

# HASIL CEK\_Sulistyani\_Catalase (CAT); glutathione peroxidase (GSH-Px); malondialdehyde (MDA); superoxide dismutase (SOD); sweet potato leaves

*by Nanik Sulistyani Sweet Potato (ipomoea Batatas L.) Leaves Ethanol*

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## Sweet Potato (*Ipomoea batatas* L.) Leaves Ethanol Extract Increases Endogenous Antioxidant Activities in Hyperlipidemic Rats

(Ekstrak Etanol Daun Ubi Keledek (*Ipomoea batatas* L.) Meningkatkan Aktiviti Antioksidan Endogen pada Tikus Hiperlipidemik)

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### ABSTRACT

Consumption of high-fat foods can induce the formation of free radicals and trigger oxidative stress. The leave of sweet potato (*Ipomoea batatas* L.) has been reported to be a potent antioxidant *in vitro*. This study investigates the effect of ethanol extract of purple sweet potato leaves on the endogenous antioxidant activity in hyperlipidemic rats. The sweet potato leaves were oven-dried and extracted using 70% ethanol in a ratio of 1:7 w/v for 24 h, followed by vacuum evaporation. The five groups of test animals were created: normal control-which was given a standard diet, negative control (high-fat diet (HFD) for 14 days), and three treated groups which treated with HFD for 14 days and start for the same time treated with sweet potato leaves extract (SPLE) at the doses of 100, 200, and 400 mg/kg BW for 28 days. The antioxidant activity was measured from liver homogenate on the 29th day. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) activities, and malondialdehyde (MDA) levels were quantified using spectrophotometry. The high-performance liquid chromatography (HPLC) analysis found rutin as a major chemical content of the SPLE. HFD significantly ( $p < 0.05$ ) decreased the activity of endogenous antioxidants (SOD, CAT, and GSH-Px) and increased the MDA level significantly ( $p < 0.05$ ) compared with the normal group. On the contrary, the treatment with the SPLE significantly ( $p < 0.05$ ) increased the activity of SOD, CAT, and GSH-Px and lowered MDA levels significantly ( $p < 0.05$ ) in a dose-dependent manner compared to the negative control. SPLE increase the activities of endogenous enzymatic antioxidants in hyperlipidemic rats.

Keywords: Catalase (CAT); glutathione peroxidase (GSH-Px); malondialdehyde (MDA); superoxide dismutase (SOD); sweet potato leaves

### ABSTRAK

Pengambilan makanan yang tinggi lemak boleh mendorong kepada pembentukan radikal bebas dan mencetuskan tekanan oksidatif. Daun ubi keledek (*Ipomoea batatas* L.) telah dilaporkan sebagai antioksidan yang kuat secara *in vitro*. Penyelidikan ini mengkaji tentang kesan ekstrak etanol daun ubi keledek ke atas aktiviti antioksidan endogen dalam tikus hiperlipidemik. Daun ubi keledek telah dikeringkan dengan ketuhar gelombang dan diekstrak menggunakan etanol 70% dalam nisbah 1:7 w/v selama 24 jam, diikuti dengan penyejatan vakum. Lima kumpulan haiwan ujian telah disediakan: kawalan normal iaitu yang diberi diet piawai, kawalan negatif (diet tinggi lemak (HFD) selama 14 hari) dan tiga kumpulan terawat yang dirawat dengan HFD selama 14 hari dan dirawat dengan ekstrak daun ubi keledek (SPLE) pada dos 100, 200 dan 400 mg/kg BW selama 28 hari. Aktiviti antioksidan diukur daripada homogenat hati pada hari ke-29. Aktiviti superoksida dismutase (SOD), katalase (CAT), glutathione peroksidase (GSH-Px), dan tahap malondialdehid (MDA) dihitung menggunakan spektrofotometri. Analisis kromatografi cecair

(HPLC) berprestasi tinggi mendapati terdapat kandungan kimia utama iaitu SPLE. HFD secara signifikan ( $p < 0.05$ ) menurunkan aktiviti antioksidan endogen (SOD, CAT & GSH-Px) dan meningkatkan tahap MDA dengan ketara ( $p < 0.05$ ) berbanding kumpulan normal. Sebaliknya, rawatan dengan SPLE ( $p < 0.05$ ) meningkatkan aktiviti SOD, CAT dan GSH-Px serta menurunkan tahap MDA dengan ketara ( $p < 0.05$ ) bergantung kepada dos berbanding kawalan negatif. SPLE meningkatkan aktiviti antioksidan enzim endogen pada tikus hiperlipidemik.

