BUKTI KORESPONDENSI ARTIKEL

JUDUL ARTIKEL : Sweet Potato (Ipomoea batatas L.) Leaves Ethanol Extract Increases Endogenous Antioxidant Activities in Hyperlipidemic Rats

NAMA JURNAL : Sains Malaysiana (Q2), Sains Malaysiana 51(9)(2022): 2873-2883

RANGKUMAN KORESPONDENSI

TANGGAL	EDITOR	AUTHOR
19-11-2021	Penerimaan submission artikel dan permintaan persetujuan dari author untuk pembayaran APC RM 1500 setelah artikel diterima	Menyetujui pembayaran RM 1500 kalau artikel diterima
23-11-2021	Pernyataan dari editor bahwa similarity dari manuskrip lebih dari 30%	Respon author akan melakukan paraphrase dan resubmit ulang
1-12-2021		Penyampaian bahwa author sudah melakukan resubmit dengan perbaikan dan menyertakan hasil similarity check sebagai supplementary file
15-1-2022	Hasil review dari reviewer C dan reviewer D	
28-1-2022		Author melakukan submit perbaikan manuskrip dan mengirimkam juga list respon (tanggapan) dari masukan reviewer
14-3-2022	Hasil review manuskrip dari reviewer A	
19-3-2022		Author melakukan resubmit manuskrip dilengkapi dengan list of respon (tanggapan)
19-3-2022	Pernyataan kalo manuskrip diterima (accepted)	

Korespondensi 19-11-2021

[Sains Malaysiana] Submission Acknowledgement - ID: 52309 External Inbox ×

Rusli Daik <jsm@ukm.edu.my> Fri, 19 Nov 2021, 15:02
to me

Dear Dr Nurkhasanah Mahfudh:

Thank you for submitting the manuscript, "Ethanol extract of sweet potato (Ipomoea batatas L) leaves increases endogenous antioxidant activities in hyperlipidemic rats" to Sains Malaysiana. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Manuscript URL: http://ejournal.ukm.my/jsm/author/submission/52309
Username: nurkhasanah

Please note that with effect from 1st July 2016 onwards, all new submission will be subjected to 1500MYR publication fee once the paper is accepted for publication. Kindly reply to this email if you agree to this commitment to avoid unnecessary delay in processing your paper.

If you have any questions, please contact me. Thank you for considering this journal as a publication venue for your work.

Nurkhasanah Mahfudh <nurkhasanah@pharm.uad.ac.id> Fri, 19 Nov 2021, 20:36
to Rusli

Dear editor of Sains Malaysiana

Regards

•••

--

Dr. Nurkhasanah, M.Si. Apt
Fakultas Farmasi Universitas Ahmad Dahlan
Jl. Prof Soepomo, Janturan, Yogyakarta

Reply Forward

Korespondensi 23-11-2021

[Sains Malaysiana] Unsuitable Submission - ID: 52309 External Inbox ×

Prof. Dr. Rusli Daik <jsm@ukm.edu.my> Tue, 23 Nov 2021, 09:06
to me

Dear Dr Nurkhasanah Mahfudh:

An initial review of "Ethanol extract of sweet potato (Ipomoea batatas L) leaves increases endogenous antioxidant activities in hyperlipidemic rats" has been made.

It has been found the similarity index of your manuscript is above 30%, thus it cannot be considered for further evaluation.

Sincerely,

Prof. Dr. Rusli Daik
jsm@ukm.edu.my
Editor-in-Chief
Sains Malaysiana
Universiti Kebangsaan Malaysia
Ph: 60389214917, Fax: 60389256086

Nurkhasanah Mahfudh <nurkhasanah@pharm.uad.ac.id> 23 Nov 2021, 10:15
to Rusli

Thank you for your email.

Could we resubmit after we make some corrections?
We will resubmit along with the similarity check

Thank you

•••

--
Dr. Nurkhasanah, M.Si. Apt
Fakultas Farmasi Universitas Ahmad Dahlan
Jl. Prof Soepomo, Janturan, Yogyakarta

Korespondensi 1-12-2021

Nurkhasanah Mahfudh <nurkhasanah@pharm.uad.ac.id> 1 Dec 2021, 15:39
to Rusli

Dear Prof, Dr. Rusli Daik

We have resubmitted the manuscript on November 26.
We have made some revisions or paraphrasing to reduce the similarity index. We also attached the similarity check result as a supplementary file along with the submission.
Hopefully, the manuscript will fulfill the requirement to be published in Sains Malaysiana.

Best Regards

•••

Korespondensi 15-1-2022

[Sains Malaysiana] Editor Decision External Inbox ×

Assoc Prof Dr Seng Joe Lim <joe@ukm.edu.my> Sat, 15 Jan 2022, 09:34

to me

Dear Dr Nurkhasanah Mahfudh:

The referee has commented on your paper titled: "Sweet potato (Ipomoea batatas L) leaves ethanol extract increases endogenous antioxidant activities in hyperlipidemic rats".

We would be glad to reconsider the paper if you are willing to amend the paper according to the recommendations by the referee.

Please find the comments with this email (located towards the end of this email, or as an email attachment, or both).

Please provide a List of Responses by answering / responding to every comments / suggestions when resubmitting the revised paper in a separate file.

Re-submission should be made online within 4 weeks.

Thank you.

44 of 58

Reviewer C:

Comments on the manuscript for authors:
All comments can be seen in the manuscript.

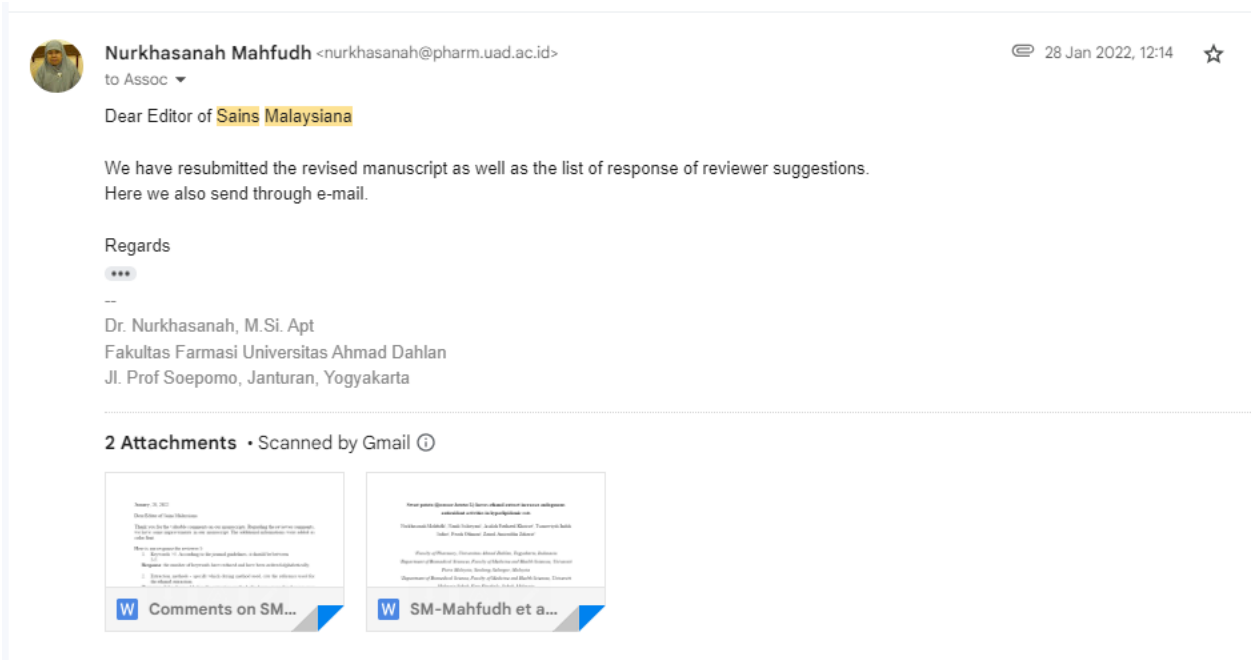
Reviewer D:

Comments on the manuscript for authors:
1. Keywords >5. According to the journal guidelines, it should be between 3-5.
2. Extraction methods - specify which drying method used, cite the reference used for the ethanol extraction.
3. What is the reference method for preparation of the high fat diet? What is the percentage of nutrient composition (fat, protein & carbohydrate) for the standard diet and high fat diet?
4. Sentence repetition in experimental design 'The treatments given to them are described below'.
5. Group 1 treatment - was the standard diet given throughout the 42 days?
6. Statement on treatment for Group II and Group III is unclear. Seems like after induction period (14 days) of high fat diet, the post diet (28 days) was only CMC-Na and SPLE, respectively.
7. Suggest to include the average daily food intake of the rats according to treatment group in the method and result.

7. Suggest to include the average daily food intake of the rats according to treatment group in the method and result.
8. After the diet induction, did the rats develop hyperlipidemia prior to the post treatment? Include the data.
9. How was the stability of the SPLE used in the post treatment? Include the information.
10. Provide supporting reference to indicate that rutin is the main phenolic compound found in sweet potato leave. From the HPLC profile around RT 7.2 min, there is another large peak. What is the possibility of it being another important phenolic compound?
11. Is the data in Table 1 based on the data from published article in International Journal of Public Health Science (2021), 10, 558-564?

Sains Malaysiana
http://ejournal.ukm.my/jsm

Korespondensi 28-1-2022



Nurkhasanah Mahfudh <nurkhasanah@pharm.uad.ac.id>
to Assoc

28 Jan 2022, 12:14

Dear Editor of Sains Malaysiana

We have resubmitted the revised manuscript as well as the list of response of reviewer suggestions.
Here we also send through e-mail.

