International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Special Issue 1, 2024

Original Article

THE EFFECT OF CATECHINS FROM PURIFIED GAMBIER (*UNCARIA GAMBIR* ROXB.) AND VITAMIN C ON MALONDIALDEHYDE (MDA) LEVELS OF MALE WHITE MICE AFTER PHYSICAL ACTIVITY

RAHMI YOSMAR, AYU AMELIA PUTRI, ALMAHDY A.*

Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Andalas University, Padang-25175, Indonesia
***Corresponding author: Almahdy A.; *Email: almahdya@phar.unand.ac.id**

Received: 17 Oct 2023, Revised and Accepted: 23 Nov 2023

ABSTRACT

Objective: The aim of this study was to assess the impact of catechins from purified gambier and vitamin C on the MDA levels of male white mice following maximum physical activity.

Methods: This research employed an experimental approach using a Post Test Only Control Group Design with male white mice. The interventions included purified gambir catechin and vitamin C alongside physical fatigue-inducing activity. The primary focus was assessing serum MDA levels in these mice, measured using the Thiobarbituric Acid Reactive Substance (TBARS) method. The test subjects were divided into five groups and average serum MDA levels were measured in each group, followed by an analysis checking for normal distribution and homogeneity. Subsequently, One Way Analysis of Variance (ANOVA) was conducted. If significant differences were observed among the groups, further analyses were performed with a significance level of $p<0.05$. All tests maintained a confidence level of 95% ($\alpha = 0.05$).

Results: The average serum MDA values for the treated groups were 1.63 nmol/ml for the negative control, 2.47 nmol/ml for the positive control, 1.75 nmol/ml for purified gambier catechin 200 mg/kgBW, 1.93 nmol/ml for Vitamin C 65 mg/kgBW, and 1.65 nmol/ml for purified gambier catechin 100 mg/kgBW and vitamin C 32.5 mg/kgBW. Based on the Kruskal-Wallis test analysis, there was a significant difference in serum MDA levels ($p<0.05$) with a significance value of 0.004 of each group. However, the Mann-Whitney test showed that the negative control group significantly differed from the positive control group and there was a significant difference between the positive control and the treatment groups.

Conclusion: The administration of catechins from purified gambier and vitamin C reduced MDA levels following maximum physical activity. Further study is recommended to investigate the efficacy of various antioxidants and their combinations in reducing MDA levels.

Keywords: Catechins from purified gambier, Vitamin C, Antioxidant, Free radicals, Physical activity, Malondialdehyde

DOI: https://dx.doi.org/10.22159/ijap.2024.v16s1.11 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Physical activity is now widely recognized as a lifestyle component, much like traditional medicine. Light exercise activities and an active lifestyle have shown numerous benefits in primary and secondary disease prevention, such as cardiovascular disease [1], type II diabetes mellitus [2], metabolic syndrome [3], and neurodegenerative diseases like Alzheimer's [4, 5]. Malondialdehyde (MDA) levels serve as a measure for assessing the body's oxidative stress. Gambier contains polyphenolic compounds which are extracted from the leaves of the Gambier plant. Gambier extract contains chemical compounds, including catechin (7-33%), katechu tannic acid (20-55%), pyrocatechol (20-30%), red katechu (3-5%) quercetin (2-4%), gambier fluorescent (1-3), wax (1-2%), fixed oil (1-2%) [5]. The main components in Gambier consist of catechins (catechin acid), catechin tanat acid (catechin anhydride), and quercetine.

The polyphenolic compounds found in gambier extract are catechins which function as antioxidant, antimicrobial and anti-inflammatory compounds. The catechins in gambier are included in the secondary metabolites of the flavonoid group. Catechins from purified gambier are obtained by isolating gambier leaf extract so that catechin isolates will be produced with levels that meet the requirements of the Indonesian Herbal Pharmacopoeia that the catechin content should not be less than 90% [6].

In addition, another source of antioxidants is vitamin C. Vitamin C as a natural antioxidant, can prevent oxidation in the body, and vitamin C is a water-soluble vitamin. Vitamin C is commonly used as an antioxidant because of its effectiveness in inhibiting free radicals, thereby preventing oxidative stress [7]. Vitamin C or ascorbic acid is a source of natural antioxidants which are synthesized *in vivo* from glucose in the liver. Humans cannot synthesize vitamin C in their own bodies due to the absence of the gulonolactone oxidase enzyme, namely 2-keto-1-gulonolactone [8].

