RED ROSELLA (*Hibiscus sabdariffa Linn.*) PETAL BREW IS ABLE TO REDUCE THE SPRAGUE DAWLEY MDA RATE IN RATS EXPOSED TO WASTE COOKING OIL

Arya Ulilalbab, Eni Maskanah
Karya Husada Nutrition Academy, Kediri, Indonesia

ABSTRACT

Food and snacks sold are usually fried using oil that has been used for frying repeatedly. Oil that is repeatedly used for frying is often called waste cooking oil. Waste cooking oil is a source of exogenous free radicals that can trigger oxidative stress. To prevent this, sufficient antioxidant intake is needed. One source of antioxidants is red rosella. The purpose of this study was to analyze the effect of giving red rosella petals on the conditions of oxidative stress in Sprague dawley rats exposed to waste cooking oil through MDA testing. The research method used was Completely Randomized Design (CRD). The sample consisted of 24 male rats which were randomly selected and divided into 4 groups: negative control (no treatment), positive control (administered with waste cooking oil of 8.92 meq/kg as much as 2 ml/kgBW), treatment 1 (administered with waste cooking oil of 8.92 meq/kg as much as 2 ml/kgBW and red rosella petal brew dosed of 540 mg/kgBW, and treatment 2 (administered with waste cooking oil of 8.92 meq/kg as much as 2 ml/kgBW and red rosella petals brew dosed of 810 mg/kgBW). The results of the one way ANOVA analysis (a=1%) and the Tukey HSD test showed the p value of MDA=0.00, indicating that all treatments had significant effect. In further tests, it was found that all treatments contained differences in MDA values. The best value in the treatment was by giving a dose of 810 mg/kgBW (serum MDA of 2.22 nmol/ml). It can be concluded that the administration of red rosella petal in doses of 540 mg/kgBW (EC50=407.52 ppm) and 810 mg/kgBW (EC50=247.82 ppm) can improve the oxidative stress of Sprague dawley rats.

***Keywords**: Antioxidant; malondialdehyde; free radicals; red rosella; oxidative stress*

ABSTRAK

Makanan dan jajanan yang dijual biasanya digoreng dengan menggunakan minyak yang telah digunakan untuk menggoreng berulangkali. Minyak yang berulangkali dipakai untuk menggoreng sering disebut jelantah. Minyak jelantah merupakan sumber radikal bebas eksogen yang dapat memicu stres oksidatif. Untuk mencegah hal tersebut maka diperlukan asupan antioksidan yang cukup. Salah satu sumber antioksidan yaitu rosella merah. Tujuan dari penelitian ini yaitu menganalisis pengaruh pemberian seduhan kelopak rosella merah terhadap kondisi stres oksidatif pada tikus Sprague dawley yang diberi minyak jelantah melalui pengujian MDA. Metode penelitian yang digunakan adalah Rancangan Acak Lengkap (RAL). Sampel terdiri dari 24 ekor tikus berkelamin jantan yang dipilih secara random dan dibagi menjadi 4 kelompok, yaitu kontrol negatif (tanpa perlakuan), kontrol positif (pemberian minyak jelantah 8,92 meq/kg sebanyak 2 ml/kgBB), perlakuan 1 (pemberian minyak jelantah 8,92 meq/kg sebanyak 2 ml/kgBB dan seduhan kelopak rosella merah dosis 540 mg/kgBB), dan perlakuan 2 (pemberian minyak jelantah 8,92 meq/kg sebanyak 2 ml/kgBB dan seduhan kelopak rosella merah dosis 810 mg/kgBB). Hasil analisa one way anova (a=1%) dan uji Tukey HSD menunjukkan p value MDA=0,00, artinya semua perlakuan berpengaruh nyata. Pada uji lanjut didapatkan bahwa semua perlakuan terdapat perbedaan nilai MDA. Nilai terbaik pada perlakuan pemberian seduhan dosis 810 mg/kg BB (MDA serum 2,22 nmol/ml). Dapat disimpulkan bahwa pemberian seduhan kelopak rosella merah dosis 540 mg/kgBB (EC50=407,52 bpj) dan 810 mg/kgBB (EC50=247,82 bpj) dapat memperbaiki kondisi stres oksidatif tikus Sprague dawley.

