_2 into organic hydroperoxide (ROOH) compounds. Many experimental reports have determined antioxidant activity to determine free radical damage and oxidative stress (Valko et al. 2006).

Bangle (*Zingiber cassumunar*) rhizome has been used as a traditional medicine in Indonesia to treat various diseases, such as headaches, fevers, constipation, asthma, rheumatism, muscle aches, and fat sloughing. Phytochemical studies have shown that *Z. cassumunar* rhizome contains phenolic groups, flavonoids, curcuminoids (Han et al. 2021), and essential oils (Bhuiyan, Chowdhury & Begum 2008). The antioxidant activity of *Z. cassumunar* has been shown *in vitro* by DPPH method and showed a potential antioxidant (Han et al. 2021; Joram et al. 2018; Marliani, Rahmawati & Sinurat 2014).

However, until now, no extensive studies have been conducted on the antihyperlipidemic and hepatoprotective effects of *Z. cassumunar* rhizome extract in the high-fat diet-induced rats. Considering this, the current study was carried out to examine the antihyperlipidemic and hepatoprotective properties of *Z. cassumunar* rhizome extract in HFD-induced rats as well as endogenous antioxidant enzymes activities that might underlying the mechanism of action of *Z. cassumunar*.

Materials and Methods

Chemicals and Reagents

Reagents for the detection of TC, TG, SGOT, and SGPT levels were purchased from Diasys, Germany, while assay kits for catalase (CAT) and glutathione peroxidase

(GSH-Px) were acquired from Elabscience Biotechnology Inc., USA.

PREPARATION OF *Z. cassumunar* RHIZOMES EXTRACT

Rhizomes of *Z. cassumunar* were collected from the local market, Yogyakarta, Indonesia, and the plant was identified at the Biology Laboratory, Universitas Ahmad Dahlan. *Z. cassumunar* rhizomes were oven dried with 50 °C temperature and ground into a powder form by using a grinder. The powder was macerated in 96% ethanol with ratio 1:3 (w/v) for 24 hours at room temperature. The extract was concentrated under reduced pressure by a vacuum at 50 °C, followed by evaporation in the fume hood to yield an ethanol extract.

ANALYSIS OF CURCUMIN *Z. cassumunar* EXTRACT CONTENT BY TLC-DENSITOMETRY

The 100 mg of *Z. cassumunar* rhizome extract was transferred to a volumetric flask 10.0 mL and diluted with ethanol. Standard solution for curcumin was prepared in ethanol at a series of concentration of 0.3, 0.6, 0.9, 1.2, and 1.5 mg/mL. Five microliters of each concentration of curcumin solution and *Z. cassumunar* extract were spotted onto the TLC silica gel 60 F254 (10 × 10 cm) plate. The plate was eluted using a mobile phase, which composed of chloroform: ethanol: glacial acetate acid (94:5:1, v/v/v). To determine the curcumin content, the developed TLC plate was subjected to densitometric scanning, which was performed using Camag TLC Scanner 4 at 425 nm. The amount of curcumin present in *Z. cassumunar* extract was calculated using the linear regression of curcumin standard (Yusuf & Nurkhasanah 2015).

EXPERIMENTAL ANIMALS

Twenty-five (25) male Wistar albino rats weighing 150-200 g were purchased from Universitas Sanata Dharma Laboratory. The rats were acclimatized for 7 days to the animal house before treatment. During the study, the rats were allowed to access food and water *ad libitum* and were maintained on a 12-h light/12h dark cycle at room temperature (25 ± 2 °C) with a controlled relative humidity (40-50%). The Research Ethical Committee of Universitas Ahmad Dahlan has authorized all of the study's protocols, and their approval number is 011904025.

PREPARATION OF HIGH FAT DIET

Hyperlipidemia was induced in twenty (20) experimental rats by feeding a high-fat diet (HFD). The HFD formula, the percentage of carbohydrate, protein and fat as well as the are listed in Table 1. All of the ingredients were mixed and moulded in the shape of animal food pellets. The pellets were then dried in the oven at 80 °C for 2 h, after which were cooled at room temperature (Rini 2012).