Physical activity is a body movement performed by skeletal muscles that requires energy in the form of daily physical activities such as walking, cycling, swimming, or sports that are carried out in a structured manner [9]. Physical exercise has many benefits for the health of the body when done regularly and properly, including reducing the risk of degenerative diseases [10]. Maximum physical activity that is carried out requires up to 100-200 times more oxygen which will later become reactive oxygen compounds [11].

Free radicals are molecules in their outer orbit that have an unpaired electron. These free radicals are reactive and can oxidize or reduce other atoms in the body. Examples of free radicals are lipid peroxyl (LOO), peroxyl (ROO-), hydroxyl (OH-), superoxide (O2-), and nitrogen dioxide (NO2-). Free radicals in moderation function as the body's defense system against pathogenic microorganisms and help in cell development. It is different if there is an increase in free radicals in the body which can cause damage to tissues [12, 13].

Oxidative stress is a condition where there the amount of oxidants (free radicals) and antioxidants in the body is not balanced, which will cause damage. Some free radicals in the body are oxygen derivatives and nitrogen derivatives. These oxygen derivatives are usually called Reactive Oxygen Species (ROS), while nitrogen derivatives are called Reactive Nitrogen Species (RNS) [14]. Excessive ROS will affect lipid peroxidation so that it can cause cell death. Lipid peroxidation is a process that involves the formation of free radicals from Polyunsaturated Fatty Acid (PUFA) or unsaturated fatty acids [15].

Antioxidants are compounds that are able to stabilize free radicals before they cause damage to normal cells, so they are needed by the body. Antioxidants in stabilizing free radicals will complement the lack of electrons from free radicals and prevent reactions that can cause oxidative stress. Antioxidants are divided into two, namely enzymatic antioxidants and non-enzymatic antioxidants [16]. Enzymatic antioxidants include the enzymes superoxide dismutase

(SOD), glutathione peroxidase (GPx), and catalase (CAT). Non-enzymatic antioxidants are further divided into two, namely first, water-soluble antioxidants, including ascorbic acid, metal-binding proteins, heme-binding proteins, and uric acid. Second, fat-soluble antioxidants, including tocopherols, carotenoids, flavonoids, bilirubin, and quinones [17].

Malondialdehyde (MDA) as a product of lipid peroxidation is a biomarker that can be used to determine the state of oxidative stress in the body. Lipid peroxidation occurs when ROS generated under conditions of oxidative stress interact with polyunsaturated fatty acids (PUFAs) in the lipid bilayer of cell membranes [18]. Increased levels of MDA in the blood indicate that there are many free radicals and unsaturated fats in the body. This makes MDA one of the parameters of antioxidant activity as an antidote to oxidative stress. Providing antioxidants will reduce MDA levels which help the body ward off free radicals [15]. The goal of this research was to evaluate how catechins sourced from purified gambier, combined with vitamin C, affect the levels of MDA in male white mice after they engage in intense physical activity. In this study, Gambier and vitamin C are natural antioxidants that serve as the body's defense system against oxidative stress conditions. It is expected that this research can demonstrate the administration of a combination of antioxidants, namely catechins from purified Gambier and vitamin C, can inhibit the formation of MDA, which means it can hinder the occurrence of oxidative stress.

MATERIALS AND METHODS

Material

The materials utilized were catechin from purified gambier containing>90% catechin obtained from PT. Andalas Sitawa Fitolab, Padang. This gambier has an analysis certificate meeting the specified requirements under No. 01/PE-FP/2020, along with vitamin C, 1% TBA reagent, 5% TCA reagent, and MDA standard with a concentration of 3.65 nmol/ml.

Methods

This research was conducted experimentally using a Post Test Only Control Group Design on male white mice. The treatments administered included purified gambir catechin and vitamin C, followed by inducing oxidative stress through physical fatigue activity involving swimming. Meanwhile, the measured outcome was the serum MDA level in male white mice.

Ethical aprroval

This research has received ethical approval for health research from the Faculty of Pharmacy, Andalas University, with no. 06/UN.16.10. D. KEPK-FF/2023.

Determination of purified gambier dosage

The dose of purified gambier given to mice test animals is in accordance with previous research that the optimal dose for reducing serum MDA levels is 200 mg/kgbb [20]. To determine the administrative volume of gambier is done using the following formula:

$$\text{Drug administration volume} = \frac{\text{BW X Dosage/KgBW}}{\text{Concentration } (\frac{\text{mg}}{\text{ml}})}$$

Determination of vitamin C dosage

The vitamin C given to the test animals is 500 mg of vitamin C which will later be converted to the mice dose [21]. After being converted, the dose of vitamin C used was 65 mg/kgBW.