Kata kunci: Daun keladi; glutathion peroksidases (GSH-Px); katalases (CAT); malondialdehid (MDA); superoksida dismutases (SOD)

## INTRODUCTION

Accumulation of fat will lead to cardiovascular and other degenerative diseases (Kumar et al. 2015; Noori 2012). Hyperlipidemic blood triggers modification of low-density lipoprotein (LDL) into its oxidized form (ox-LDL), which can increase reactive oxygen species (ROS) and cause oxidative stress (Halder & Bhattacharyya 2014). The oxidative stress could cause the accumulation of lipid peroxidation products, such as malondialdehyde (MDA), and reduces endogenous antioxidants, such as superoxide dismutase (SOD) (Barkas et al. 2020).

The body has a natural defense system against free radical molecules because of their potential toxicity in the form of endogenous antioxidant enzymes, namely superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT). They prevent damage to macromolecular components in cells by neutralizing and speeding up the breakdown of free radical molecules (Valko et al. 2007). SOD works by catalyzing the dismutation of superoxide into hydrogen peroxide ( $H_2O_2$ ) (Birben et al. 2012). Then, CAT neutralizes the resultant  $H_2O_2$  by catalyzing its decomposition reaction into  $H_2O$  and  $O_2$  (Kodydková et al. 2014; Switala & Loewen 2002). However, the activities of these intracellular antioxidant enzymes can decrease due to oxidative stress (Araujo et al. 1995; Halder & Bhattacharyya 2014).

Purple sweet potatoes (*Ipomoea batatas* L.) are widely grown root crops in the worldwide for their nutritional benefits. Many scholars have reported the antioxidative and free-radical scavenging activities of the tuberous roots that help prevent various types of degenerative diseases. The leaves contain several nutrients and phytochemical compounds, such as fiber, vitamin C, flavonoids, terpenoids, saponins, polyphenols, tannins, and alkaloids (Islam 2006; Meira et al. 2012; Rumbaoua et al. 2009).

For these reasons, the pharmacological effect of purple sweet potato (*Ipomoea batatas* L.) leaves is fascinating to study. According to previous reports,

treatments with the leaf extract can lower cholesterol and triglyceride levels while increasing plasma high-density lipoprotein (HDL) in diabetic rats (Heriwijaya et al. 2020; Kurata et al. 2017). Furthermore, high-fat diet induced rat treated with sweet potato leaves for 35 days has been found to affect lipid metabolism (Kurata et al. 2017). The leaves also prove effective in decreasing oxidative stress in hyperlipidemic rats (Chen et al. 2011). The objective of the present study was to explore the effect of purple sweet potato leaf extract in increasing the antioxidant activity in hyperlipidemic rats.

## MATERIALS AND METHODS

### MATERIALS

The leave of sweet potato used in this study were collected from Magelang, Central Java, Indonesia. The sample was authenticated and deposited at Universitas Ahmad Dahlan's Laboratory of Biology, with an identification number of 135/Lab.Bio/VIII/2019. The Wistar rats tested in this study were purchased from the Integrated Research Laboratory, Universitas Gadjah Mada. The reagents used in the endogenous antioxidant analysis were purchased from Elabscience, namely SOD Elabscience assay kit (E-BC-K022-S), CAT Elabscience assay kit (E-BC-K031-S), GSH-Px Elabscience assay kit (E-BC-K096-S), and MDA Elabscience assay kit (E-BC-K025-S). The solvent used in the high-performance liquid chromatography (HPLC) analysis was methanol (E-Merck, analytical grade).

### EXTRACTION

The leaves were removed from the stem and rinsed in water. The leaves were dried using oven with 50 °C temperature and then crushed using a blender. The sweet potato leaf powder was sifted using a 40-mesh sieve. Afterward, a powder sample weighing 400 g was extracted using 70% ethanol in a ratio of 1:7 w/v for 24 h by maceration. To get a concentrated extract, the filtrate

was evaporated using a vacuum rotary evaporator at a temperature of 60 °C (Mahfudh et al. 2021).

#### HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

The sweet potato leaf extract (SPLE, 10 µL) was analyzed in an HPLC system (Shimadzu LC2010) using a C18 column (Shim-pack), methanol as the mobile phase, and flow rate of 1 mL/min. The UV detector was set at 370 nm wavelength. For comparison, rutin and standard quercetin solutions of approximately 10 µL were used.

#### ETHICAL APPROVAL

The Research Ethics Committee of Universitas Ahmad Dahlan have accepted the animal handling protocol, which has the reference number 011904031. The test animals were two-month-old male Wistar rats, weighing between 130 and 150 g. They were acclimatized for seven days and administered standard brailer-II (BR II) pellets prior to the treatment. The BR II contain corn, soybean meal, wheat pollard, coconut meal, fish meal, meat meal, rice flour, tapioca, coconut oil, and fish oil premix. The nutrition content of BR II is protein 19.0-20.0%, lipid 5%, carbohydrate 50%, and the calorie are 310 cal/100 gram. During the experiment, the animals

2 were kept in a well-ventilated room with a light cycle (12 hours light and 12 hours dark), humidity and room temperature were maintained. The number of animals is 5 animals in each cage.

#### 1 PREPARATION OF THE HIGH-FAT DIET

The high-fat diet was prepared by mixing 300 g of regular food BR II, 20 g of chicken egg yolk, 100 g of butter, 10 g of beef fat, and 0.05% propylthiouracil (PTU). After carefully mixing all of the ingredients, pellet were formed and dried in a 50 °C oven (Sari et al. 2020). The nutrition content of HFD are carbohydrate 36.57%, protein 14.41% and lipid 43.25%. The calorie of HFD was 434.35 cal/100 gram.

#### EXPERIMENTAL DESIGN

The extract was homogenized by suspending it in 1% natrium carboxy methyl cellulose (CMC Na). The suspension of sample was prepared daily before treatment, to maintain from microbe contamination. The rats were divided into five groups, each consisting of five randomly selected rats. The number of animals in each groups were calculated using Federer formula (Ihwah et al. 2018). The treatment duration is described in Figure 1, and the group dividing of animal treatment were presented in Figure 2.

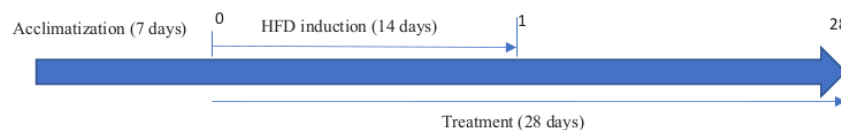


FIGURE 1. Desain for HFD induction and treatments

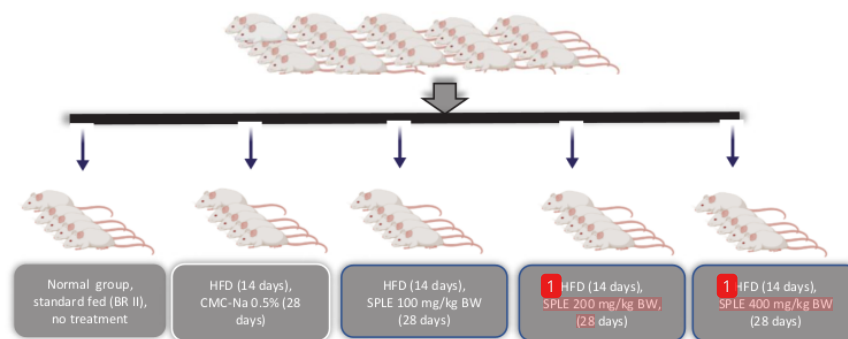


FIGURE 2. The group dividing of animal treatment

The body weight was monitored weekly. The test rats were anesthetized using carbon dioxide (CO<sub>2</sub>) gas on the 29<sup>th</sup> day (Canadian Council of Animal Care 2010), and an abdominal incision was made to extract the liver organ. Finally, after the extraction of the liver organ, they were sacrificed using an incinerator.

#### SAMPLE PREPARATION

The liver tissue (1 g) was sliced and added with 9 mL of phosphate buffer (pH 7.4), then homogenized in an ice bath using pestle and centrifuged at 10000 rpm and 4 °C for 10 min. SOD, CAT, and GSH-Px activity assays as well as MDA level analysis, were performed on the supernatant.