Regards

--
Dr. Nurkhasanah, M.Si. Apt
Fakultas Farmasi Universitas Ahmad Dahlan
Jl. Prof Soepomo, Janturan, Yogyakarta

2 Attachments • Scanned by Gmail

Comments on SM...

SM-Mahfudh et a...

Revised manuskrip R1 (warna biru adalah perbaikan)

Sweet potato (*Ipomoea batatas* L) leaves ethanol extract increases endogenous antioxidant activities in hyperlipidemic rats

Nurkhasanah Mahfudh[1], Nanik Sulistyani[1], Asiilah Fatihatul Khoirot[1], Tsanawiyah Indah Safira[1], Fezah Othman[2], Zainul Amiruddin Zakaria[3]

[1]*Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia*

[2]*Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*

[3]*Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia*

***corresponding author**: Nurkhasanah Mahfudh: nurkhasanah@pharm.uad.ac.id

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 hours, 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 sweet potato leaves extract (SPLE) treated groups 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.

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 and 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 (Switala and Loewen 2002; Kodydková et al. 2014). However, the activities of these intracellular antioxidant enzymes can decrease due to oxidative stress (Araujo et al. 1995; Halder and 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 (Rumbaoa, Cornago, and Geronimo 2009; Islam 2006; Meira et al. 2012).

For the above 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, Jawi, and Satriyasa 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 Method

Materials

The leave of sweet potato used in this study were collected from Magelang, Central Java, Indonesia. The sample was authenticated and deposited in the 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 ^{o}C temperature and then crushed. 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 hours by maceration. To get a concentrated extract, the filtrate was evaporated using a vacuum rotary evaporator at a temperature of 60 ^{o}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 brailler-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. During the experiment the animals were kept in a well-ventilated room with a light cycle (12 hours light and 12 hours dark), humidity and room temperature were maintained.

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, Nurkhasanah, and Sulistyani 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 are described in Figure 1.

Group I is normal control group, the rats were given standard diet (BR II pellets).

Group II is negative control group, the rats were fed a HFD for 14 days and 1% sodium carboxymethylcellulose (CMC-Na) for 28 days.

Group III, IV, V are treated groups: the rats were given a HFD for 14 days and SPLE orally at a dose of 100, 200, and 400 mg/kg BW for 28 days.

After HFD induction in group II-V, beside treatment, the rats also were feed by standard diet.

The treatment in group II-V were given along the 28 days of studies.

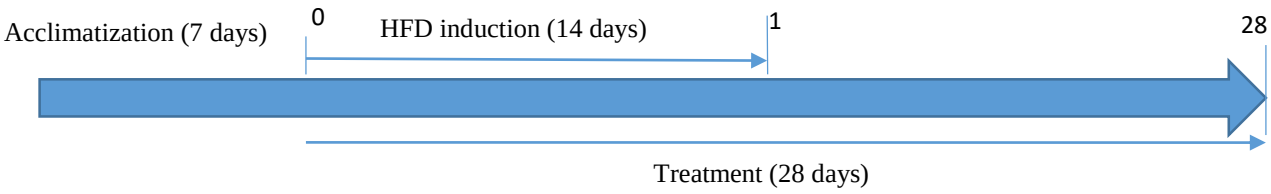


Figure 1. Desain for HFD induction and treatments

The body weight was monitored weekly. The test rats were anesthetized using carbondioxyde (CO_2) gas on the 29th 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 minutes. 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 minutes. 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 Elabscience 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_2*^-), 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 Elabscience assay kit (E-BC-K031-S) according to the procedure described in the manufacturer's instruction. The enzyme CAT works by decomposing H_2O_2 into H_2O. The remaining H_2O_2 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 Elabscience 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 Elabscience 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 anlysis 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 2 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 it's area under curve (AUC), it makes up 17.4% of all the components in this extract.

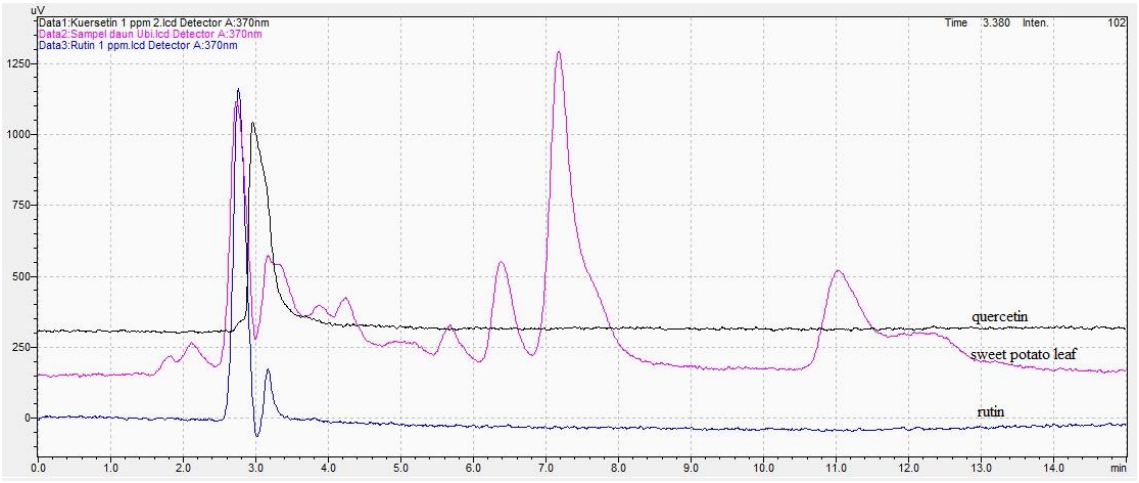


Figure 2. The HPLC chromatogram of sweet potato leaf extract in comparison with rutin and quercetin.

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.

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
SPLE 200 mg/ kg BW	148.82 ± 16.96	118.16 ± 13.11	-30.66
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 significantly 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 BW. However, the increasing

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

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 ± SD)**
Normal	76.73 ± 12.91[#]
Negative control	44.29 ± 22.13 [*]
SPLE 100 mg/kg BW	94.39 ± 22.54[#]
SPLE 200 mg/ kg BW	100.55 ± 19.15[#]
SPLE 400 mg/kg BW	140.31 ± 24.55[*#]

* different from the normal group significantly ($p \leq 0.05$), [#] different from the negative control group significantly ($p \leq 0,05$). The statistical analysis were carried out by ANOVA and followed by LSD. The data presented as mean from 5 rat per groups.

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 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 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 ± SD)**
Normal	204.32± 21.43[#]
Negative control	75.21 ± 26.7[*]
SPLE 100 mg/kg BW	115.41 ± 17.65[*#]
SPLE 200 mg/ kg BW	249.19 ± 16.94[*#]
SPLE 400 mg/kg BW	263.63 ± 27.02[*#]

* different from the normal group significantly ($p \leq 0.05$), [#] different from the negative control group significantly ($p \leq 0.05$). 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 GHS-Px activities in the normal (BR II pellet), negative control (high-fat diet), and SLPE-treatment groups (high-fat diet and SLPE). 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.

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$^{\#}$
Negative control	84.21 ± 23.62*
SPLE 100 mg/kg BW	114.28 ± 8.24*
SPLE 200 mg/ kg BW	172.68 ± 30.54$^{\#}$
SPLE 400 mg/kg BW	184.87 ± 2.25$^{\#}$

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

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 give the best result in this study.

The MDA level in this group decrease until normal level.

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[#]
Negative control	86.88 ± 2.8[*]
SPLE 100 mg/kg BW	70.15 ± 9.57[#*]
SPLE 200 mg/ kg BW	45.61 ± 0.60[#*]
SPLE 400 mg/kg BW	18.92 ± 1.63[#]

*significantly different from the normal group ($p \leq 0.05$), [#]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 also was 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 and 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 (Lattimer and Haub 2010; Isken et al. 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 and 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, Boyce, and Somasundram 2011; Zhang et al. 2019) and in vivo (Koncic, Petlevski, and Kalodera 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, Navarro, and Pérez 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 derivate with hydroxy group at position C-3 substituted with glucose and rhamnose sugar groups. Quercetin derivate 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, Nie, and Zhu 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 derivates (Manhivi and Sivakumar 2021). A previous in vitro test of SPLEs has shown higher antioxidant activities than α-tocopherol and higher radical scavenging activities than ethylenediaminetetraacetic acid (EDTA) (Rumbaoa, Cornago, and Geronimo 2009). Furthermore, as one of the major compounds in the SPLE (see 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, Guo, and Yuan 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, Stanely, and Prince 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-tricaffeoylquinic acid (Zhang et al. 2019); quercetin 3-O-β-D-sophoroside, quercetin, benzyl β-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 and Jawi 2012). In an in vitro study, elevated GSH levels were observed after applying high-concentration SPLEs to treat Hep G2 cells (Koncic, Petlevski, and Kalodera 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, Ansarin, and Jouyban 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 and 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 conclude 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

Acknowledgment

The authors are grateful to the Ministry of Education and Culture of the Republic of Indonesia for funding this study through the Postgraduate Research Scheme 2020 with the contract number PTM034/SKPP.TT/LPPM UAD/VI/2020.

References

Aguilar, Tomás Alejandro Fregoso, Brenda Carolina Hernández Navarro, and Jorge Alberto Mendoza Pérez. 2016. "Endogenous Antioxidants: A Review of Their Role in Oxidative Stress." *A Master Regulator of Oxidative Stress - The Transcription Factor Nrf2*, no. December. https://doi.org/10.5772/65715.

Al-rejaie, Salem S, Abdulaziz M Aleisa, Mohamed M Sayed-ahmed, Othman A Al-shabanah, Hatem M Abuohashish, Mohammed M Ahmed, Khaled A Al-hosaini, and Mohamed M Hafez. 2013. "Protective Effect of Rutin on the Antioxidant Genes Expression in Hypercholestrolemic Male Westar Rat." *BMC Complementary & Alternative Medicine* 13 (136).