ANIMAL TREATMENT

The experimental animals were divided into five groups of five rats each at random. The sample size was calculated using the Federer formula and the optimum number of samples in each group was ≥4.75 (Ihwah et al. 2018). The *Z. cassumunar* extract was diluted in CMC-Na 0.5% solution to assure the homogeneity. The experimental used was as follows: Group I: normal control group, received CMC-Na 0.5%; Group II: HFD induced hyperlipidemic rats, treated with CMC-Na 0.5%; Group III, IV and V were HFD-induced hyperlipidemic rats and treated with ZCRE at doses 100, 200 and 400 mg/kg BW, respectively.

The CMC-Na 0.5% was given in the control group, as the CMC-Na is a solvent to dilute the ZCRE before treatment. The condition of hyperlipidemia was induced in all the groups except normal control group along the study. The administration of *Z. cassumunar* extract was done orally by oral gavage starting on day 15th and continued for two weeks. Throughout the experiment, the rats' body weight gain was monitored weekly and the food intake was monitored daily.

TABLE 1. The composition of high fat diet and the calorie

Composition of HFD	weight (g)	Composition per 100 g			Total calorie
		Carbohydrate	Protein	Fat	
BR II standard feed	300	181.5	57	12	1062
Chicken egg yolk	20	3.6	16	27	64.2
Butter	100	1.4	0.5	81.6	742
Beef fat	10			100	90.2
Propylthiouracil (PTU)	0.21				
Total	430	186.5	73.5	220.6	1958.4
Composition per 100 g of HFD		43.37	17.09	51.30	455.44

COLLECTION AND TREATMENT OF BLOOD SAMPLES

Twenty-four (24) h after the last treatment, the blood samples were collected from orbital sinus using capillary tubes. The blood was then centrifuged and serum was separated. The serum was used for biochemical (total cholesterol, triglycerides, SGOT and SGPT) analysis. Animals were then slaughtered, and the liver was removed for an examination of antioxidants.

PREPARATION OF TISSUE HOMOGENATES

The liver tissue was divided into small pieces, weighed and homogenized with Phosphate Buffer Saline (PBS) (Sigma-Aldrich) 0.01 M, pH 7.4 on ice. The ratio of PBS volume (mL) to the weight of the tissue (g) was 9:1. After that, the tissue homogenate was centrifuged for 15 min at 3000 rpm. Supernatants were maintained frozen until required for further analysis.

LIPID PROFILE ANALYSIS

The total cholesterol (TC) and triglycerides (TG) were determined using Diasys® kit and followed the manufacturer's instructions. Measurement of total cholesterol levels using the enzymatic photometric test cholesterol oxidase-peroxidase aminoantipyrin (CHOD-PAP) method. The quantitative analysis was carried out using a spectrophotometric method at 546 nm.

As for the measurement of triglyceride levels, it was carried out using the enzymatic photometric test glycerol-3 phosphate oxidase-phenol aminoantipyrin (GPO-PAP) method, and the quantitative analysis was carried out by spectrophotometric method at 546 nm.

DETERMINATION OF SERUM LEVELS OF SGOT AND SGPT

The SGPT and SGOT levels were determined using the Diasys® kit following the manufacturer's instruction by kinetic method. The 100 µL of blood serum was added by 1 mL of reagent 1 and then incubated at 37 ºC for 5 min, then added 250 µL of reagent 2. The absorbance of mixture was read 4 times at a wavelength of 365 nm, every 1 min.

MEASUREMENT OF THE LEVELS OF CATALASE (CAT)

The analysis of CAT activity was carried out using a catalase assay kit (Elabsicience, E-BC-K031). This method was based on catalase (CAT) decomposed by H_2O_2 that can be quickly stopped by ammonium molybdate. A yellowish complex was produced by the reaction of the remaining H_2O_2 with ammonium molybdate. A UV-Vis spectrophotometer (Shimadzu 1700 PC) can be used to determine CAT activity by measuring the formation of the yellowish complex at 405 nm.