#### PROTEIN LEVEL DETERMINATION

The Bradford protein assay was used to determine the protein concentration in the liver homogenate (Walker 1996). Approximately 0.1 mL of the supernatant was added with 5 mL of Bradford's reagent, vortexed, and then incubated at room temperature for 10-60 min. The absorbance of the protein sample solution was read at a wavelength of 595 nm. The bovine serum albumin (BSA) was used for preparing as standard curve. The concentration series of BSA were 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/mL.

#### SOD ACTIVITY ASSAY

The SOD activity was evaluated using the Elabsience assay kit (E-BC-K022-S) according to the procedure described in the manufacturer's instruction. This assay measured the activity of the T-SOD (Total SOD) enzyme. SOD enzyme in this sample has inhibitory specific effect on superoxide anion (O<sub>2</sub><sup>•-</sup>), which is produced by xanthine and xanthine oxidase system and can oxidize hydroxylamine to form nitrite. Nitrite appears purplish after reacting with the chromogenic reagent. The absorbance was measured at 550 nm, and the difference in absorbance value between the control and the sample indicates the activity of SOD.

#### CAT ACTIVITY ASSAY

The CAT activity was measured using the Elabsience assay kit (E-BC-K031-S) according to the procedure described in the manufacturer's instruction. The enzyme CAT works by decomposing H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O. The remaining H<sub>2</sub>O<sub>2</sub> then interacts with the ammonium molybdate to form a yellowish complex. The absorbance

was measured at 405 nm, and the difference in absorbance between the control and the sample indicates the level of CAT enzyme activity.

#### GSH-PX ACTIVITY ASSAY

The GSH-Px activity was determined using the Elabsience assay kit (E-BC-K096-S) according to the procedure described in the manufacturer's instruction. GSH can react with dinitrobenzoic acid to form a persistent yellow 5-thio-dinitrobenzoic acid anion. The absorbance was measured at 412 nm, and the difference in absorbance value between the control and the sample indicates the activity of the GSH-Px enzyme in the sample.

#### MDA CONCENTRATION ASSESSMENT

The MDA in the liver homogenate was measured using the Elabsience assay kit (E-BC-K025-S) according to the procedure provided in the manufacturer's instruction. MDA can react with thiobarbituric acid (TBA) and produce a red compound with a maximum absorbance at 532 nm.

#### STATISTICAL ANALYSIS

The quantitative data were analysed statistically using SPSS 16.0 for windows. The analysis was carried out by ANOVA and followed by post hoc test (LSD) with significant level 0.05 for comparing treatment group with control group.

## RESULTS

#### HPLC PROFILE OF THE SWEET POTATO LEAF EXTRACT

The HPLC chromatogram of the SPLE presented in Figure 3 shows several peaks labeled with retention time. The peak labeled 2.7 min represents the presence of rutin in the SPLE sample instead of quercetin, which would otherwise peak at 2.9 min. This result indicates that rutin is a chemical content of the SPLE, and based on its area under curve (AUC), it makes up 17.4% of all the components in this extract.

#### BODYWEIGHT REDUCTION AFTER TREATMENT WITH THE SWEET POTATO LEAF EXTRACT

The results showed that feeding the test animals with HFD increased their body weight while treatment with the SPLE was found to decrease it, as evident in the initial and final body weights presented in Table 1.

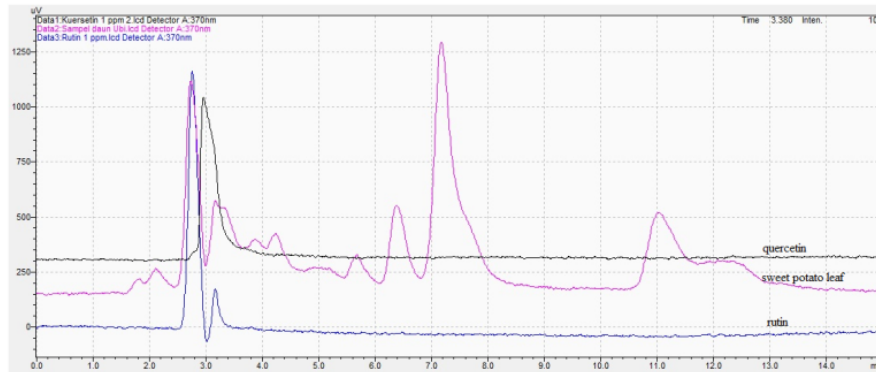


FIGURE 3. The HPLC chromatogram of sweet potato leaf extract in comparison with rutin and quercetin