Araujo, Fernanda B., Décio S. Barbosa, Chang Y. Hsin, Raul C. Maranhão, and Dulcineia S.P. Abdalla. 1995. "Evaluation of Oxidative Stress in Patients with Hyperlipidemia." *Atherosclerosis* 117 (1): 61–71. https://doi.org/10.1016/0021-9150(94)05558-Z.

Barkas, Fotios, Tzortzis Nomikos, Evangelos Liberopoulos, and Demosthenes Panagiotakos. 2020. "Diet and Cardiovascular Disease Risk among Individuals with Familial

Hypercholesterolemia: Systematic Review and Meta-Analysis." *Nutrients* 12 (8): 1–22. https://doi.org/10.3390/nu12082436.

Birben, Esra, Umit Murat Sahiner, Cansin Sackesen, Serpil Erzurum, and Omer Kalayci. 2012. "Oxidative Stress and Antioxidant Defense." *WAO Journal* 5: 9–19.

Canadian Council of Animal Care. 2010. *Canadian Council on Animal Care in Science CCAC Guidelines on : Euthanasia of Animals Used*. Ottawa, Canada.

Chang, Hao-hsiang, Yi-chan Lan, Shiu-dong Chung, and Chiang-ting Chien. 2021. "Sweet Potato Leaf Feeding Decreases Cholesterol , Oxidative Stress and Thrombosis Formation in Syrian Hamsters with a High-Cholesterol Diet." *Life* 11 (802): 1–11.

Chao, Pi-yu, Wei-yu Huang, Shene-pin Hu, Hsiao-feng Lo, Kuan-hung Lin, and Meng-yuan Huang. 2013. "Indigenous Purple Vegetable Extracts Protect against Hydrogen Peroxide-Induced DNA Damage in Human Lymphocytes." *Food and Nutrition Sciences* 4: 62–70. https://doi.org/http://dx.doi.org/10.4236/fns.2013.48A008 Published.

Chen, W., Tong-jun Mao, L. Fan, Yuhan Zhou, Jing Yu, Y. Jin, and Peng-chao Hou. 2011. "Effect of Purple Sweet Potato on Lipid Metabolism and Oxidative Stress in Hyperlipidemic Rats." *Journal of Zhejiang University. Medical Sciences* 40 (4): 360–64.

Ebele, Ikekpeazu Joy, Ikekpeazu Adanna Jenniger, Eke Christian Nnabugo, Ogbu Innocent Sidney, Onyekwelu Kenechukwu Chibuike, Orji Oliver Chukwuma, Nwachukwu Daniel, and Eze Anthonius Anayochukwu. 2016. "Oxidative Stress/Lipid Peroxidation and Antioxidant Enzymes in Apparently Healthy Individuals Involved in Physical Exercise." *Asian Journal of Medical Sciences* 7 (6): 16–19. https://doi.org/10.3126/ajms.v7i6.15245.

Enogieru, Adaze Bijou, William Haylett, Donavon Charles Hiss, Soraya Bardien, and Okobi Eko Ekpo. 2018. "Review Article Rutin as a Potent Antioxidant : Implications for

Neurodegenerative Disorders." *Oxidative Medicine and Cellular Longevity* 2018: 1–17. https://doi.org/https://doi.org/10.1155/2018/6241017.

Girsang, Ermi, I Nyoman Ehrich Lister, Chrismis Novalinda Ginting, Ika Adhani Sholihah, Martinus Ahmad Raif, and Sidharta Kunardi. 2020. "Antioxidant and Antiaging Activity of Rutin and Caffeic Acid." *Pharmaciana* 10 (2): 147–56. https://doi.org/10.12928/pharmaciana.v10i2.13010.

Gusti, Amani M.T., Safaa Y. Qusti, Eida M. Alshammari, Eman A. Toraih, and Manal S. Fawzy. 2021. "Antioxidants-Related Superoxide Dismutase (Sod), Catalase (Cat), Glutathione Peroxidase (Gpx), Glutathione-s-Transferase (Gst), and Nitric Oxide Synthase (Nos) Gene Variants Analysis in an Obese Population: A Preliminary Case-Control Study." *Antioxidants* 10 (4). https://doi.org/10.3390/antiox10040595.

Halder, Suranjana, and Maitree Bhattacharyya. 2014. "Oxidative Stress: Lipid Peroxidation Products as Predictors in Disease Progression." *Journal of Experimental and Integrative Medicine* 4 (3): 151. https://doi.org/10.5455/jeim.210414.rw.007.

Hariri, Niloofar, and Louise Thibault. 2010. "High-Fat Diet-Induced Obesity in Animal Models." *Nutrition Research Reviews* 23 (2): 270–99. https://doi.org/10.1017/S0954422410000168.

Heriwijaya, I Pande Putu Deny, I Made Jawi, and Bagus Komang Satriyasa. 2020. "Uji Efektivitas Ekstrak Air Daun Ubi Jalar Ungu (Ipomoea Batatas) Terhadap Profil Lipid Tikus Putih Jantan Galur Wistar Yang Diinduksi Pakan Dislipidemia." *Intisari Sains Medis* 11 (2): 452–56. https://doi.org/10.15562/ism.v11i2.584.

Hue, Seow-mun, Amru Nasrulhaq Boyce, and Chandran Somasundram. 2011. "Comparative Study on the Antioxidant Activity of Leaf Extract and Carotenoids Extract from Ipomoea Batatas Var. Oren (Sweetpotato) Leaves." *International Journal of Nutrition and Food*

Engineering 5 (10): 604–7.

Ihwah, A, P Deoranto, S Wijana, and IA Dewi. 2018. "Comparative Study between Federer and Gomez Method for Number of Replication in Complete Randomized Design Using Simulation : Study of Areca Palm (Areca Catechu) as Organic Waste for Producing Handicraft Paper Comparative Study between Federer and Gomez." *IOP Conf. Series: Earth and Environmental Science 131* 131 (012049): 0–6. https://doi.org/doi :10.1088/1755-1315/131/1/012049.

Isken, Frank, Susanne Klaus, Martin Osterhoff, Andreas F.H. Pfeiffer, and Martin O. Weickert. 2010. "Effects of Long-Term Soluble vs. Insoluble Dietary Fiber Intake on High-Fat Diet-Induced Obesity in C57BL/6J Mice." *The Journal of Nutritional Biochemistry* 21 (4): 278–84. https://doi.org/10.1016/j.jnutbio.2008.12.012.

Islam, Shahidul. 2006. "Sweetpotato (Ipomoea Batatas L.) Leaf: Its Potential Effect on Human Health and Nutrition." *Journal of Food Science* 71 (2): R13–121. https://doi.org/10.1111/j.1365-2621.2006.tb08912.x.

Islam, Shahidul, M Yoshimoto, K Ishiguro, and O Yamakawa. 2016. "Bioactive Compounds in Ipomoea Batatas Leaves." In *Proc XXVI IHC - Issues and Advances in Postharvest Hort*, 693–99. https://doi.org/10.17660/ActaHortic.2003.628.88.

Jang, In-Sil, and Mj-Ja Choi. 2019. "Effects of Isoflavone Supplementation on Lipid Profiles and Antioxidant Enzyme Activities in Growing Rats Fed High Fat Diet." *Clinical Nutrition Research* 8 (4): 296–306. https://doi.org/10.7762/cnr.2019.8.4.296.

Jawi, I M, and A Wiwiek Indrayani. 2015. "Aqueous Extract of Balinese Purple Sweet Potato (Ipomoea Batatas l .) Prevents Oxidative Stress and Decreases Blood Interleukin-1 in Hypercholesterolemic Rabbits." *Bali Medical Journal* 4 (1): 37–40.

Kamalakkannan, Narasimhanaidu, Ponnaian Stanely, and Mainzen Prince. 2006. "Antihyperglycaemic and Antioxidant Effect of Rutin , a Polyphenolic Flavonoid , in Streptozotocin-Induced Diabetic Wistar Rats." *Pharmacology & Toxicology* 98: 97–103.

Khoubnasab Jafari, Maryam, Khalil Ansarin, and Abolghasem Jouyban. 2015. "Comments on 'Use of Malondialdehyde as a Biomarker for Assesing Oxidative Stress in Different Disease Pathologies: A Review.'" *Iranian Journal of Public Health* 44 (5): 714–15.

Kodydková, J, L Vávrová, M Kocík, and A Žák. 2014. "Review Article Human Catalase , Its Polymorphisms , Regulation and Changes of Its Activity in Different Diseases." *Folia Biologica (Praha)* 60: 153–67.

Koncic, Marijana Zovko, Roberta Petlevski, and Zdenka Kalodera. 2013. "Antioxidant Activity of Ipomoea Batatas L. Lam. Leaf Grown in Continental Croatia and Its Effect on Glutathione Level in Glucose-Induced Oxidative Stress." *International Journal of Food Properties* 16 (5): 964–73. https://doi.org/10.1080/10942912.2011.573117.

Krochmal-marczak, Barbara, Tomasz Cebulak, Ireneusz Kapusta, Joanna Kaszuba, and Natalia Zurek. 2020. "The Content of Phenolic Acids and Flavonols in the Leaves of Nine Varieties of Sweet Potatoes (Ipomoea Batatas L .) Depending on Their Development , Grown in Central Europe." *Molecules* 25 (3473). https://doi.org/https://doi.org/10.3390/molecules25153473.

Kumar, Vinay, Ahmed Abdullah Khan, Anu Tripathi, Praveen K Dixit, and U K Bajaj. 2015. "Role of Oxidative Stress in Various Diseases : Relevance of Dietary Antioxidants." *The Journal of Phytopharmacology* 4 (2): 126–32.