Grouping and treating the test subjects

25 male white mice (*Mus musculus L.*) were split into 5 groups for varied treatments. The first group acted as the control, receiving no experimental treatment. The second group solely engaged in physical fatigue activity. The other groups underwent experimental treatments involving purified gambir and vitamin C, combined with physical fatigue activity. The third group had purified gambir at 200 mg/kgBW, the fourth group received vitamin C at 65 mg/kgBW, and the fifth group had purified gambir at 100 mg/kgBW combined with vitamin C at 32.5 mg/kgBW. The dosage of the test formulation administered was half of the dosage given in groups 3 and 4 to prevent the occurrence of prooxidants. Prooxidants, in this context, refer to the test formulations administered that do not potentially reduce MDA levels but rather increase them, leading to oxidative stress. The physical fatigue involved swimming until signs of exhaustion and reduced limb movement appeared, lasting a maximum of about 45 min. Test solutions were orally given for 7 d, and on day eight, groups 2 to 5 engaged in physical fatigue activity. Following this, the mice were dried in sunlight anesthetized with inhaled ether, and blood samples were collected from their carotid arteries to measure serum MDA levels by using Thiobarbituric Acid Reactive Substance (TBARS) Method.

Assessment of MDA levels with the *Thiobarbituric Acid Reactive Substance* (TBARS) method

- Preparation of Test Animal Blood Serum

Blood samples flowing it into a centrifugation tube than centrifuge for 10 min at 3000 rpm. Then, the serum was taken and put into a microtube, which was stored at-20oC until testing for MDA levels was carried out

- Procedure for Inspection of MDA Levels

Serum sample of 0.2 ml is then taken put into a test tube. Add 1 ml of 5% TCA reagent then mix using a vortex for 1 minute until homogeneous. After that, centrifuge the solution at 3000 rpm for 10 min. Then, take the supernatant2 and put it in another test tube, then add 1 ml of 1% TBA reagent. Heat the solution in a water bath at 95 °C-100 °C for 30 min. The solution is cooled to room temperature. After that, measure the absorbance using a UV-Vis spectrophotometer at a wavelength of 532 nm [23]. Then, determine the value of MDA levels using the formula [22]:

$$\text{MDA levels} = \frac{\text{Absorbance Sample}}{\text{Absorbance Standard}} \text{ x Konsentrasi Standar (nmol/ml)}$$

Data analysis

After obtaining the serum MDA levels for each treatment group, the average and standard deviation of these serum MDA levels were then calculated. The assessment commenced by examining the serum MDA level data for normal distribution and homogeneity. Following this, a One-Way Analysis of Variance (ANOVA) was conducted for normally distributed data or Kruskal-Wallis for non-normally distributed data. If significant differences were observed among the groups, further analyses were carried out at a significance level of $p<0.05$. All assessments maintained a confidence level of 95% ($\alpha = 0.05$).

Table 1: The average serum MDA levels for each treatment group

Group	Treatment	N	The average serum MDA levels+SD (nmol/ml)
I (Negative Control)	No experimental treatment	5	1.63+0.17
II (Positive Control)	Swimming	5	2.47+0.30
III	Purified gambir at 200 mg/kgBW and swimming	5	1.75+0.16
IV	Vitamin C at 65 mg/kgBW and swimming	5	1.93+0.24
V	Purified gambir at 100 mg/kgBW combined with vitamin C at 32.5 mg/kgBW and swimming	5	1.65+0.19

RESULTS

According to table 1, the highest average serum MDA levels were observed in the second (II) group, reaching 2.47 nmol/ml+0.30. This group, identified as the positive control, was solely engaged in physical fatigue activity. This indicates that such activity might trigger oxidative stress, evident from the elevated serum MDA levels. Following this, the fourth group received vitamin C at 65 mg/kgBW alongside physical activity, resulting in an average serum MDA level of 1.93 nmol/ml+0.24, which is lower than the levels observed in the positive control. This suggests that vitamin C might inhibit oxidative stress, consequently reducing serum MDA levels.