MEASUREMENT OF THE LEVELS OF GLUTATHIONE PEROXIDASE (GSH-Px)

A glutathione peroxidase (GSH-Px) assay kit was used to measure the GSH-Px activity (Elabsicience, E-BC-K096). GSH-Px is a glutathione peroxidase that can facilitate the reaction of hydrogen peroxide (H_2O_2) with reduced glutathione to form H_2O and oxidized glutathione, which is the basis for the GSH-Px activity assay (GSSG). The rate of the enzymatic reaction can be used to express the glutathione peroxidase's activity. The consumption of reduced glutathione can be used to calculate the activity of glutathione. Since reduced glutathione can react with hydrogen peroxide (H_2O_2) without the help of GSH-Px, the amount of GSH reduced through non-enzymatic means should be deducted. A UV-Vis spectrophotometer (Shimadzu 1700 PC) can measure the steady yellow color of the 5-thio-dinitrobenzoic acid anion, which is produced when GSH reacts with dinitrobenzoic acid to determine the change in GSH level.

STATISTICAL ANALYSIS

Statistical analysis was carried out using the SPSS software (Version 22). One-way analysis of variance (ANOVA) was used to evaluate the data, and the Least Significant Difference (LSD) test with the significant level of 95% was used to determine whether there were any differences in the group means.

RESULTS

DETERMINATION OF CURCUMIN CONTENT IN *Z. cassumunar* RHIZOME EXTRACT

Results from the TLC-densitometry analysis shows that extract of *Z. cassumunar* rhizome contains curcumin. Curcumin is one of the main compositions in *Z. cassumunar* rhizome extract. Qualitative and quantitative are performed to determine the curcumin content in *Z. cassumunar* rhizome extract. The present study indicates that *Z. cassumunar* rhizome extract contains curcumin compounds (Figure 1), the retardation factor (Rf) of curcumin is 0.75 equal with the curcumin standard. The concentration of curcumin is 5.56 ± 1.97%. Curcumin is considered as major bioactive compounds in *Z. cassumunar* rhizome, which possesses potential effects as an antioxidant. This finding was met with previous studies that reported the curcuminoids content in the *Z. cassumunar* rhizome (Rafi et al. 2011).

Z. cassumunar EXTRACT INHIBITS THE INCREASE OF BODY WEIGHT IN HIGH-FAT-DIET RAT

After four weeks of treatment, all rats gained weight, as shown in Figure 2. The bodyweight of rats in the HFD

control group has increased significantly, higher than in the normal group (p=0.003). Treatment of *Z. cassumunar* rhizome extract significantly reduced the average of weight gain (Table 2). Previous research also reported rat body weight increased highly after 2 weeks of feeding with HFD (Mahfudh et al. 2021). Consumption of HFD will continuously lead to an increase in visceral fat deposits to obesity and hyperlipidemia (Hariri & Thibault 2010). These data were in agreement with the results of the increased levels of total cholesterol and triglyceride (Table 3). This study proved that *Z. cassumunar* can inhibit excessive weight gain in rat fed a HFD. The average weight gain of treated group was decreased in dose dependent, but statistically not significant (p>0.05).

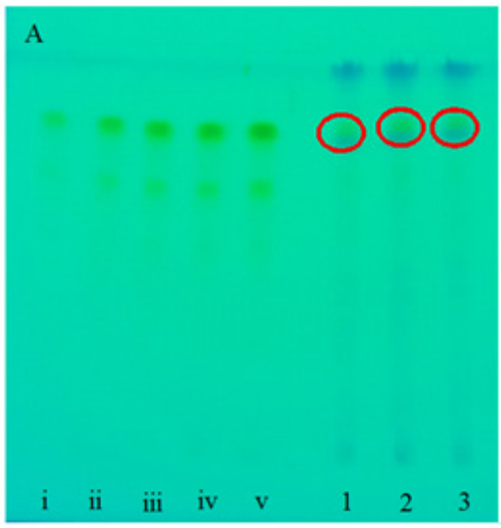

FIGURE 1. The TLC profile of *Z. cassumunar* extract using curcuminoids as a reference under UV 254 nm. i-v were curcuminoids standard; 1-3: *Z. cassumunar* extract