Kurata, Rie, Tooru Kobayashi, Takanori Ishii, Hiroshi Niimi, Seiji Nisaka, Masaomi Kubo, and Masaoki Kishimoto. 2017. "Influence of Sweet Potato (Ipomoea Batatas L.) Leaf Consumption on Rat Lipid Metabolism." *Food Science and Technology Research* 23 (1):

57–62. https://doi.org/10.3136/fstr.23.57.

Lattimer, James M., and Mark D. Haub. 2010. “Effects of Dietary Fiber and Its Components on Metabolic Health.” *Nutrients* 2 (12): 1266–89. https://doi.org/10.3390/nu2121266.

Lee, Chia Lin, Shou Lun Lee, Chao Jung Chen, Hsin Chun Chen, Ming Ching Kao, Chuan Hao Liu, Jau Yang Chen, Yen Ting Lai, and Yang Chang Wu. 2016. “Characterization of Secondary Metabolites from Purple Ipomoea Batatas Leaves and Their Effects on Glucose Uptake.” *Molecules* 21 (6): 1–14. https://doi.org/10.3390/molecules21060745.

Mahfudh, Nurkhasanah, Nanik Sulistyani, Muhammad Syakbani, and Athifah Candra Dewi. 2021. “The Antihyperlipidaemic and Hepatoprotective Effect of Ipomoea Batatas L . Leaves Extract in High-Fat Diet Rats.” *International Journal of Public Health Science (IJPHS)* 10 (3): 558–64. https://doi.org/10.11591/ijphs.v10i3.20777.

Manhivi, Vimbainashe E, and Dharini Sivakumar. 2021. “An Evaluation of Phenolic Compounds , Carotenoids , and Antioxidant Properties in Leaves of South African Cultivars , Peruvian 199062 . 1 and USA ’ s Beauregard.” *Frontiers in Nutrition* 8 (November): 1–10. https://doi.org/10.3389/fnut.2021.773550.

Meira, Marilena, Eliezer Pereira da Silva, Jorge M. David, and Juceni P. David. 2012. “Review of the Genus Ipomoea: Traditional Uses, Chemistry and Biological Activities.” *Brazilian Journal of Pharmacognosy* 22 (3): 682–713. https://doi.org/10.1590/S0102-695X2012005000025.

Nagai, Miu, Mariko Tani, Yoshimi Kishimoto, Maki Iizuka, Emi Saita, Miku Toyozaki, Tomoyasu Kamiya, Motoya Ikeguchi, and Kazuo Kondo. 2011. “Sweet Potato (Ipomoea Batatas L.) Leaves Suppressed Oxidation of Low Density Lipoprotein (LDL) in Vitro and in Human Subjects.” *J. Clin. Biochem. Nutr* 48 (3): | 203–208. https://doi.org/doi: 10.3164/jcbn.10_84.

Noori, S. 2012. “An Overview of Oxidative Stress and Antioxidant Defensive System.” *Open Access Sci Reports* 1 (8): 1–9.

Poli, Andrea, Carlo M. Barbagallo, Arrigo F.G. Cicero, Alberto Corsini, Enzo Manzato, Bruno Trimarco, Franco Bernini, et al. 2018. “Nutraceuticals and Functional Foods for the Control of Plasma Cholesterol Levels. An Intersociety Position Paper.” *Pharmacological Research* 134 (May): 51–60. https://doi.org/10.1016/j.phrs.2018.05.015.

Rodríguez, E., J. L. Arqués, R. Rodríguez, M. Nuñez, M. Medina, T. L. Talarico, I. A. Casas, et al. 1989. “Antioxidant Compounds and Their Antioxidant Mechanism.” *Intech Open* 32: 137–44. https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics.

Rumbaoa, Rowena Grace O., Djanna F. Cornago, and Inacrist M. Geronimo. 2009. “Phenolic Content and Antioxidant Capacity of Philippine Sweet Potato (Ipomoea Batatas) Varieties.” *Food Chemistry* 113 (4): 1133–38. https://doi.org/10.1016/J.FOODCHEM.2008.08.088.

Sari, Novita, Nurkhasanah, and Nanik Sulistyani. 2020. “The Antioxidant Effect of Bangle (Zingiber Cassumunar) Rhizome Extract on Superoxide Dismutase (Sod) Activity in Hyperlipidemic Rats.” *Research Journal of Chemistry and Environment* 24 (1): 78–81.

Singh, Sneha, Vandana Panda, S. Sudhamani, and Payal Dande. 2020. “Protective Effect of a Polyherbal Bioactive Fraction in Propylthiouracil-Induced Thyroid Toxicity in Ratsby Modulation of the Hypothalamic–Pituitary–Thyroid and Hypothalamic–Pituitary–Adrenal Axes.” *Toxicology Reports* 7 (2020): 730–42. https://doi.org/10.1016/j.toxrep.2020.06.002.

Sumardika, I Wayan, and I Made Jawi. 2012. “Water Extract of Sweet Potato Leaf Imrpoved Lipid Profile and Blood SOD Content of Rats with High Cholesterol Diet.” *Medicina* 43

(2): 67–70.

Switala, Jacek, and Peter C Loewen. 2002. “Diversity of Properties among Catalases.” *Archives of Biochemistry and Biophysics* 401: 145–54.

Valko, Marian, Dieter Leibfritz, Jan Moncol, Mark T.D. Cronin, Milan Mazur, and Joshua Telser. 2007. “Free Radicals and Antioxidants in Normal Physiological Functions and Human Disease.” *International Journal of Biochemistry and Cell Biology* 39 (1): 44–84. https://doi.org/10.1016/j.biocel.2006.07.001.

Walker, John M. 1996. *The Protein Protocols Handbook*. Edited by John M Walker. Second edi. Hatfield: University of Hertfordshire.

Wang, Sunan, Shaoping Nie, and Fan Zhu. 2016. “Chemical Constituents and Health Effects of Sweet Potato.” *Food Research International* 89: 90–116. https://doi.org/10.1016/j.foodres.2016.08.032.

Yang, Jian xiong, Juan Guo, and Jiangfeng Yuan. 2008. “In Vitro Antioxidant Properties of Rutin.” *LWT - Food Science and Technology* 41 (6): 1060–66. https://doi.org/https://ddoi.org/10.1016/j.lwt.2007.06.010.

Zhang, Chengcheng, Daqun Liu, Liehong Wu, Zhang Jianming, Xiaoqiong Li, and Weicheng Wu. 2019. “Chemical Characterization and Antioxidant Properties of Ethanolic Extract and Its Fractions from Sweet Potato (Ipomoea Batatas L) Leaves.” *Foods* 9 (15): 1–14.

Respon to reviewer

January, 28, 2022

Dear Editor of Sains Malaysiana

Thank you for the valuable comments on our manuscripts. Regarding the reviewer comments, we have some improvements in our manuscript. The additional informations were added as color font.

Here is our response for reviewer 1:

1. Keywords >5. According to the journal guidelines, it should be between 3-5.

Response: the number of keywords have reduced and have been ordered alphabetically.

2. Extraction methods - specify which drying method used, cite the reference used for the ethanol extraction.

Response: It has been added in the extraction method: the leaves were dried using oven with 50 ^{o}C temperature. The reference also has been added.

3. What is the reference method for preparation of the high fat diet? What is the percentage of nutrient composition (fat, protein & carbohydrate) for the standard diet and high fat diet?

Response: The composition of HFD has been presented in the method for preparation of high fat diet. The reference also has added.

4. Sentence repetition in experimental design 'The treatments given to them are described below.

Response: The repetition has been deleted

5. Group 1 treatment - was the standard diet given throughout the 42 days?

Response: Group 1 is the normal group: the standard diet given throughout the study (28 days). We added figure 1, to make it more clear.

6. Statement on treatment for Group II and Group III is unclear. Seems like after the induction period (14 days) of high fat diet, the post diet (28 days) was only CMC-Na and SPLE, respectively.

Response: We added the sentence : After HFD induction in group II-V, beside treatment, the rats were fed by standard diet. The treatment in group II-V were given along the 28 days of studies

7. Suggest to include the average daily food intake of the rats according to the treatment group in the method and result.

Response: The average daily of food intake did not record in this study. The food provided daily is 30g/rat, but usually food is left over.

8. After the diet induction, did the rats develop hyperlipidemia prior to the post treatment? Include the data.

Response: We reported this result in another study. So we add in the discussion. The HFD induction model used in this study also was 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).

9. How was the stability of the SPLE used in the post treatment? Include the information.

Response: we add in the experimental design: The suspension of sample was prepared daily before treatment, to maintain from microbe contamination.

10. Provide supporting reference to indicate that rutin is the main phenolic compound found in sweet potato leave. From the HPLC profile around RT 7.2 min, there is another large peak. What is the possibility of it being another important phenolic compound?

Response: We added some information in the discussion section as well as references to support this finding: Rutin is quercetin derivate with a hydroxy group at position C-3 substituted with glucose and rhamnose sugar groups. Quercetin derivates 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, Nie, and Zhu 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 derivates (Manhivi and Sivakumar 2021).

11. Is the data in Table 1 based on the data from published article in International Journal of Public Health Science (2021), 10, 558-564?

Response: The source of data is the same. But we analyzed and presented the data in different ways.

Response for Reviewer 2 comments (in the manuscripts)

1. Suggestion to paraphrase the sentence in the abstract has been done and written with blue fonts.

2. Suggestion to add lowere or increase in how many percentage or fold
   Response: As the limitation in number of words in abstract, the percentage of increasing or lowering were added in the discussion session.

3. Keywords should be alphabetical order
   Response: It has been done.

4. The additional information of extraction procedure
   Response: we added the sentence "The leaves were dried using oven with 50 ^{o}C temperature and then crushed"
5. What is the percentage of fats and kcal per 100g? state the percentage of each protein, carbohydrate and fats, and also the kilocalorie.
   Response: we added in the preparation of HFD: 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.