***Kata kunci**: Antioksidan; malondialdehid; radikal bebas; rosella merah; stres oksidatif*

Correspondence: Arya Ulilalbab, Karya Husada Nutrition Academy, Jalan Soekarno Hatta No. 7, Pare Sub-District, Kediri, East Java, Indonesia. Phone: +6285755211490. E-mail: arya17051990@gmail.com

pISSN:2355-8393 ● eISSN: 2599-056x ● doi: http://dx.doi.org/10.20473/fmi.v54i3.10005
● Fol Med Indones. 2018;54:167-171 ● Received 28 Aug 2017 ● Accepted 1 Feb 2018

INTRODUCTION

In the era of unfavorable economic conditions, people have tendency to use cooking oil repeatedly (repeatedly heated). Cooking oil is usually used for 3-4 times frying and after that, it is not used anymore and is often referred to as waste cooking oil (Rifqi et al 2012). The average use of cooking oil is around 137,309 per year and will continue to increase at certain events, such as Eid, Christmas and New Year (Mansyur & Fauzan

1999). According to Food Consumption Statistics data (Ministry of Agriculture of RI 2012), the consumption of pieced fried foods increased from 2010 to 2011. In 2010, fried food consumption was 94.744 pieces per capita. Whereas, in 2011, it was 101.105 per capita. According to Suleeman and Sulastri (2006), of the 22 types of snacks asked in Susenas, fried food are the most preferred snacks in Indonesia. The 2002 consumption module of Susenas data stated that fried food are chosen by almost half of households in Indonesia (49%).

In general, fried food vendors do not control the frying temperature and use frying oil repeatedly, so it can accelerate the damage of cooking oil. According to Birowo (2000), this is very dangerous for health. Damaged (waste) cooking oil triggers degenerative diseases, such as hyperlipidemia, cardiovascular disease and fatty liver. The disease is associated with excessive consumption of saturated fat and exposure to free radicals. Peroxide numbers are used as a marker of oxidative damage to oil and show free radicals measured as toxic peroxides. In a study, the provision of waste cooking oil with peroxide number of 118 mek/kg in the group of rats given with 1 ml/day waste cooking oil for 28 days produced the highest MDA (Malondialdehyde) value which was 0.189 mg/ml. In the normal group, the concentration was 0.078 mg/ml. This shows that natural antioxidants in experimental animals are not sufficient to prevent free radicals in the positive control group (Ulilalbab et al 2012). According to Suryohudoyo (2000), an increase in MDA is a sign of oxidative damage by free radicals in cell membranes.

Effervescent purple rosella antioxidants have been shown to be effective in counteracting free radicals from waste cooking oil with biomarkers of serum MDA (Ulilalbab et al 2012), so further studies are needed to determine whether antioxidant steeping powder of red rosella petals can counteract free radicals in Sprague dawley rats that are administered with cooking oil. Red rosella petals are chosen since the anthocyanin content is highest compared to other parts of the plant (Esa et al 2010). This study examined the benefits of 540 mg/kgBW and 810 mg/kgBW red rosella petal powder on its ability to counteract free radicals by observing serum MDA biomarkers in Sprague Dawley rats that were exposed to waste cooking oil.

MATERIALS AND METHODS

The instruments used in this study included spatulas, glass beakers, measuring cups, dark bottles, digital scales, rat cages, special animal drinking bottles, sonde, and spectrophotometer. The materials used were Sprague dawley rats, waste cooking oil, standard feed, mineral water and red rosella petals.

This was a True Experimental Laboratory study with post test only control group design. The design of the treatment in this study was Completely Randomized Design (CRD). The sample consisted of 24 healthy male Sprague dawley rats aged 3-4 months with weight of 170-200 g, selected by random sampling to be divided into four groups. Each group consisted of 6 rats, namely: Group I: negative control (normal group), receiving standard feed and distilled water in the morning (as placebo), not receiving waste cooking oil and not receiving rosella petal powder; Group II: positive control (exposure group), receiving standard feed, distilled water in the morning (as placebo) and then receiving waste cooking oil as much as 2 ml/kgBW during the day; Group III: treatment group that received standard feed, brew of red rosella powder of 540 mg/kgBW p.o in the morning and 2 ml waste cooking oil/kgBW during the day; and Group IV: treatment group that received standard feed, brew of red rosella powder of 810 mg/kgBW p.o in the morning and 2 ml/kgBW waste cooking oil during the day.