The negative control group displayed an average serum MDA level of 1.63 nmol/ml, notably distinct from the positive control group engaged in physical fatigue activities, showing 2.47 nmol/ml. This suggests swimming can heighten MDA levels. In the treatment groups, those receiving the purified Gambir test showed 1.75 nmol/ml, Vitamin C administration reached 1.93 nmol/ml, and the combination yielded 1.65 nmol/ml. These findings imply that the test preparation pre-physical fatigue might possess antioxidants, mitigating the rise in free radicals. This is supported by lower serum MDA levels in the test groups compared to the solely physically fatigued positive control.

Based on the Kruskal-Wallis test analysis, there was a significant difference in serum MDA levels ($p<0.05$) with a significance value of 0.004 of each group. The analysis proceeded with the Mann-Whitney test to determine the significance of each group. Results showed that the negative control group significantly differed from the positive control group. The treatment group given a dose of 65 mg/kgBW of vitamin C exhibited no significant difference compared to the negative control group. Similarly, the treatment group receiving 200 mg/kgBW of Gambier did not significantly differ from the negative control group. Likewise, the treatment group receiving a combination of 100 mg/kgBW of Gambier and 32.5 mg/kgBW of vitamin C showed similar results. The positive control group significantly differed from both the negative control group and the treatment groups administered with 200 mg/kgBW of Gambier, 65 mg/kgBW of vitamin C, and the combined doses of 100 mg/kgBW of Gambier and 32.5 mg/kgBW of vitamin C. These findings indicate that the administration of purified gambir catechins and vitamin C, either individually or in combination, effectively reduced serum MDA levels.

DISCUSSION

Catechins function by impeding MDA formation through the initiation of free radicals, halting the oxidative reaction. They hinder both the progression and cessation phases in the typical lipid peroxidation process, promoting a non-radical process [25]. Structurally, catechins feature a hydroxyl functional group (-OH) that can scavenge free radicals by donating hydrogen. The-OH group binds with the lipid radical, releasing its H+to create an RH complex that inhibits the initiation phase. In other processes, peroxyl radicals also bond with H+groups, forming ROOH or hydroperoxide complexes, subsequently decomposed by metal chelating agents to thwart the propagation process. These actions highlight how the functional groups in catechins function as antioxidants, countering the impact of free radicals [26]. The treatment group 4 which was given vitamin C at a dose of 65 mg/kgbb had an average serum MDA level lower than the positive control group. This proves that vitamin C can reduce serum MDA levels of mice subjected to physical fatigue. In accordance with research conducted by Ghanwat *et al.* (2016) showed that administration of vitamin C as an exogenous antioxidant helps reduce oxidative stress [21]. Research conducted by Yimcharoen *et al.* (2019) also showed the same thing that Vitamin C reduces free radicals which can inhibit the process of lipid peroxidation thereby preventing cell damage [27]. Vitamin C in its active form, namely ascorbic acid, will donate its electrons to radical compounds, in this case, vitamin C acts as a free radical reducing agent. The antioxidant properties of vitamin C come from the hydroxyl group it has by donating H+along with electrons so that it counteracts the occurrence of free radicals [25].

Malondialdehyde is formed from unsaturated fats (poly unsaturated fatty acids) which have double bonds in the cell membrane. When the levels of free radicals in the blood are high, exceeding the endogenous antioxidants, this condition is called oxidative stress. The free electrons in these free radicals will bind to unsaturated fats in the cell membrane so that the double bond becomes a saturated double bond. Under these conditions, free radicals, such as reactive peroxide, reactive oxygen, reactive hydrogen, and other free radicals will bind to the lipid bilayer of the cell membrane so that they will form MDA which is a marker of oxidative stress [15]. The decrease in MDA levels could have been caused by a decrease in free radical production, an increase in antioxidant activity or it could be due to both so as to prevent or reduce oxidative stress.

When the body's MDA level is elevated, there's a tendency for increased interaction between free radicals and unsaturated fats. Conversely, a reduced MDA level indicates a lesser interaction between these elements. This suggests a direct relationship between MDA levels and the quantity of free radicals in the body [18]. Vigorous physical activity elevates oxygen demand, leading to the release of free radicals, particularly superoxide radicals [23]. From the results obtained the average value of MDA levels in the positive control group was higher than the negative control group. This indicates that the physical fatigue activity given to the positive control group test animals causes an increase in free radicals so that higher antioxidants are needed as an antidote to oxidative stress. In accordance with research conducted by Sandhiutami *et al.* (2016) also stated that there was an increase in free radicals in mice test animals after being given maximum physical activity in the form of swimming [24].