6. Do you mean that after 14 days, on day 15 until end of study, all groups were given normal diet instead of the HFD? If yes, this must be mentioned clearly.
   Response: we added in the experimental design "After HFD induction in group II-V, beside treatment, the rats also were feed by standard diet". We also added the Figure 1 to make the experimental design more clear.

7. What is the range of concentration BSA used? Please state
   Response: We added the information in the protein determination level "The concentration series of BSA were 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml".

8. How about statistical analysis? how you analyse your result?
   Response: We added the statistical analysis: "The quantitative data were analysed statistically using SPSS 16.0 for windows. The anlysis was carried out by ANOVA and followed by post hoc test (LSD) with significant level 0.05 for comparing treatment group with control group.

9. Should run t-test to see the different between initial and final weight of each group.
   Response: It has been done we added the significant data with *.

10. it's good to also report increase or decrease in % or fold, so that reader can see more clearly.
    Response: We added in the discussion: 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.

11. (table 2). What statistical test are you using? please indicate. ANOVA?what is your post hoc test? how many rats per group also should be stated. tables must be self explanatory.
    Response: we added in the note of table 2: The statistical analysis were carried out by ANOVA and followed by LSD. The data presented as mean from 5 rat per groups.

12. Why you do 3 different doses? i believe that you want to see if there is any different between them. the way you analyse your result, i can't see their difference statistically. This is because it's also crucial to see and come to conclusion which dose is the best dose (and to get this conclusion, you need to have statistical data between the 3 different doses).
    Response: We added in the discussion of SOD, CAT, GSH-Px and MDA the statistical analysis between the SPLE treated groups. We wrote with the font color.

Regards
Nurkhasanah, (Ph.D)
Profesor
Faculty of Pharmacy, Universitas Ahmad Dahlan
Yogyakarta

Korespondensi 14 Maret 2022

[Sains Malaysiana] Editor Decision External Inbox ×

Assoc Prof Dr Seng Joe Lim <joe@ukm.edu.my> 14 Mar 2022, 15:05
to me

Dear Dr Nurkhasanah Mahfudh:

The referee has commented on your paper titled: "Sweet potato (Ipomoea batatas L) leaves ethanol extract increases endogenous antioxidant activities in hyperlipidemic rats".

We would be glad to reconsider the paper if you are willing to amend the paper according to the recommendations by the referee.

Please find the comments with this email (located towards the end of this email, or as an email attachment, or both).

Please provide a List of Responses by answering / responding to every comments / suggestions when resubmitting the revised paper in a separate file.

Re-submission should be made online within 4 weeks.

Thank you.

Assoc Prof Dr Seng Joe Lim
Department of Food Sciences,
Faculty of Science and Technology,
Universiti Kebangsaan Malaysia,
43600 UKM Bangi, Selangor,
Malaysia
joe@ukm.edu.my

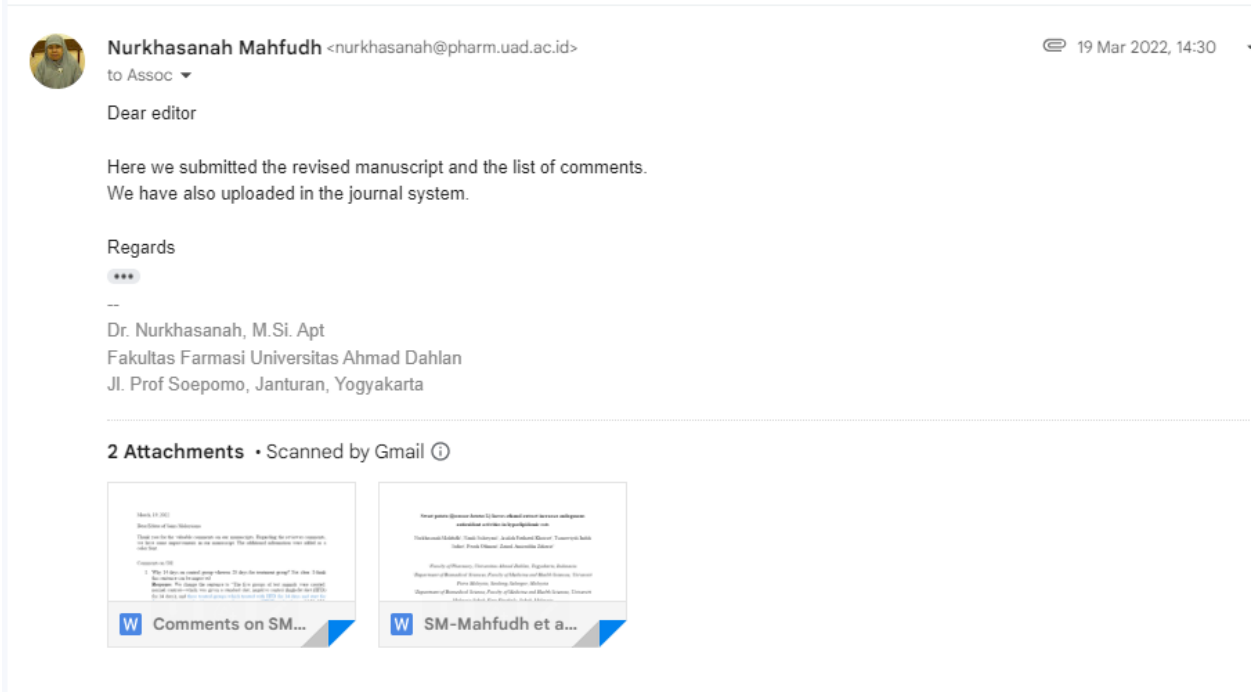
Reviewer A:

Comments on the manuscript for authors:
My comments can be viewed in the manuscript.

Sains Malaysiana
http://ejournal.ukm.my/jsm

One attachment • Scanned by Gmail

W 52439-179263-1-...



Perbaikan manuskrip ke-2 (perbaikan diberi warna biru)

Sweet potato (*Ipomoea batatas* L) leaves ethanol extract increases endogenous antioxidant activities in hyperlipidemic rats

Nurkhasanah Mahfudh[1], Nanik Sulistyani[1], Asiilah Fatihatul Khoirot[1], Tsanawiyah Indah Safira[1], Fezah Othman[2], Zainul Amiruddin Zakaria[3]

[1]*Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia*
[2]*Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*
[3]*Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia*

***corresponding author**: Nurkhasanah Mahfudh: nurkhasanah@pharm.uad.ac.id

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 hours, 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.

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 and 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 (Switala and Loewen 2002; Kodydková et al. 2014). However, the activities of these intracellular antioxidant enzymes can decrease due to oxidative stress (Araujo et al. 1995; Halder and 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 (Rumbaoa, Cornago, and Geronimo 2009; Islam 2006; Meira et al. 2012).

For the above 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, Jawi, and Satriyasa 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 Method

Materials

The leave of sweet potato used in this study were collected from Magelang, Central Java, Indonesia. The sample was authenticated and deposited in the 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 ^{o}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 hours by maceration. To get a concentrated extract, the filtrate was evaporated using a vacuum rotary evaporator at a temperature of 60 ^{o}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 brailler-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 are protein 19.0-20.0%, lipid 5%, carbohydrate 50%, and the calorie are 310 cal/100 gram. During the experiment the animals 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 animal is 5 animals in each cages.

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, Nurkhasanah, and Sulistyani 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 are described in Figure 1, and the group dividing of animal treatment were presented in Figure 2.

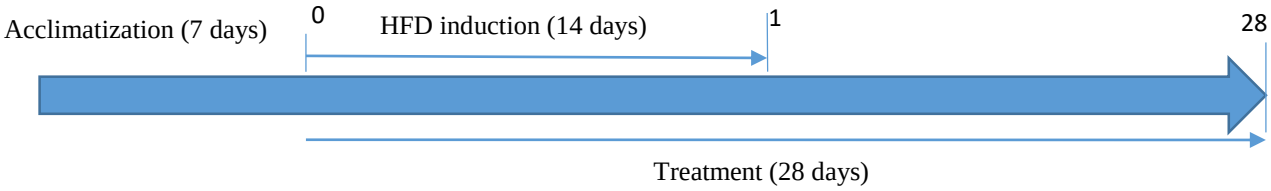


Figure 1. Desain for HFD induction and treatments

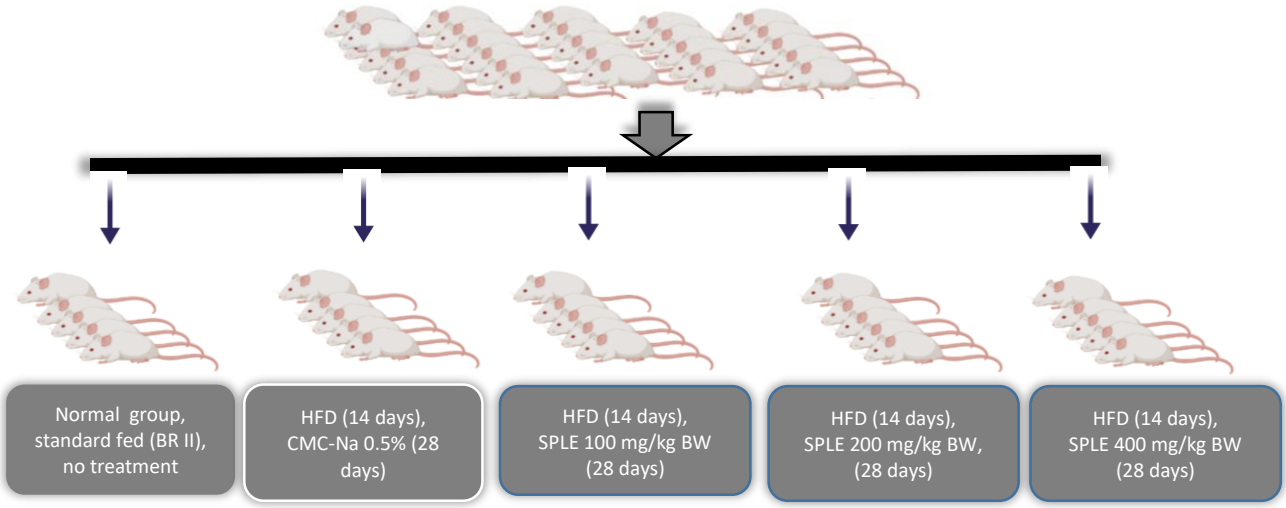


Figure 2. The group dividing of animal treatment

The body weight was monitored weekly. The test rats were anesthetized using carbondioxyde (CO_2) gas on the 29th 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 minutes.