The feed of experimental animals used POKPHAND CP 591 brand with composition of 13.0% water, 18.0-20.0% protein, 3.0% fat, 6.0% fiber, 7.0% ash, 0.9% calcium, and 0.6% phosphorus. The ingredients used in feed formulations included corn, bran, fish flour, soybean meal, coconut cake, meat and bone flour, wheat shards, peanut meal, leaf flour, canola, vitamins, calcium, phosphate and trace minerals. The provision of waste cooking oil and administration of red rosella petals was done for 21 days. The study was conducted at the Gadjah Mada University Inter-University Center Laboratory, Central for Drug Evaluation and Analysis (C-DEA) Laboratory of the University of Surabaya Pharmacy Faculty, and the UB Food Quality and Food Safety Testing Laboratory.

In the initial stage, normality analysis was carried out with the Shapiro-Wilk test and homogeneity test with Levene's test. The data were normal and homogeneous, then an analysis of comparison between groups with One Way Anova test was carried out. There was a significant difference, then the test continued with the Tukey HSD test to determine further the difference in each treatment group.

RESULTS

Antioxidant testing was done by DPPH (a, a diphenyl picryl hydrazil) method with the absorbance used of λ=516.5 nm. The results of the analysis of antioxidant

activity (DPPH test) from red rosella petal brew is presented in Table 1.

Table 1. Analysis of antioxidant activity (DPPH test) of red rosella petal brew

Code	Sample	EC_{50}
V-9-B2	540 mg/kgBW	407.52 bpj (mg/L)
V-9-B1	810 mg/kgBW	247.82 bpj (mg/L)

Table 1 shows that the antioxidant activity (DPPH test) successively from the highest is the red rosella petal boil dosed of 810 mg/kgBW (247.82 bpj) and then 540 mg/kgBW (407.52 bpj).

Based on the results of the study, serum MDA values were obtained. The results of serum MDA analysis are presented in Fig. 1.

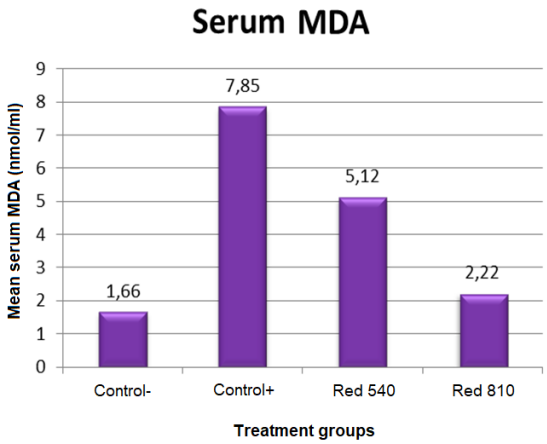

Fig. 1. Mean serum MDA value.

Fig. 1 shows that the mean value of the MDA in the control group is negative at 1.66 nmol/ml. Mean MDA value of the positive control group was 7.85 nmol/ml. Mean MDA value in group receiving injection of red rosella petals in a dose of 540 mg/kgBW was 5.12 nmol/ml. Mean MDA value group in group receiving red rosella petal in a dose of 810 mg/kgBW was 2.22 nmol/ml. The highest MDA value was in positive control, while the lowest was in negative control. In treatment group, the lowest MDA value approaching that of the negative control group was on the treatment of giving red roselle petals in a dose of 810 mg/kgBW.

Table 2 shows that the serum MDA values in all groups have significant differences. MDA values from the highest to the lowest were those in positive control group, group receiving 540 mg/kgBW red rosella petals, 810 mg/kgBW red rosella petals, and in negative control, consecutively.

Table 2. Differences in serum MDA values in different groups

Groups	Mean serum MDA values
Negative control	1.66 ± 0.11 nmol/ml (a)
Positive control	7.85 ± 0.29 nmol/ml (b)
Receiving 540 mg/kgBW red rosella petal brew	5.12 ± 0.23 nmol/ml (c)
Receiving 810 mg/kgBW red rosella petal brew	2.22 ± 0.10 nmol/ml (d)

Note: groups marked with different letters indicate significant difference

DISCUSSION

This study proved that the antioxidant activity of red rosella petals at 810 mg/kgBW was higher than the dose of 540 mg/kgBW. According to Adenipekun (1998), red rosella contains citric acid of 4 gr/100 g. According to Gordon (1990), citric acid is a secondary antioxidant that provides synergistic effect by donating H+ ions which can regenerate primary antioxidants, thereby increasing the effectiveness of the primary antioxidants. Esa et al's (2010) study stated that rosella petals extracted using water have the highest antioxidant activity compared to other parts of this plant. The highest total value of antioxidant activity to the lowest in each part of the plant are petals (54.1%), seeds (45.9%), leaves (27.9%), and stems (10.7%). This study used rosella petals as it the highest antioxidant activity when brewed using water compared to other parts of rosella plant.