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TABLE 1. The effects of treatment of sweet potato leaf extract on the body weight of high-fat diet rat

Groups	Initial body weights (g)	Final body weights (g)	Bodyweight changes (g)
Normal	130.85 ± 4.17	129 ± 1.41	-0.46
Negative control	146.66 ± 20.83	174.88 ± 16.71	28.22
SPLE 100 mg/kg BW	137.2 ± 30.69	135.8 ± 24.71	-1.46
1 SPLE 200 mg/ kg BW	148.82 ± 16.96	118.16 ± 13.11	-30.66
1 SPLE 400 mg/kg BW	151.32 ± 11.60	109.8 ± 16.5*	-41.52

\*significant different with initial body weight

#### EFFECTS OF THE SWEET POTATO LEAF EXTRACT ON SOD ACTIVITIES IN HFD RATS

Table 2 shows the SOD activities in rats consuming a high-fat diet for 14 days (Day 0 to 14) and those with supplemental SPLE until Day 28. The SOD activities of negative control groups were significantly lower ( $p < 0.05$ ) than normal groups. But significant increase ( $p < 0.05$ ) in those receiving supplemental SPLE. The treatment of SPLE on HFD diet rats with dose of 100, 200, and 400 mg/kg BW could increase the SOD activities 2.1; 2.3 and 3.2-fold compare to negative control. The SOD activities increased in a dose-dependent manner. The post hoc test using LSD showed that SOD activities in SPLE 400 mg/kg BW group was significant different ( $p < 0.05$ ) compare SPLE 100 mg/kg BW and SPLE 200 mg/kg

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BW. However, the increasing activity of SOD in SPLE 200 mg/kg BW was not significantly different compare to SPLE 100 mg/kg BW.

#### EFFECTS OF THE SWEET POTATO LEAF EXTRACT ON CAT ACTIVITIES IN HIGH-FAT DIET FED RATS

Table 3 shows the CAT activities in high-fat diet fed rats for 14 days and those treated with supplemental SPLE until day 28. The test rats in the negative control group showed considerably lower CAT activities significantly ( $p < 0.05$ ) than the normal group. Meanwhile, those in the treatment groups exhibited significantly higher CAT activities ( $p < 0.05$ ) than the normal and negative control groups. These activities increased in a dose-dependent

manner after treatment with the SPLE. The post hoc test showed that increasing of CAT activity in SPLE 200 mg/kg BW was significant difference with SPLE 100 mg/kg

kg BW groups, but not significantly different compare to 400 mg/kg BW. It showed that SPLE treatment with dose of 200 mg/kg BW is the optimum dose in increasing of CAT activity.

TABLE 2. The effects of sweet potato leaf extract treatment on the SOD activities in rats fed a high-fat diet

Groups	SOD activities
	(U/mg protein, mean $\pm$ SD)
Normal	76.73 $\pm$ 12.91 <sup>#</sup>
Negative control	44.29 $\pm$ 22.13 <sup>*</sup>
SPLE 100 mg/kg BW	94.39 $\pm$ 22.54 <sup>#</sup>
SPLE 200 mg/ kg BW	100.55 $\pm$ 19.15 <sup>#</sup>
SPLE 400 mg/kg BW	140.31 $\pm$ 24.55 <sup>#</sup>

\* different from the normal group significantly ( $p < 0.05$ ), <sup>#</sup> different from the negative control group significantly ( $p < 0.05$ ). The statistical analysis was carried out by ANOVA and followed by LSD. The data presented as mean from 5 rat per groups

TABLE 3. The effects of sweet potato leaf extract treatment on CAT activities in high-fat diet fed rat

Groups	CAT activities
	(U/mg protein, mean $\pm$ SD)
Normal	204.32 $\pm$ 21.43 <sup>#</sup>
Negative control	75.21 $\pm$ 26.7 <sup>*</sup>
SPLE 100 mg/kg BW	115.41 $\pm$ 17.65 <sup>#</sup>
SPLE 200 mg/ kg BW	249.19 $\pm$ 16.94 <sup>#</sup>
SPLE 400 mg/kg BW	263.63 $\pm$ 27.02 <sup>#</sup>