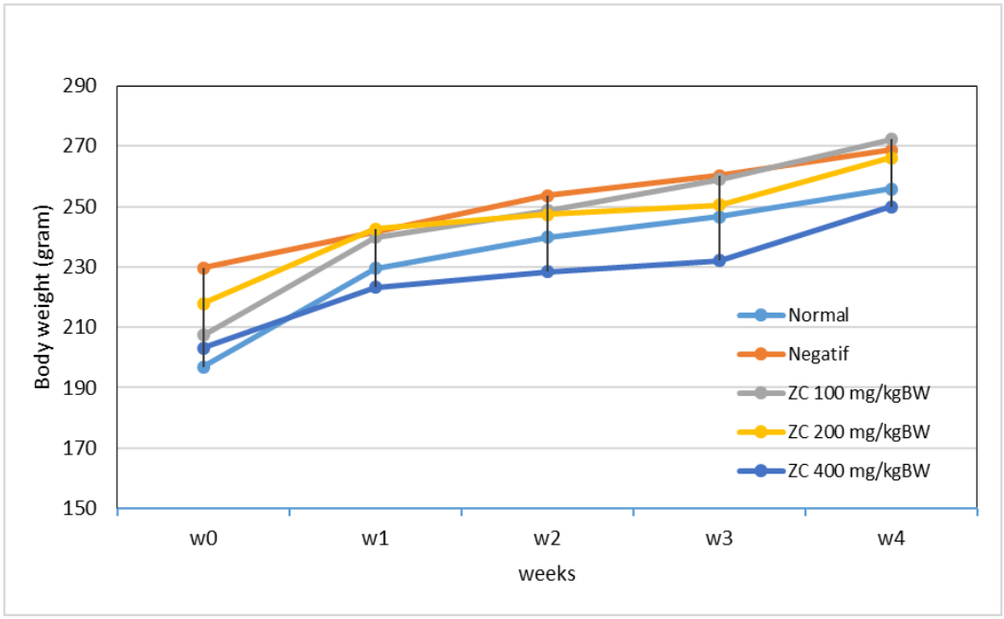

FIGURE 2. The increasing bodyweight of animal rats induced by high fat diet and treated by *Z. cassumunar* extract

DECREASING OF TOTAL CHOLESTEROL AND TRIGLYCERIDE

Z. cassumunar extract treatment resulted in a significant decrease in total cholesterol and triglycerides level in hyperlipidemic rats compared to the negative control (Table 3). Previous studies showed that after four weeks of treatment, *Z. cassumunar* extract decreased LDL levels (Paramita et al. 2019).

THE SGPT AND SGOT LEVEL

The present study found that HFD induction could increase the SGOT and SGPT activity level significantly (Table 4), and the treatment of ZCRE was reduced the SGOT and SGPT level significantly.

TABLE 2. The effect of *Z. cassumunar* extract treatment in high fat diet induced rat on rat weight gain

Group	Initial bodyweight (g), Mean (SD)	Final bodyweight (g), Mean (SD)	Weight gain (g/week)
Normal	229.6 (30.84)	255.8 (17.12)	9.8 (4.77)
Negative control	196.8 (28.77)	272.2 (20.11)	16.2 (3.12)[a]
ZCRE 100 mg/kg BW	207.4 (13.33)	268.8 (6.97)	14.75 (2.99)
ZCRE 200 mg/kg BW	217.8 (18.14)	266.2 (34.68)	12.1 (5.22)
ZCRE 400 mg/kg BW	203.2 (21.24)	250 (26.61)	11.7 (4.38)

Note: [a] = significant difference compared to the normal group (p =0.003), data was found from 5 replicates each groups

TABLE 3. The total cholesterol and triglyceride level of high fat diet induced rat treated by *Z. cassumunar* extract

Group	Cholesterol (mg/dL) Mean (SD)	Triglyceride (mg/dL) Mean (SD)
Normal	42.56 (9.16)[a]	58.04 (6.79)[a]
Negative control	109.17 (11.99)[b]	186.99 (9.69)[b]
ZCRE 100 mg/kg BW	84.21 (12.60)[ab]	186.38 (14.15)[a]
ZCRE 200 mg/kg BW	80.75 (11.36)[ab]	103.31 (12.43)[ab]
ZCRE 400 mg/kg BW	43.01 (10.14)[b]	55.34 (14.80)[b]

Data was found from 5 replicates each group. a = compared to the normal group, there is a significant difference ($p < 0.05$), b = compared to the negative control group, there is a significant difference ($p < 0.05$)