The average value of MDA levels in the treatment group 3 was lower than that of the positive control group which was given a physical fatigue activity in the form of swimming. This shows that the purified gambier given has antioxidant activity which can counteract the occurrence of free radicals so that MDA levels decrease in the body. In accordance with research conducted by Irramah *et al.* (2017) that administration of standardized gambier can reduce ethanol-induced serum MDA levels. Irramah *et al.*'s study used variations in doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg, so that the results of purified Gambir at a dose of 200 mg/kg could reduce MDA levels more than the other two variations of doses [20].

Based on a comparison of the average serum MDA levels between the other two treatment groups, namely treatment group 3 and treatment group 4, treatment group 5 was able to reduce MDA levels higher. The combination dose of purified gambir catechins and vitamin C which was carried out in this study showed a lower decrease in serum MDA levels than single dose administration.

The treatment group that was given gambir at a dose of 200 mg/kgbb was not significantly different from the treatment group that was given vitamin C at a dose of 65 mg/kgbb, and the treatment group that was given a combination of gambir at a dose of 100 mg/kgbb and vitamin C at a dose of 32.5 mg/kgbb. This shows that the three treatment groups can have the same effect on reducing MDA levels in the body. The treatment group given a dose of 65 mg/kgbb of vitamin C was not significantly different from the treatment group given a combination of 100 mg/kgbb of gambir and 32.5 mg/kgbb of vitamin C. From the Mann Whitney test, it was found that the group that provided the best antioxidant activity to ward off free radicals was the treatment group that was given a combination test preparation of purified gambier at a dose of 100 mg/kgbb and vitamin C at a dose of 32.5 mg/kgbb.

CONCLUSION

Physical fatigue activity increased serum MDA levels in male white mice, whereas catechins from purified gambier, vitamin C, and their combinations reduced these levels in mice subjected to physical fatigue. The combination dose exhibited a more substantial reduction in MDA levels compared to the others. Further research is recommended to investigate the efficacy of various antioxidants and their combinations in reducing MDA levels.

ANKNOWLEDGMENT

The authors thank to Faculty Pharmacy Andalas University for its free funding (Grant No. 11/UN16.10. D/PJ.01./2023).

AUTHORS CONTRIBUTIONS

RY: Supervision, writing manuscript, Revision and Editing, AA: Conceptualization, Supervision, Resources, Review and Editing; and AAP: Data Collection and Writing manuscript. All authors approved the final version of the manuscript.

CONFLICT OF INTERESTS

The authors declare that thre is no conflict of interest in publishing the manuscript.