\* different from the normal group significantly ( $p < 0.05$ ), <sup>#</sup> different from the negative control group significantly ( $p < 0.05$ ). The data presented as mean from 5 rat per groups

#### EFFECTS OF HYPERLIPIDEMIC TREATMENT WITH THE SWEET POTATO LEAF EXTRACT ON GSH-PX ACTIVITIES IN HIGH-FAT DIET FED RATS

Table 4 shows the mean GSH-Px activities in the normal (BR II pellet), negative control (high-fat diet), and SPLE-treatment groups (high-fat diet and SPLE). Compared with the normal group, the test rats in the negative control exhibited substantially lower GSH-Px activities ( $p < 0.05$ ). Meanwhile, the hyperlipidemic test rats treated with SPLE

had increased GSH-Px activities significantly ( $p < 0.05$ ) than the negative control group. These activities increased with the dose of the given SPLE. The post hoc test on GSH-Px activities showed that increasing of GSH-Px activity in SPLE 200 mg/kg BW was significant difference with SPLE 100 mg/kg BW groups, but not significantly different compare to 400 mg/kg BW. It showed that SPLE treatment with dose of 200 mg/kg BW is the optimum dose in increasing of GSH-Px activity.

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TABLE 4. The effects of sweet potato leaf extract treatment on GSH-Px activities in high-fat diet fed rats

Groups	GSH-Px activities (U/mg protein, mean ± SD)
Normal	185.67± 24.17 <sup>#</sup>
Negative control	84.21 ± 23.62 <sup>*</sup>
SPLE 100 mg/kg BW	114.28 ± 8.24 <sup>*</sup>
SPLE 200 mg/ kg BW	172.68 ± 30.54 <sup>#</sup>
1 SPLE 400 mg/kg BW	184.87 ± 2.25 <sup>#</sup>

\*different from the normal group significantly ( $p \leq 0.05$ ), <sup>#</sup> different from the negative control group significantly ( $p \leq 0.05$ ). The data presented as mean from 5 rat per groups

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EFFECTS OF HYPERLIPIDEMIC TREATMENT WITH THE  
SWEET POTATO LEAF EXTRACT ON MDA LEVELS IN  
HIGH-FAT DIET FED RATS

Table 5 shows the mean MDA levels of five test rats in the normal, negative control, and treatment groups during the 28-day experiment. The test rats in the negative control, whose hyperlipidemic conditions were induced with a high-fat diet, showed a significantly high mean MDA level ( $p < 0.05$ ) than the normal group. Meanwhile, those treated with SPLE for 28 days had significantly

high mean MDA levels ( $p < 0.05$ ) than the normal group, the negative control, or both. These MDA concentrations decreased in a dose-dependent manner (Table 5). The post hoc test using LSD showed that MDA level in SPLE 400 mg/kg BW group was significant different ( $p < 0.05$ ) compare to both of treated groups i.e., SPLE 100 mg/kg BW and SPLE 200 mg/kg BW. The SPLE treatment with dose 400 mg/ kg BW gives the best result in this study. The MDA level in this group decrease until normal level.

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TABLE 5. The effects of sweet potato leaf extract treatment on MDA levels in rats fed a high-fat diet

Groups	MDA concentrations (nmol/mg protein, mean ± SD)
Normal	14.14± 0.47 <sup>#</sup>
Negative control	86.88 ± 2.8 <sup>*</sup>
1 SPLE 100 mg/kg BW	70.15 ± 9.57 <sup>#</sup>
SPLE 200 mg/ kg BW	45.61 ± 0.60 <sup>#</sup>
SPLE 400 mg/kg BW	18.92 ± 1.63 <sup>#</sup>

\*significantly different from the normal group ( $p \leq 0.05$ ), <sup>#</sup>significantly different from the negative control group ( $p \leq 0.05$ )

DISCUSSION

In a normal condition, metabolism generates free radicals, which is very reactive in the body. They denature biological molecules like proteins, lipids, and carbohydrates by reacting with them. As a result, critical

cellular structures and functions are destroyed, leading to various of clinical disease (Ebele et al. 2016). The presence of free radicals and highly content of blood lipid will lead to lipid peroxidation and cause severe oxidative stress.