Rosella is a source of phenols (Owoade et al 2015). The highest component of phenol is flavonoids. According to Setijowati et al (1998), flavonoids are secondary antioxidants. Secondary antioxidants have a function by composing fat peroxide into a stable end product. Secondary antioxidants have a function to counteract free radicals and prevent chain reactions. Besides containing flavonoids, rosella also contains ascorbic acid 6.7 mg/100gr (Morton 1999) and citric acid 4 gr/100 g (Adenipekun 1998). According to Mahdavi et al (1995), oxygen catcher, such as ascorbic acid, reacts with free oxygen and transfers it to a closed system. Ascorbic acid and citric acid can be classified as oxygen catcher or chelate. This antioxidant can act as a hydrogen donor to the oxidizing radical so that it can renew the primary antioxidant. Therefore, phenolic antioxidants can be used at low concentrations if it is synergistical and added simultaneously.

Malondialdehyde (MDA) is one of the end products of lipid peroxidation which is toxic to living cells. In addition, MDA is a measure of free radicals contained in the body and is considered a biomarker that is often

used to determine the level of oxidative stress (Wulandari 2002). The results showed that the negative control group had the lowest MDA value (1.66 nmol/ml) compared to other groups. This is because in this group the cooking oil was not given so that the resulting MDA values tended to be lower than in other groups. The highest MDA value was found in the positive control group (7.85 nmol/ml) because the positive control group was exposed to the source of free radicals in the form of waste cooking oil without being given any antioxidant intake. Waste cooking oil contained triglycerides which have unsaturated (double) chains that experience autooxidation to form free radicals (Ketaren 1986), which can cause oxidative stress.

Positive control groups showed conditions of oxidative stress characterized by free radicals. High free radicals are characterized by increased serum MDA values. According to Favier (1995), MDA is the end product of lipid peroxidation process. The high value of serum MDA is influenced by lipid peroxidation caused by free radicals. The presence of antioxidants will be able to reduce the number of free radicals. Cotran and Kumar (1999) stated that a situation where there is an imbalance between the production of oxygen-derived compounds and the body's antioxidant system is the situation that triggers stress oxidative conditions, where one of the parameters is the increase in the production of free radical derivatives.

Further test results showed that all groups were significantly different. This is because the negative control group was only treated with standard feed without the use of waste cooking oil or the provision of red roselle petals so that the serum MDA value tended to be normal (1.66 nmol/ml) and lowest compared to other groups. Whereas, the positive control group had the highest serum MDA value (7.85 nmol/ml) compared to other groups. This indicated that in the positive control group oxidative stress occurred because this group received only cooking oil and standard feed without the provision of antioxidant intake.

In positive control group, the absence of antioxidant intake caused free radicals in the blood to increase. This was characterized by an increase in the value of serum MDA. The high serum MDA value is caused by the absence of antioxidant intake that can neutralize fat peroxidation reaction. Fat peroxidation is a chain reaction to unsaturated fatty acids (PUFAs) triggered by free radicals (Suryohudoyo, 1997). Arief (2000) in his study also stated that the presence of free radicals in the absence of antioxidants as an inhibitor, causing the rat serum MDA value to increase. The prevention of oxidative stress is caused by inhibiting the increase in MDA value as a biomarker of the amount of free radicals present in the body.

The brew of red rosella petals in doses of 540 mg/kgBW and 810 mg/kgBW provided a significant antioxidant effect in preventing oxidative stress. This was indicated by the MDA value in doses of 540 mg/kgBW and 810 mg/kgBW which was lower than the that in positive control group. According to Favier (1995), MDA is the final product of lipid oxidation. High MDA value is influenced by lipid peroxidation, which indirectly shows the high number of free radicals. The presence of antioxidant performance in both doses of red rosella petals can reduce the effects of free radicals which can cause oxidative stress. Red roselle petal steeping in dose of 810 mg/kgBW (EC50=247.82 bpj) showed the best results in lowering MDA value because its antioxidant activity was higher than that in the rosella petal in a dose of 540 mg/kgBW (EC50=407.52 bpj).

CONCLUSION

Red rosella petal brew dosed of 540 mg/kgBW had EC50 value=407.52 ppm, while the red rosella petal brew dosed of 810 mg/kgBW had EC50=247.82 ppm. Waste cooking oil used in this study had peroxide number of 8.92 meq/kg. Red roselle petal steeping in doses of 540 mg/kgBW and 810 mg/kgBW is effectively able to reduce the MDA serum of Sprague dawley rats. Roselle petal steeping dose of 810 mg/kgBW was most effective in reducing the MDA serum of Sprague dawley rats. In the negative control group, the MDA value was the lowest, whereas in the positive control group the MDA value was the highest compared to other groups.