TABLE 4. The SGOT and SGPT level in HFD induced rat and treated by *Z. cassumunar* rhizome extract

Group	SGPT (U/L) Mean (SD)	SGOT (U/L) Mean (SD)
Normal	21.67 (6.12)	65.51 (12.12)
Negative control	49.82 (11.80)[a]	93.82 (14.94)[a]
ZCRE 100 mg/kg BW	40.98 (13.44)	44.93 (13.57)[b]
ZCRE 200 mg/kg BW	37.02 (11.53)	52.12 (20.24)[b]
ZCRE 400 mg/kg BW	31.63 (1.25)[b]	52.48 (8.17)[b]

Data was found from 5 replicates each groups. a = compared to the normal group, there is significant difference ($p < 0.05$), b = compared to the negative control group, there is significant difference ($p < 0.05$)

INCREASING OF CAT AND GSH-PX ACTIVITY

The endogenous antioxidant activity was found to decrease ($p<0.05$) in the HFD induced hyperlipidemic rat and found to increase ($p<0.05$) in the ZCRE treated groups as presented in Table 5.

DISCUSSION

Hyperlipidemia is characterized by an elevation of any or all lipid profiles or lipoproteins in the blood (Gadde et al. 2018). A lifestyle diet rich in calories, fat, and cholesterol plays a vital role in causing hyperlipidemia. A high-fat diet (HFD) as a major cause of developing this complication (Barkas et al. 2020). The previous studies reported that HFD can induce dyslipidemia in animals and increase the expression of genes related to lipid synthesis. The rat given HFD experienced a very significant increase in total cholesterol and triglyceride levels (Zhang et al. 2018). Some previous studies have reported the potential of medicinal plants to develop as anti-hyperlipidemia.

The research found that high-fat diet-induced rats had significantly higher serum levels of both total cholesterol and triglycerides (Table 3). The hyperlipidemia effect may be ascribed to the high-fat diet. The increase of serum total cholesterol and triglyceride, which was caused by the consumption of high-fat, will increase acetyl-CoA levels as the lipid catabolism will lead to the formation of acetyl-CoA, the intermediate metabolic of cholesterol biosynthesis. The induction of HFD increases the serum total cholesterol ($p=0.000$) and triglyceride ($p=0.000$) significantly. It is caused by excessive fat accumulation due to HFD (Md Abdullah et al. 2019). The increase of serum total cholesterol and triglyceride is an important indicator of a successful hyperlipidemic rats model. Curcumin one of the main compound in this extract was reported to have hypocholesterolemic through the activation of cholesterol 7a-hydroxylase (CYP7A1), a rate limiting enzyme in the biosynthesis of bile acid from cholesterol, at the mRNA level (Kim & Kim 2010).

The previous research demonstrated that high-fat diet can result in liver dysfunction, one of which is the accumulation of fat in the liver's hepatocytes (fatty liver disease) (Kumar et al. 2019; Md Abdullah et al. 2019) and can cause the fatty liver disease like steatohepatitis which is caused by an imbalance between the liver's production and secretion of triglycerides. Overproduction of fatty acids in the liver, which is the most frequent cause of fatty liver accumulation. It will cause the creation of free radicals, which may result in the necrosis of hepatocytes and other types of liver damage, increasing the activity of the SGOT and SGPT enzymes, a sign of liver damage (Widarti & Nurqaidah 2019).

The current study discovered that SGOT and SGPT activity levels might be elevated by HFD induction (Table 4). Due to the accumulation of lipids in the liver, the increase in SGOT and SGPT led to the production of free radical species that damaged the liver (Rindler et al. 2013). This effect might be related to the curcumin content in the *Z. cassumunar* extract. Curcumin previously reported as hepatoprotective agent against paracetamol (Tung, Hai & Son 2017) and carbon tetrachloride (CCl_4) (Ibrahim et al. 2020) induced hepatotoxicity. Some phenylbutanoids compounds have been successfully elucidated form *Z. cassumunar* including cassumunols I-M (Nakamura et al. 2022). Some chemical content from essential oil of *Z. cassumunar* extract was also reported including sabinen, terpinen, terpinen-4-ol, and (E)-1-(3,4-dimethoxyphenyl) butadiene (Sukatta et al. 2009). These compounds have been reported to have some pharmacological effect including anti-inflammatory effect (Ramadhan, Mahfudh & Sulistyani 2020), inhibition of nitric oxide production (Adhila, Nurkhasanah & Sulistyani 2019).