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 minutes. 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 Elabscience 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_2*^-), 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 Elabscience assay kit (E-BC-K031-S) according to the procedure described in the manufacturer's instruction. The enzyme CAT works by decomposing H_2O_2 into H_2O. The remaining H_2O_2 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 Elabscience 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 Elabscience 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 anlysis 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 it's area under curve (AUC), it makes up 17.4% of all the components in this extract.

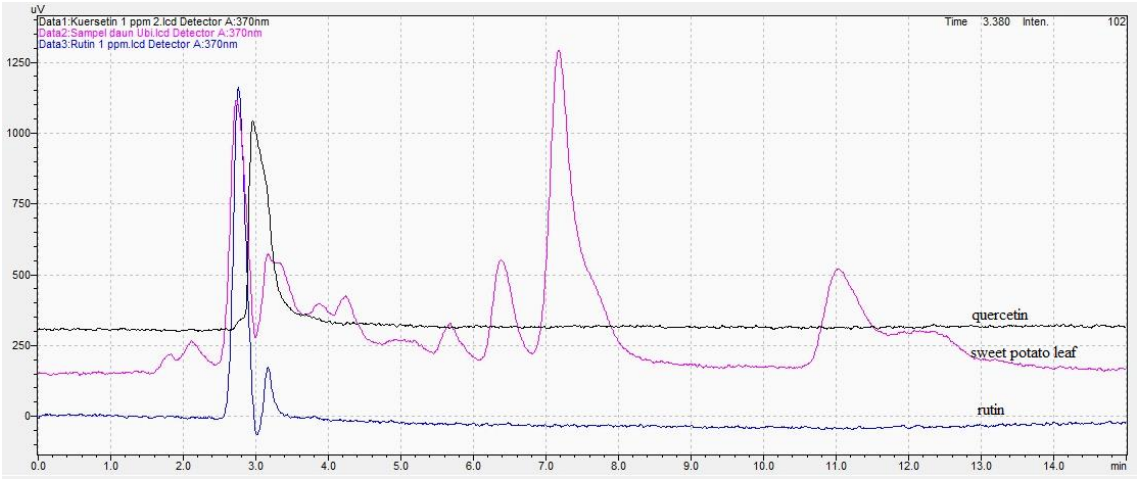


Figure 3. The HPLC chromatogram of sweet potato leaf extract in comparison with rutin and quercetin.

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.

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
SPLE 200 mg/ kg BW	148.82 ± 16.96	118.16 ± 13.11	-30.66
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 significantly 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 BW. However, the increasing activity of SOD in SPLE 200 mg/kg BW was not significantly different compare to SPLE 100 mg/kg BW.

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 ± SD)**
Normal	$76.73 \pm 12.91^{\#}$
Negative control	$44.29 \pm 22.13^{*}$
SPLE 100 mg/kg BW	$94.39 \pm 22.54^{\#}$
SPLE 200 mg/ kg BW	$100.55 \pm 19.15^{\#}$
SPLE 400 mg/kg BW	$140.31 \pm 24.55^{*\#}$

* different from the normal group significantly ($p\leq0.05$), # different from the negative control group significantly ($p\leq0,05$). The statistical analysis were carried out by ANOVA and followed by LSD. The data presented as mean from 5 rat per groups.

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 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 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 ± SD)
Normal	204.32± 21.43$^{\#}$
Negative control	75.21 ± 26.7*
SPLE 100 mg/kg BW	115.41 ± 17.65$^{*\#}$
SPLE 200 mg/ kg BW	249.19 ± 16.94$^{*\#}$
SPLE 400 mg/kg BW	263.63 ± 27.02$^{*\#}$

* different from the normal group significantly ($p \leq 0.05$), $^{\#}$ different from the negative control group significantly ($p \leq 0.05$). 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 GHS-Px activities in the normal (BR II pellet), negative control (high-fat diet), and SLPE-treatment groups (high-fat diet and SLPE). 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.

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$^{\#}$
Negative control	84.21 ± 23.62*
SPLE 100 mg/kg BW	114.28 ± 8.24*
SPLE 200 mg/ kg BW	172.68 ± 30.54$^{\#}$
SPLE 400 mg/kg BW	184.87 ± 2.25$^{\#}$

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

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 give the best result in this study. The MDA level in this group decrease until normal level.

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	$14.14 \pm 0.47^{\#}$
Negative control	$86.88 \pm 2.8^{*}$
SPLE 100 mg/kg BW	$70.15 \pm 9.57^{\#*}$
SPLE 200 mg/ kg BW	$45.61 \pm 0.60^{\#*}$
SPLE 400 mg/kg BW	$18.92 \pm 1.63^{\#}$

*significantly different from the normal group ($p \leq 0.05$), #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 also was 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 and 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 (Lattimer and Haub 2010; Isken et al. 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 and 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, Boyce, and Somasundram 2011; Zhang et al. 2019) and in vivo (Koncic, Petlevski, and Kalodera 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, Navarro, and Pérez 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 derivate with hydroxy group at position C-3 substituted with glucose and rhamnose sugar groups. Quercetin derivate 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, Nie, and Zhu 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 derivates (Manhivi and Sivakumar 2021). A previous in vitro test of SPLEs has shown higher antioxidant activities than α-tocopherol and higher radical scavenging activities than ethylenediaminetetraacetic acid (EDTA) (Rumbaoa, Cornago, and Geronimo 2009). Furthermore, as one of the major compounds in the SPLE (see 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, Guo, and Yuan 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, Stanely, and Prince 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-tricaffeoylquinic acid (Zhang et al. 2019); quercetin 3-O-β-D-sophoroside, quercetin, benzyl β-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 and Jawi 2012). In an in vitro study, elevated GSH levels were observed after applying high-concentration SPLEs to treat Hep G2 cells (Koncic, Petlevski, and Kalodera 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, Ansarin, and Jouyban 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 and 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 conclude 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

Acknowledgment

The authors are grateful to the Ministry of Education and Culture of the Republic of Indonesia for funding this study through the Postgraduate Research Scheme 2020 with the contract number PTM034/SKPP.TT/LPPM UAD/VI/2020.

References

Aguilar, Tomás Alejandro Fregoso, Brenda Carolina Hernández Navarro, and Jorge Alberto Mendoza Pérez. 2016. "Endogenous Antioxidants: A Review of Their Role in Oxidative Stress." *A Master Regulator of Oxidative Stress - The Transcription Factor Nrf2*, no. December. https://doi.org/10.5772/65715.

Al-rejaie, Salem S, Abdulaziz M Aleisa, Mohamed M Sayed-ahmed, Othman A Al-shabanah, Hatem M Abuohashish, Mohammed M Ahmed, Khaled A Al-hosaini, and Mohamed M Hafez. 2013. "Protective Effect of Rutin on the Antioxidant Genes Expression in Hypercholestrolemic Male Westar Rat." *BMC Complementary & Alternative Medicine* 13 (136).

Araujo, Fernanda B., Décio S. Barbosa, Chang Y. Hsin, Raul C. Maranhão, and Dulcineia S.P. Abdalla. 1995. "Evaluation of Oxidative Stress in Patients with Hyperlipidemia."

Atherosclerosis 117 (1): 61–71. https://doi.org/10.1016/0021-9150(94)05558-Z.

Barkas, Fotios, Tzortzis Nomikos, Evangelos Liberopoulos, and Demosthenes Panagiotakos. 2020. "Diet and Cardiovascular Disease Risk among Individuals with Familial Hypercholesterolemia: Systematic Review and Meta-Analysis." *Nutrients* 12 (8): 1–22. https://doi.org/10.3390/nu12082436.

Birben, Esra, Umit Murat Sahiner, Cansin Sackesen, Serpil Erzurum, and Omer Kalayci. 2012. "Oxidative Stress and Antioxidant Defense." *WAO Journal* 5: 9–19.

Canadian Council of Animal Care. 2010. *Canadian Council on Animal Care in Science CCAC Guidelines on : Euthanasia of Animals Used*. Ottawa, Canada.

Chang, Hao-hsiang, Yi-chan Lan, Shiu-dong Chung, and Chiang-ting Chien. 2021. "Sweet Potato Leaf Feeding Decreases Cholesterol , Oxidative Stress and Thrombosis Formation in Syrian Hamsters with a High-Cholesterol Diet." *Life* 11 (802): 1–11.

Chao, Pi-yu, Wei-yu Huang, Shene-pin Hu, Hsiao-feng Lo, Kuan-hung Lin, and Meng-yuan Huang. 2013. "Indigenous Purple Vegetable Extracts Protect against Hydrogen Peroxide-Induced DNA Damage in Human Lymphocytes." *Food and Nutrition Sciences* 4: 62–70. https://doi.org/http://dx.doi.org/10.4236/fns.2013.48A008 Published.