ACKNOWLEDGMENT

This study was funded by the Directorate of Research and Community Service Directorate General of Research and Development Strengthening Ministry of Research, Technology and Higher Education based on Decree Number 012/SP2H/K2/KM/2017 on the Beginner Lecturer Research scheme.

REFERENCES

Adenipekun IT (1998) Extraction and colours of roselle (*Hibiscus sabdariffa*) Juice. A Thesis. University of Ibadan, Ibadan, Nigeria

Arief A (2000), Pengaruh antioksidan pada tempe kedelai terhadap kadar superoksid dismutase (SOD)

dan malondialdehid (MDA) serum tikus wistar dipapar dengan asap rokok sub-akut. Faculty of Medicine, Universitas Brawijaya, Malang

Birowo A (2000). Minyak jelantah berbahaya, dalam Suirta IW (2009). Preparasi biodiesel dari minyak jelantah kelapa sawit. Jurnal Kimia 3, 1-6

Cotran J, Kumar V (1999). Basic Pathology of Disease. New York, McGraw Hill Publisher

Esa NM, Hern FS, Ismail A, Yee CL (2010) Antioxidant activity in different parts of roselle (*Hibiscus sabdariffa L.*) extracts and potential exploitation of the seeds. Food Chemistry 122, 1055-1060

Favier, et al (1995). Analysis of free radical in biological system. Switzerland, Birkhauser Verlag-Basel

Gordon MH (1990). The mechanism of antioxidants action in vitro. In B.J.F. Hudson, editor. Food Antioxidants. London, Elvesier Applied Science

Ketaren S (1986). Pengantar teknologi minyak dan lemak pangan. Jakarta, UI Press

Mahdavi DL, Deshpande SS, Salunke DK (1995). Food antioxidants. New York, Marcel Dekker

Mansyur, Fauzan (1999). KDI lakukan operasi pasar minyak goreng. Harian Kompas, May 1, 1999

Ministry of Agriculture of RI (2012). Statistik konsumsi pangan 2012. Pusat Data dan Sistem Informasi Pertanian. Sekretariat Jenderal Kementerian Pertanian.

Morton JF (1999). *Roselle Hibiscus sabdariffa L*. Available from http://www.transgenica.com/ database/ h/hibiscus.htm.

Owoade AO, Lowe GM, Khalid R (2015). the in vitro antioxidant properties of Hibiscus Anthocyanins Rich Extract (HAE). Nature and Science 13, 22-29

Rifqi, Khaerur, Kadarwati S, Wahyuni S (2012). Preparasi, karakteristik, dan uji aktivitas ni-mo/zeolit alam dalam proses catalytc cracking jelantah menjadi biogasoline. Indonesian Journal of Chemical Science

Setijowati N, Sujudi H, Astuti TW, Widodo A (1998). Pengaruh radikal bebas dan vitamin E terhadap jumlah circulating endothel pada darah tikus yang dipapar asap rokok kretek secara kronik. Majalah Kedokteran Universitas Brawijaya XIV

Suleeman E, Sulastri E (2006). jajanan favorit separuh rumah tangga di Indonesia mengandung zat berbahaya. Suara Pembaharuan

Suryohudoyo P (1997). Oksidan dan antioksidan pada diabetes melitus. Di dalam Tjokroprawiro A, et al. Proceedings of The 3rd Surabaya Diabetes Update. Surabaya.

Suryohudoyo P (2000). Kapita selekta ilmu kedokteran molekuler. Perpustakaan Nasional RI. Jakarta, Penerbit CV Sagung Seto, p 31-47

Ulilalbab A, Priyanto AD, Maulana HI, Fitriani E, Resti F, Estiasih T (2012). Pemberian tablet effervescent rosella ungu menurunkan nilai mda (malondialdehid) tikus wistar yang dipapar minyak jelantah. The Indonesian Journal of Public Health 9, 81-86

Wulandari SS (2002). Pengaruh pemberian dekok dan instant jahe (*Zingiber officinate Rocs*.) terhadap kadar mda serum, hepar, dan paru tikus (*Rattus norvegicus*) yang dipapar asap rokok kretek sub akut. A Thesis. Faculty of Medicine, Universitas Brawijaya, Malang