A high-fat diet not only contributes to increasing body weight and lipid profile but also decreases enzymatic antioxidant activities. Further, it is claimed as a major risk factor of degenerative diseases, including diabetes, hypertension, and other cardiovascular illnesses (Poli et al. 2018). The HFD induction model used in this study was also found to increase the cholesterol and triglyceride level of rat. The increasing of cholesterol and triglyceride was followed by increasing of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase which showed the hepatotoxicity (Mahfudh et al. 2021).

This study has found that consumption of a high-fat diet increases the body weight of the test rats. This corresponds to a previous study that links metabolic changes to diet-induced obesity in mice (Hariri & Thibault 2010). Increased body weight is also followed by elevated lipid profiles in the blood. Administration of propylthiouracil (PTU) also accelerates the accumulation of blood lipids by suppressing thyroid function in cholesterol synthesis (Singh et al. 2020). The treatment with the SPLE on high fat diet fed rat decreases the body weight gain compared with the negative control. This finding has been confirmed with a previous study that reported supplementing a high-fat diet with sweet potato leaves in rats reduces weight gain and lowers adipose tissue, cholesterol, and triglyceride levels (Mahfudh et al. 2021). Purple sweet potatoes that are rich in polyphenols and dietary fiber can reduce lipid levels and weight gain in a dose-dependent manner. The mechanism of dietary fibre in SPLEs are thought to be due to the inhibition or blocking lipid absorption in the small intestine (Isken et al. 2010; Lattimer & Haub 2010).

The current study has associated a high-fat diet with the increasing of free radicals in the rat bodies. The endogenous antioxidants decrease in rats receiving a high-fat diet (Jang & Choi 2019). Meanwhile, treatments with the SPLE increase SOD, CAT, and GSH-Px activities and lowers the concentrations of MDA, the end product of lipid peroxidation. These findings correspond to several previous studies that have confirmed the antioxidant activity of SPLEs both *in vitro* (Hue et al. 2011; Zhang et al. 2019) and *in vivo* (Koncic et al. 2013; Lee et al. 2016).

Superoxide dismutase (SOD) is an endogenous antioxidant that regulates ROS levels. It catalyzes the efficient disposal of superoxide anions and converts them to hydrogen peroxide. Enzymatic and non-enzymatic production of superoxide anions are both possible. When free radicals are detected in an unusually high

amount in the body, they cause oxidative stress which leads to oxidative damage at the cellular, tissue, and organ levels (Aguilar et al. 2016; Gusti et al. 2021). The SOD activities in rats with high-fat-diet-induced hyperlipidemia (negative control) are lower than the normal control (standard BR II food). Elevated lipid concentration in the blood is thought to trigger lipid peroxidation and increase superoxide radicals, whose high presence suppresses the SOD activities. Treatments with the SPLE prove effective to significantly increase the SOD activities in hyperlipidemic rats in a dose-dependent manner. High flavonoid contents are behind the extract's ability to scavenge and neutralize superoxide radicals and reduce lipid peroxidation. These activities depend on the conjugated double bond and the hydroxyl group, which neutralize free radicals by donating the electron.

The current study found that the SPLE contains rutin in high quantity. Rutin is quercetin derivative with hydroxy group at position C-3 substituted with glucose and rhamnose sugar groups. Quercetin derivative are commonly identified in sweet potato leave (Chao et al. 2013; Krochmal-marczak et al. 2020). Many scholars have reported that SPLEs have considerably high phenolic content which is responsible for their antioxidant effect (Wang et al. 2016; Zhang et al. 2019). Another studies on several sweet potato leaves cultivars from South Africa using UPLC/QTOF-MS also found the high content of rutin and some of quercetin derivatives (Phahlane et al. 2021). A previous *in vitro* test of SPLEs has shown higher antioxidant activities than  $\alpha$ -tocopherol and higher radical scavenging activities than ethylenediaminetetraacetic acid (EDTA) (Rumbaoa et al. 2009). Furthermore, as one of the major compounds in the SPLE (Figure 2), rutin is deemed responsible for increasing endogenous antioxidant activities. Rutin is widely reported as a potent antioxidant *in vitro*. Moreover, it has higher DPPH scavenging activities than butylated hydroxytoluene (BHT) and proves effective to inhibit lipid peroxidation (Yang et al. 2008). Many suggest making use of its antioxidant properties in therapeutical management of neurodegenerative disorders (Enogieru et al. 2018), in antiaging (Girsang et al. 2020) and antihyperglycemic treatments (Kamalakkannan et al. 2006), and in inducing hepatoprotective effects against hypercholesterolemia (Al-Rejaie et al. 2013). Apart from rutin, the antioxidant properties of SPLEs have also been attributed to several flavonoids (Islam et al. 2016), including 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid,