REFERENCES

1. Daviglus ML, Lloyd Jones DM, Pirzada A. Preventing cardiovascular disease in the 21st century: therapeutic and preventive implications of current evidence. Am J Cardiovasc Drugs. 2006;6(2):87-101. doi: 10.2165/00129784-200606020-00003, PMID 16555862.
2. Bertram MY, Lim SS, Barendregt JJ, Vos T. Assessing the cost-effectiveness of drug and lifestyle intervention following opportunistic screening for pre-diabetes in primary care. Diabetologia. 2010;53(5):875-81. doi: 10.1007/s00125-010-1661-8, PMID 20135088.
3. Church T. Exercise in obesity, metabolic syndrome, and diabetes. Prog Cardiovasc Dis. 2011;53(6):412-8. doi: 10.1016/j.pcad.2011.03.013, PMID 21545927.
4. Prevention DS. Activity is the best medicine. Nature. 2011;475(7355).
5. Hurley BF, Hanson ED, Sheaff AK. Strength training as a countermeasure to aging muscle and chronic disease. Sports Med. 2011;41(4):289-306. doi: 10.2165/11585920-000000000-00000, PMID 21425888.
6. 6. Isnawati TA, Rainil M, Sampumo OD, Mutiatikum D, Widowati L, Gitawati R. Characterization of 3 types gambir extract (Uncaria gambir Roxb) from sumatera barat. Boll Penelit Kesihat. 2012;40:201-8.
7. Pathy K. Process for preparation of vitamin C and method for determination of vitamin C in tablets. SCSOAJ. 2018;1(3):1-14. doi: 10.32474/SCSOAJ.2018.01.000114.
8. Telang PS. Vitamin C in dermatology. Indian Dermatol Online J. 2013;4(2):143-6. doi: 10.4103/2229-5178.110593, PMID 23741676.
9. Simioni C, Zauli G, Martelli AM, Vitale M, Sacchetti G, Gonelli A. Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. Oncotarget. 2018;9(24):17181-98. doi: 10.18632/oncotarget.24729, PMID 29682215.
10. McArdle WD, Katch FI KV. Exercise physiology: nutrition, energy, and human performance. Edisi ke 7. Philadelphia: Lippincott Williams & Wilkins; 2010.
11. Fernandes CF, LC, Cyrino ESS. Production of reactive oxygen species during the aerobic and anaerobic exercise. Rev Bras Cineantropom Desempenho Hum. 2006;8(1):102-9.
12. Elsayed Azab A, Adwas Almokhtar A, Ibrahim Elsayed AS, Adwas AA, Ibrahim Elsayed ATA Sedik, Quwaydir FA. Oxidative stress and antioxidant mechanisms in human body. J Appl Biotechnol Bioeng. 2019;6(1):43-7.
13. Ammar A, Trabelsi K, Boukhris O, Glenn JM, Bott N, Masmoudi L. Effects of aerobic, anaerobic and combined-based exercises on plasma oxidative stress biomarkers in healthy untrained young adults. Int J Environ Res Public Health. 2020;17(7). doi: 10.3390/ijerph17072601, PMID 32290148.
14. Dewa I, Susilawati A. Kajian pustaka: sumber reactive oxygen species (ROS) vaskular [Review: vascular sources of reactive oxygen species]. Stomatognatic (JKG Unej). 2021;18(1):1-10.
15. Dewi IP, Ulinnuha JU, Holidah D. The effect of sugarcane leaf extract on malondialdehyde plasma levels in carbon tetrachloride-induced rats. JFM. 2022;5(1):99-106. doi: 10.35451/jfm.v5i1.1340.
16. Ribeiro JS, Santos MJMC, Silva LKR, Pereira LCL, Santos IA, da Silva Lannes SC. Natural antioxidants used in meat products: a brief review. Meat Sci. 2019;148:181-8. doi: 10.1016/j.meatsci.2018.10.016, PMID 30389412.
17. Shalaby EA, Shanab SMM. Antioxidant compounds, assays of determination and mode of action. African Journal of Pharmacy and Pharmacology 2013;7(10):528-39.
18. Ayala A, Munoz MF, Arguelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014;2014:360438. doi: 10.1155/2014/360438, PMID 24999379.
19. Intan PR, Khariri. The use of laboratory animals in supporting the development of the medical world. Semin Sains. 2020;1(1):141-4.
20. Irramah M. Pengaruh uncaria gambir roxb terhadap ulkus gaster dan kadar malondialdehid hewan coba yang diinduksi etanol. MKA. 2017;40(1):1. doi: 10.22338/mka.v40.i1.p1-10.2017.
21. Ghanwat G, Patil AJ, Patil J, Kshirsagar M, Sontakke A, Ayachit RK. Effect of vitamin C supplementation on blood lead level, oxidative stress and antioxidant status of battery manufacturing workers of western Maharashtra, India. J Clin Diagn Res. 2016;10(4):BC08-11. doi: 10.7860/JCDR/2016/15968.7528, PMID 27190789.
22. Zeb A, Ullah F. A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances in fried fast foods. J Anal Methods Chem. 2016;2016:9412767. doi: 10.1155/2016/9412767, PMID 27123360.
23. Haris NH, Nugroho T, Utomo AW, Nindita Yora. The effect of red cabbage extract to serum MDA levels in rats after maximum physical activity. IJPTher. 2021;2(3). doi: 10.22146/ijpther.1117.
24. Sandhiutami N, Made Dwi, Yesi Desmiaty AA. Efek antioksidan ekstrak etanol biji pepaya (*carica papaya l.*) terhadap aktivitas enzim superoksida dismutase dan kadar malondialdehid pada mencit stress oksidatif dengan perenangan. J Ilmu Kefarmasian Indones. 2016;14(1):26-32.
25. Lieberman M. Mad marks' basic medical biochemistry a clinical approach. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2013.
26. Ningsih E, Rahayuningsih S. Extraction, isolation, characterisation and antioxidant activity assay of catechin gambir (Uncaria gambir (Hunter). Roxb Kimia. 2019;7(2).
27. Yimcharoen M, Kittikunnathum S, Suknikorn C, Nak-On W, Yeethong P, Anthony TG. Effects of ascorbic acid supplementation on immune status in healthy women following a single bout of exercise. Sports Sci Health. 2019;17(3):635-45.