TABLE 5. The increase of CAT and GSH-Px activity in *Z. cassumunar* extract treated in hyperlipidemic rat

Group	CAT activity (U/mgprot) Mean (SD)	GSH-Px activity (U/mgprot) Mean (SD)
Normal	50.87 (3.87)^{b}	52.68 (1.8)^{b}
Negative control	21.07 (3.86)^{a}	24.17 (6.5)^{a}
ZCRE 100 mg/kg BW	31.56 (5.01)^{ab}	29.27 (2.8)^{a}
ZCRE 200 mg/kg BW	65.97 (5.75)^{ab}	43.86 (7.1)^{ab}
ZCRE 400 mg/kg BW	81.55 (11.78)^{ab}	59.10 (5.5)^{b}

Data was found from 5 replicates each groups. a = compared to the normal group, there is significant difference ($p<0.05$), b = compared to the negative control group, there is significant difference ($p<0.05$)

Hyperlipidemia increases reactive oxygen species (ROS) and leads to oxidative stress (Sikder et al. 2018). The present study found that HFD induction decrease the Catalase (CAT) and Gluthation peroxidase (GSH-Px) activity significantly ($p<0.05$). Treatment with *Z. cassumunar* extract could increase the CAT ($p=0.035$) and GSH-Px activity ($p=0.041$) significantly in dose-dependent manner.

Oxidative stress is the imbalance between the production and accumulation of reactive oxygen species (ROS) (Nita & Grzybowski 2016). Under normal conditions, intracellular ROS is physiologically required at low levels. Oxidative stress caused by a HFD has been shown in experimental models and patients with clinical conditions. The levels of malondialdehyde products of lipid peroxidation products, nitric oxide, and advanced protein oxidation products were increased in rat fed a HDF (Lasker et al. 2019). Due to increased generation of free radicals and impaired antioxidant capacity, notably the suppression of antioxidant enzymes, hyperlipidemia is linked to increased oxidative stress. The hyperlipidemic condition is associated by decreased antioxidant enzyme activity and glutathione levels, which correlate with increased levels of MDA, as oxidative metabolic product (Karam et al. 2017). Decreased antioxidants caused by impaired metabolism or poor lifestyle (e.g., consumption of HFD) can lead to cancer, neurodegeneration, cardiovascular disease, diabetes or kidney disease (Lasker et al. 2019).

Hyperlipidemia increased leptin levels also promoted inflammation reactions and increase in cytokine-induced of NADPH oxidase activation. This condition increases the ROS production which turn to oxidation of lipids and proteins (Yang et al. 2008). Several reports have shown the alterations in the antioxidant enzymes during hyperlipidemia condition. The anti-oxidative defense system including SOD, CAT, and GSH-Px, showed lower activities during hyperlipidemia (Kumar et al. 2015). Antioxidant will help to restore the imbalance.

Several medicinal plant have been reported to have strong antioxidants effect as exogenous antioxidants (Skowron et al. 2018). In the present study, *Z. cassumunar* extract act as exogenous antioxidant which help the endogenous antioxidant to scavenge free radical species produced by hyperlipidemic condition. The previous study reported that SOD activity was decreased in HFD fed rat. The increased generation of ROS in this study was the reason for the decline in the SOD enzyme activity. However, SOD enzyme activity was increased in HFD rat treated by *Z. cassumunar* extract (Sari, Nurkhasanah & Sulistyani 2020).

In the present study, it is proven the decrease in the CAT and GSH-Px activity in hyperlipidemia rat induced by a HFD (Table 5). The decrease in antioxidant enzymes is associated with an increase in ROS production. The increasing of ROS is caused by high levels of LDL in hyperlipidemia conditions that will be easily oxidized (ox-LDL), resulting in oxidative stress. Hyperlipidemia conditions increase NADPH oxidase 4 (NOX4) which plays an important role in the formation of H_2O_2. The excessive formation of H_2O_2 will decrease CAT and GSH-Px activity (Landmesser et al. 2000). H_2O_2 is resulted by decomposition of the superoxide (O_2^-) compound by the SOD enzyme. H_2O_2 compounds are non-radical compounds but can be transformed into hydroxyl radicals (OH^-) by reduced metal anions (Fe/Cu) (Halliwell 2015).