and 3,4,5-tricafeoylquinic acid (Zhang et al. 2019); quercetin 3-O- $\beta$ -D-sophoroside, quercetin, benzyl  $\beta$ -D-glucoside, 4-hydroxy-3-methoxybenzaldehyde, and methyl decanoate (Lee et al. 2016) and caffeic acid and caffeoylquinic acids—viz. 3-O-chlorogenic acid, 3,4-di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, 4,5-di-O-caffeoylquinic acid, and 3,4,5-tri-O-caffeoylquinic acid (Kurata et al. 2017).

The enzyme CAT breaks down the hydrogen peroxide ( $H_2O_2$ ) produced by the activation of SOD into water ( $H_2O$ ) and oxygen ( $O_2$ ). CAT finalized the detoxification process initiated by SOD (Rodríguez et al. 1989). GSH-Px is also responsible for inactivating  $H_2O_2$  by catalyzing an oxidation mechanism, i.e., the oxidation of glutathione (GSH) into oxidized glutathione (GSSG) and  $H_2O_2$  reduction into water. Glutathione reductase converts back GSSG to GSH by using NADPH as an energy source or electron donor. The current study has found that increasing of SPLE dose treatment increases CAT and GSH-Px activities significantly. The GSH-Px, SOD, and CAT activities are positively intercorrelated.

The activity of SPLE in scavenging of free radical molecule and ability to suppress oxidation of LDL, which lead to the prolongation of a lag time in the oxidation of lipoprotein *in vitro* (Nagai et al. 2011). In hyperlipidemic rats, the treatment with SPLEs effectively lowers the LDL and triglyceride levels and increases high-density lipoprotein (HDL) and SOD level significantly ( $p=0.001$ ) (Sumardika & Jawi 2012). In an *in vitro* study, elevated GSH levels were observed after applying high-concentration SPLEs to treat Hep G2 cells (Koncic et al. 2013). This confirms the extract's ability to increase endogenous antioxidants in high glucose-induced oxidative stress. This process is thought to be the result of flavonoids, especially rutin and caffeic acid that can act as antioxidants (Girsang et al. 2020) to prevent oxidation of LDL by binding free radicals and transition metal ions that promote lipid peroxidation. A previous study has also found that treating high-fat-diet-induced hyperlipidemia in rats with SPLEs can lower total cholesterol and triglyceride levels significantly (Mahfudh et al. 2021). Similarly, SPLEs have been considered responsible for decreasing the ROS levels, prolonging the formation of  $FeCl_3$ -induced thrombosis, and increasing the SOD contents of the liver in Syrian hamsters fed high-cholesterol foods (Chang et al. 2021). It is suggested that treatment with sweet potato leaves provides a nutritional strategy for dyslipidemia and prevents cardiovascular diseases.

Another biomarker of oxidative damage is malondialdehyde (MDA) levels. MDA results from protein damage due to ROS in the peroxidation of lipids and carbonyl compounds (Khoubnasab Jafari et al. 2015). In the current study, the SPLE significantly lowers the MDA levels in hyperlipidemic rats ( $p<0.05$ ) in a dose-dependent manner, indicating the extract's ability to decrease lipid peroxidation. This finding is comparable to the results of a previous study that attributes SPLE to the lowered total blood cholesterol, MDA, and interleukin-1 levels in hypercholesterolemic rabbits (Jawi & Indrayani 2015). It also confirms the potency of SPLEs in inhibiting oxidative stress resulting from hyperlipidemic conditions. The current study recommended that sweet potato leaves could be developed as a nutritional strategy for balancing a diet with high fat consumption.

#### CONCLUSION

The present study concludes the activity of sweet potato leaf extract in increasing the activities of endogenous antioxidants (SOD, CAT, GSH-Px) and decreasing the metabolite oxidation (MDA) in hyperlipidemic rats.

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