Chen, W., Tong-jun Mao, L. Fan, Yuhan Zhou, Jing Yu, Y. Jin, and Peng-chao Hou. 2011. "Effect of Purple Sweet Potato on Lipid Metabolism and Oxidative Stress in Hyperlipidemic Rats." *Journal of Zhejiang University. Medical Sciences* 40 (4): 360–64.

Ebele, Ikekpeazu Joy, Ikekpeazu Adanna Jenniger, Eke Christian Nnabugo, Ogbu Innocent Sidney, Onyekwelu Kenechukwu Chibuike, Orji Oliver Chukwuma, Nwachukwu Daniel, and Eze Anthonius Anayochukwu. 2016. "Oxidative Stress/Lipid Peroxidation and Antioxidant Enzymes in Apparently Healthy Individuals Involved in Physical Exercise." *Asian Journal of Medical Sciences* 7 (6): 16–19. https://doi.org/10.3126/ajms.v7i6.15245.

Enogieru, Adaze Bijou, William Haylett, Donavon Charles Hiss, Soraya Bardien, and Okobi Eko Ekpo. 2018. "Review Article Rutin as a Potent Antioxidant : Implications for Neurodegenerative Disorders." *Oxidative Medicine and Cellular Longevity* 2018: 1–17. https://doi.org/https://doi.org/10.1155/2018/6241017.

Girsang, Ermi, I Nyoman Ehrich Lister, Chrismis Novalinda Ginting, Ika Adhani Sholihah, Martinus Ahmad Raif, and Sidharta Kunardi. 2020. "Antioxidant and Antiaging Activity

of Rutin and Caffeic Acid." *Pharmaciana* 10 (2): 147–56. https://doi.org/10.12928/pharmaciana.v10i2.13010.

Gusti, Amani M.T., Safaa Y. Qusti, Eida M. Alshammari, Eman A. Toraih, and Manal S. Fawzy. 2021. "Antioxidants-Related Superoxide Dismutase (Sod), Catalase (Cat), Glutathione Peroxidase (Gpx), Glutathione-s-Transferase (Gst), and Nitric Oxide Synthase (Nos) Gene Variants Analysis in an Obese Population: A Preliminary Case-Control Study." *Antioxidants* 10 (4). https://doi.org/10.3390/antiox10040595.

Halder, Suranjana, and Maitree Bhattacharyya. 2014. "Oxidative Stress: Lipid Peroxidation Products as Predictors in Disease Progression." *Journal of Experimental and Integrative Medicine* 4 (3): 151. https://doi.org/10.5455/jeim.210414.rw.007.

Hariri, Niloofar, and Louise Thibault. 2010. "High-Fat Diet-Induced Obesity in Animal Models." *Nutrition Research Reviews* 23 (2): 270–99. https://doi.org/10.1017/S0954422410000168.

Heriwijaya, I Pande Putu Deny, I Made Jawi, and Bagus Komang Satriyasa. 2020. "Uji Efektivitas Ekstrak Air Daun Ubi Jalar Ungu (Ipomoea Batatas) Terhadap Profil Lipid Tikus Putih Jantan Galur Wistar Yang Diinduksi Pakan Dislipidemia." *Intisari Sains Medis* 11 (2): 452–56. https://doi.org/10.15562/ism.v11i2.584.

Hue, Seow-mun, Amru Nasrulhaq Boyce, and Chandran Somasundram. 2011. "Comparative Study on the Antioxidant Activity of Leaf Extract and Carotenoids Extract from Ipomoea Batatas Var. Oren (Sweetpotato) Leaves." *International Journal of Nutrition and Food Engineering* 5 (10): 604–7.

Ihwah, A, P Deoranto, S Wijana, and IA Dewi. 2018. "Comparative Study between Federer and Gomez Method for Number of Replication in Complete Randomized Design Using Simulation : Study of Areca Palm (Areca Catechu) as Organic Waste for Producing Handicraft Paper Comparative Study between Federer and Gomez." *IOP Conf. Series: Earth and Environmental Science 131* 131 (012049): 0–6. https://doi.org/doi :10.1088/1755-1315/131/1/012049.

Isken, Frank, Susanne Klaus, Martin Osterhoff, Andreas F.H. Pfeiffer, and Martin O. Weickert. 2010. "Effects of Long-Term Soluble vs. Insoluble Dietary Fiber Intake on High-Fat Diet-Induced Obesity in C57BL/6J Mice." *The Journal of Nutritional Biochemistry* 21 (4): 278–84. https://doi.org/10.1016/j.jnutbio.2008.12.012.

Islam, Shahidul. 2006. “Sweetpotato (Ipomoea Batatas L.) Leaf: Its Potential Effect on Human Health and Nutrition.” *Journal of Food Science* 71 (2): R13–121. https://doi.org/10.1111/j.1365-2621.2006.tb08912.x.

Islam, Shahidul, M Yoshimoto, K Ishiguro, and O Yamakawa. 2016. “Bioactive Compounds in Ipomoea Batatas Leaves.” In *Proc XXVI IHC - Issues and Advances in Postharvest Hort*, 693–99. https://doi.org/10.17660/ActaHortic.2003.628.88.

Jang, In-Sil, and Mj-Ja Choi. 2019. “Effects of Isoflavone Supplementation on Lipid Profiles and Antioxidant Enzyme Activities in Growing Rats Fed High Fat Diet.” *Clinical Nutrition Research* 8 (4): 296–306. https://doi.org/10.7762/cnr.2019.8.4.296.

Jawi, I M, and A Wiwiek Indrayani. 2015. “Aqueous Extract of Balinese Purple Sweet Potato (Ipomoea Batatas l .) Prevents Oxidative Stress and Decreases Blood Interleukin-1 in Hypercholesterolemic Rabbits.” *Bali Medical Journal* 4 (1): 37–40.

Kamalakkannan, Narasimhanaidu, Ponnaian Stanely, and Mainzen Prince. 2006. “Antihyperglycaemic and Antioxidant Effect of Rutin , a Polyphenolic Flavonoid , in Streptozotocin-Induced Diabetic Wistar Rats.” *Pharmacology & Toxicology* 98: 97–103.

Khoubnasab Jafari, Maryam, Khalil Ansarin, and Abolghasem Jouyban. 2015. “Comments on ‘Use of Malondialdehyde as a Biomarker for Assesing Oxidative Stress in Different Disease Pathologies: A Review.’” *Iranian Journal of Public Health* 44 (5): 714–15.

Kodydková, J, L Vávrová, M Kocík, and A Žák. 2014. “Review Article Human Catalase , Its Polymorphisms , Regulation and Changes of Its Activity in Different Diseases.” *Folia Biologica (Praha)* 60: 153–67.

Koncic, Marijana Zovko, Roberta Petlevski, and Zdenka Kalodera. 2013. “Antioxidant Activity of Ipomoea Batatas L. Lam. Leaf Grown in Continental Croatia and Its Effect on Glutathione Level in Glucose-Induced Oxidative Stress.” *International Journal of Food Properties* 16 (5): 964–73. https://doi.org/10.1080/10942912.2011.573117.

Krochmal-marczak, Barbara, Tomasz Cebulak, Ireneusz Kapusta, Joanna Kaszuba, and Natalia Zurek. 2020. “The Content of Phenolic Acids and Flavonols in the Leaves of Nine Varieties of Sweet Potatoes (Ipomoea Batatas L .) Depending on Their Development , Grown in Central Europe.” *Molecules* 25 (3473). https://doi.org/https://doi.org/10.3390/molecules25153473.

Kumar, Vinay, Ahmed Abdullah Khan, Anu Tripathi, Praveen K Dixit, and U K Bajaj. 2015. "Role of Oxidative Stress in Various Diseases : Relevance of Dietary Antioxidants." *The Journal of Phytopharmacology* 4 (2): 126–32.

Kurata, Rie, Tooru Kobayashi, Takanori Ishii, Hiroshi Niimi, Seiji Nisaka, Masaomi Kubo, and Masaoki Kishimoto. 2017. "Influence of Sweet Potato (Ipomoea Batatas L.) Leaf Consumption on Rat Lipid Metabolism." *Food Science and Technology Research* 23 (1): 57–62. https://doi.org/10.3136/fstr.23.57.

Lattimer, James M., and Mark D. Haub. 2010. "Effects of Dietary Fiber and Its Components on Metabolic Health." *Nutrients* 2 (12): 1266–89. https://doi.org/10.3390/nu2121266.

Lee, Chia Lin, Shou Lun Lee, Chao Jung Chen, Hsin Chun Chen, Ming Ching Kao, Chuan Hao Liu, Jau Yang Chen, Yen Ting Lai, and Yang Chang Wu. 2016. "Characterization of Secondary Metabolites from Purple Ipomoea Batatas Leaves and Their Effects on Glucose Uptake." *Molecules* 21 (6): 1–14. https://doi.org/10.3390/molecules21060745.

Mahfudh, Nurkhasanah, Nanik Sulistyani, Muhammad Syakbani, and Athifah Candra Dewi. 2021. "The Antihyperlipidaemic and Hepatoprotective Effect of Ipomoea Batatas L . Leaves Extract in High-Fat Diet Rats." *International Journal of Public Health Science (IJPHS)* 10 (3): 558–64. https://doi.org/10.11591/ijphs.v10i3.20777.

Manhivi, Vimbainashe E, and Dharini Sivakumar. 2021. "An Evaluation of Phenolic Compounds , Carotenoids , and Antioxidant Properties in Leaves of South African Cultivars , Peruvian 199062 . 1 and USA ' s Beauregard." *Frontiers in Nutrition* 8 (November): 1–10. https://doi.org/10.3389/fnut.2021.773550.

Meira, Marilena, Eliezer Pereira da Silva, Jorge M. David, and Juceni P. David. 2012. "Review of the Genus Ipomoea: Traditional Uses, Chemistry and Biological Activities." *Brazilian Journal of Pharmacognosy* 22 (3): 682–713. https://doi.org/10.1590/S0102-695X2012005000025.