Z. cassumunar is thought to be used as a source of antioxidants. Several studies have proven the antioxidant activity of Bengle rhizomes using the DPPH test. The ethanol extract of bangle rhizome (*Zingiber cassumunar* Roxb.) has the effect of free radical scavengers (Marliani, Rahmawati & Sinurat 2014).

Catalase is an enzyme that will catalyze the decomposition of hydrogen peroxide, a reactive oxygen species, which is a toxic product of normal aerobic metabolism. CAT activity was found to decrease significantly ($p<0.05$) in HFD induced rat. Increased CAT activity in the *Z. cassumunar* extract group showed that extract had antioxidant activity and was able to improve the effects of ROS in biological systems. CAT catalyze the breakdown of H_2O_2 into H_2O and O_2. The H_2O_2 can be converted to water by catalase up to a certain point. The catalase's capacity to function will be diminished by high levels of H_2O_2. The rate of enzyme activity will slow down due to an overabundance of substrate. The presence of *Z. cassumunar* maintains the rate and ability of CAT in reducing H_2O_2 (Rivera-Mancia et al. 2018).

One of the enzymes responsible for preserving glutathione in its reduced state is glutathione peroxidase (GSH-Px). Glutathione is crucial for preserving healthy function and reducing oxidative stress in cells. It can function as different electrophiles, singlet oxygen, and hydroxyl radical scavengers. The GSH-Px enzyme catalyzes the reduction of H_2O_2 or oxygen hydroperoxide (ROOH) to water (H_2O) and alcohol (ROH) (Zhan et al. 2004).

Z. cassumunar contains flavonoid compounds, curcuminoids, and essential oils, which are known as potential compounds as antioxidants. The content of curcumin is one of the main compounds in *Z. cassumunar*

rhizome extract (Figure 1). Curcumin has previously reported to have the effect of capturing free radicals. Curcumin could act antioxidant by inhibiting ROS formation, ROS scavenging and increasing antioxidant response. Curcumin has been reported to increase CAT, SOD, and GSH-Px activity for scavenging ROS (Lin et al. 2019). Curcumin is known to give antioxidant and immunostimulant effects by increasing T-cell proliferation, natural killer (NK) cell activity, and the activity of NO (Ramadhan, Mahfudh & Sulistyani 2020). The Nrf2 gene's induction process results in increased regulation (Nuclear factor-erythroid 2-related factor-2). The antioxidant pathway will be initiated when Nrf2 translocates from the cytoplasm into the nucleus. The activity of the enzymes NADPH oxidase, lipoxygenase or cyclooxygenase, xanthine dehydrogenase, and nitric oxide synthase, as well as cellular activity that results in ROS, are also inhibited by curcumin, which increases the bioavailability of cellular antioxidant enzymes (Rivera-Mancia et al. 2018).

Previous studies of curcumin has been reported the properties of rapid metabolism and low bioavailability in oral administration (Dei Cas & Ghidoni 2019). Some strategy in formulation were needed to give a higher concentration in the blood, and will give a better effect including. The co-administration of curcumin with other compound such as piperin could increase the bioavailability (Hegde et al. 2023). The absorption of curcumin was found to increase in mixture with lecithin (Cuomo et al. 2011). Lecithin can be found in various foodstuffs such as eggs and nuts. It was recommended to formulate the *Z. cassumunar* extract in the form of combination with other material.

Conclusions

The study found that *Z. cassumunar* rhizome extract significantly lowered the serum total cholesterol (TC) and triglyceride (TG) levels. The SGPT and SGOT level was also found to decrease, indicating that the hepatoprotective effect of *Z. cassumunar* extract in high-fat diet induced rat. The antihyperlipidemic and hepatorotective effect of *Z. cassumunar* rhizome extract is considered by its antioxidant activity which presented by increasing of antioxidant enzymes CAT and GSH-Px activity following the *Z. cassumunar* rhizome extract treatment.

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