Nagai, Miu, Mariko Tani, Yoshimi Kishimoto, Maki Iizuka, Emi Saita, Miku Toyozaki, Tomoyasu Kamiya, Motoya Ikeguchi, and Kazuo Kondo. 2011. "Sweet Potato (Ipomoea Batatas L.) Leaves Suppressed Oxidation of Low Density Lipoprotein (LDL) in Vitro and in Human Subjects." *J. Clin. Biochem. Nutr* 48 (3): | 203–208. https://doi.org/doi: 10.3164/jcbn.10_84.

Noori, S. 2012. “An Overview of Oxidative Stress and Antioxidant Defensive System.” *Open Access Sci Reports* 1 (8): 1–9.

Poli, Andrea, Carlo M. Barbagallo, Arrigo F.G. Cicero, Alberto Corsini, Enzo Manzato, Bruno Trimarco, Franco Bernini, et al. 2018. “Nutraceuticals and Functional Foods for the Control of Plasma Cholesterol Levels. An Intersociety Position Paper.” *Pharmacological Research* 134 (May): 51–60. https://doi.org/10.1016/j.phrs.2018.05.015.

Rodríguez, E., J. L. Arqués, R. Rodríguez, M. Nuñez, M. Medina, T. L. Talarico, I. A. Casas, et al. 1989. “Antioxidant Compounds and Their Antioxidant Mechanism.” *Intech Open* 32: 137–44. https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics.

Rumbaoa, Rowena Grace O., Djanna F. Cornago, and Inacrist M. Geronimo. 2009. “Phenolic Content and Antioxidant Capacity of Philippine Sweet Potato (Ipomoea Batatas) Varieties.” *Food Chemistry* 113 (4): 1133–38. https://doi.org/10.1016/J.FOODCHEM.2008.08.088.

Sari, Novita, Nurkhasanah, and Nanik Sulistyani. 2020. “The Antioxidant Effect of Bangle (Zingiber Cassumunar) Rhizome Extract on Superoxide Dismutase (Sod) Activity in Hyperlipidemic Rats.” *Research Journal of Chemistry and Environment* 24 (1): 78–81.

Singh, Sneha, Vandana Panda, S. Sudhamani, and Payal Dande. 2020. “Protective Effect of a Polyherbal Bioactive Fraction in Propylthiouracil-Induced Thyroid Toxicity in Ratsby Modulation of the Hypothalamic–Pituitary–Thyroid and Hypothalamic–Pituitary–Adrenal Axes.” *Toxicology Reports* 7 (2020): 730–42. https://doi.org/10.1016/j.toxrep.2020.06.002.

Sumardika, I Wayan, and I Made Jawi. 2012. “Water Extract of Sweet Potato Leaf Imrpoved Lipid Profile and Blood SOD Content of Rats with High Cholesterol Diet.” *Medicina* 43 (2): 67–70.

Switala, Jacek, and Peter C Loewen. 2002. “Diversity of Properties among Catalases.” *Archives of Biochemistry and Biophysics* 401: 145–54.

Valko, Marian, Dieter Leibfritz, Jan Moncol, Mark T.D. Cronin, Milan Mazur, and Joshua Telser. 2007. “Free Radicals and Antioxidants in Normal Physiological Functions and Human Disease.” *International Journal of Biochemistry and Cell Biology* 39 (1): 44–84.

https://doi.org/10.1016/j.biocel.2006.07.001.

Walker, John M. 1996. *The Protein Protocols Handbook*. Edited by John M Walker. Second edi. Hatfield: University of Hertfordshire.

Wang, Sunan, Shaoping Nie, and Fan Zhu. 2016. "Chemical Constituents and Health Effects of Sweet Potato." *Food Research International* 89: 90–116. https://doi.org/10.1016/j.foodres.2016.08.032.

Yang, Jian xiong, Juan Guo, and Jiangfeng Yuan. 2008. "In Vitro Antioxidant Properties of Rutin." *LWT - Food Science and Technology* 41 (6): 1060–66. https://doi.org/https://ddoi.org/10.1016/j.lwt.2007.06.010.

Zhang, Chengcheng, Daqun Liu, Liehong Wu, Zhang Jianming, Xiaoqiong Li, and Weicheng Wu. 2019. "Chemical Characterization and Antioxidant Properties of Ethanolic Extract and Its Fractions from Sweet Potato (Ipomoea Batatas L) Leaves." *Foods* 9 (15): 1–14.

Respon to reviewer

March, 19, 2022

Dear Editor of Sains Malaysiana

Thank you for the valuable comments on our manuscripts. Regarding the reviewer comments, we have some improvements in our manuscript. The additional information were added as a color font.

Comments on SM:

1. Why 14 days on control group whereas 28 days for treatment group? Not clear. I think this sentence can be improved
   Response: We change the sentence to "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"

2. …and then crushed using?
   Response: …and then crushed using a blender

3. State how many rats in a cage, the nutritional content of standard diet. Ex: carbohydrate xx%, protein xx% etc and also the kcal
   Response: we added in the method " The nutrition content of BR II are protein 19.0-20.0%, lipid 5%, carbohydrate 50%, and the calorie are 310 cal/100 gram. The number of animals is 5 animals in each cage.

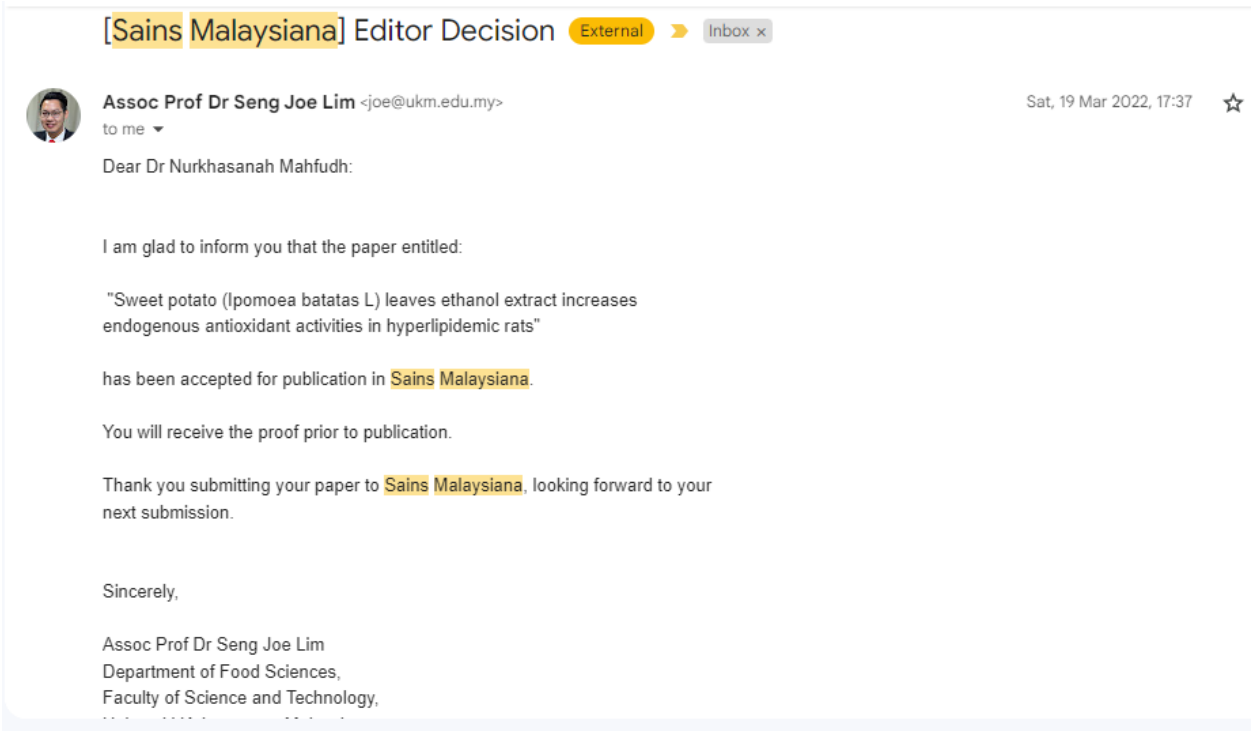
4. Better all these change to 1 figure. This way, its not very clear
   Response: It has been added the Figure 2

5. The hfd was given only for 14 days? And the remaining 28 days was 1% sodium carboxymethylcellulose (CMC-Na) + normal diet? Nee to be clearly stated.
   Response: It has been added in Figure 2.

6. How do you know the cholesterol level increase? You did not measure cholesterol level. How do you justify that the rats is a hyperlipidemic rats?
   Response: We cited from the previous studies (Mahfudh et al, 2021).

7. I think the sentences in red is more like an introduction and statement that your sample contain rutin. And this statement is a repeated statement
   Response: This sentence is based on the study. We studied the chemical content using HPLC. And the HPLC result was also presented in this manuscript.

Korespondensi 19-3-2022 (pernyataan accepted dari editor)



[Sains Malaysiana] Editor Decision External Inbox ×

Assoc Prof Dr Seng Joe Lim <joe@ukm.edu.my> Sat, 19 Mar 2022, 17:37

to me

Dear Dr Nurkhasanah Mahfudh:

I am glad to inform you that the paper entitled:

"Sweet potato (Ipomoea batatas L) leaves ethanol extract increases endogenous antioxidant activities in hyperlipidemic rats"

has been accepted for publication in Sains Malaysiana.

You will receive the proof prior to publication.

Thank you submitting your paper to Sains Malaysiana, looking forward to your next submission.

Sincerely,

Assoc Prof Dr Seng Joe Lim
Department of Food Sciences,
Faculty of Science and Technology,