



The influence of roselle-based wedang uwuh (*Hibiscus sabdariffa* L.) on SOD (superoxide dismutase) and MDA (malondialdehyde) of cigarette smoke-exposed rat

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KEYWORDS

Cigarette
Malonaldehyde
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ABSTRACT

Cigarette smoke is the leading cause of several respiratory diseases because it contains high free radical compounds. These free radical compounds can be neutralized with antioxidants obtained from natural ingredients such as roselle. Roselle contains various bioactive compounds and consumes in the form of infusion. Mixing roselle with various herbs as wedang uwuh can increase the antioxidant content. Wedang uwuh is a beverage consisting of various combinations of herbs such as sappan wood, cloves, and cinnamon. This study aims to analyze levels of MDA and SOD in rats exposed to cigarette smoke and given wedang uwuh. This research was conducted on 30 white male Wistar rats that divided into six groups- P0 (normal control), P1 (roselle-based wedang uwuh), P2 (commercial wedang uwuh), P3 (smoke control), P4 (smoke + roselle-based wedang uwuh), P5 (smoke + commercial wedang uwuh). The dose of wedang uwuh is 2.7 ml/kg BW, and the dose of exposure to cigarette smoke is one cigarette for 5 minutes for 30 days. The differences between each group were analyzed using the one way ANOVA test and HSD advanced test. Statistical results showed that giving roselle-based wedang uwuh (P4) significantly reduced MDA levels and increased SOD than the smoke control group (P3). Roselle-based wedang uwuh reduce the MDA levels and increase SOD better than commercial wedang uwuh.

Introduction

Smoking is the leading cause of several respiratory diseases, a chronic obstructive pulmonary disease (COPD) (Soriano et al., 2020). Exposure to cigarette smoke can form free radicals in the body (Żukowski et al., 2018). In very high amounts, free radicals are caused by cigarette smoke and can cause disturbances or abnormalities in the respiratory tract ranging from the trachea, bronchi and bronchioles to the pulmonary alveoli (Santoso, 2012). In addition, the high amount of free radicals can trigger the formation of oxidative stress by an increase in MDA levels as the result of lipid peroxide (Ayala et al., 2014) and a decrease in systemic endogenous antioxidant capacity in the form of enzymes such as SOD and glutathione peroxidase (GPx) (Szczeklik et al., 2016).

Roselle (*H. sabdariffa* L.) is a plant from the Malvaceae family and is widely grown in Indonesia. People use roselle calyx as herbal tea because it contains several substances such as phenolic acids, organic acids, fatty acids, flavonoids and anthocyanins (Salem et al., 2020) that giving roselle antioxidant properties. Several studies have shown that roselle effectively lowers blood pressure, lowers fat profile, has anti-inflammatory, antimicrobial, anti-anaemia, anticarcinogenic, hepatoprotective, and improves fertility disorder (Riaz and Chopra, 2018). Another study showed that roselle extract prevented the increase in MDA (Ulilalbab et al., 2015); thus preventing and improving rat hepatocyte degeneration in rats exposed to cigarette smoke (Ulilalbab et al., 2018).

Mixing roselle with herbs in making beverage can increase these antioxidant levels

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(Wijaya, 2017). Wedang uwuh is a traditional beverage made from a mixture of typical Indonesian herbs, giving it a spicy-sweet taste, a distinctive aroma of herbs, and a red colour (Herdiana et al., 2014). The ingredients used in making wedang uwuh are ginger, sappanwood, cloves, cinnamon, nutmeg, lemongrass leaves and cardamom (Pudjowati et al., 2021).

Wedang uwuh based on roselle is thought to be able to reduce oxidative stress and increase antioxidant levels. Thus, the purpose of this study was to determine the effect of roselle-based wedang uwuh on serum SOD and MDA levels in rats exposed to the cigarette smoke.

Research Methods

Preparation of wedang uwuh

The dried roselle calyx, dried clove flowers, dried sappan wood, and cinnamon powder were obtained from a local market in Kediri, East Java, Indonesia. Dried roselle, clove flower, and sappan wood were ground into a powder (60 mesh) and put in a teabag (mix of 1.891 g of roselle, 1.340 g of sappan wood, 0.206 g of cinnamon, and 0.063 g of clove flower powder). Wedang Weeka (commercially dried herbs of ginger, nutmeg leaves, cinnamon leaves, clove leaves, clove flowers, sappan wood and cardamom) used as control (commercial wedang uwuh).

Roselle-based wedang uwuh (RWU) and commercial wedang uwuh (CWU) were prepared according to Widyaningsih et al. (2020) by extracting 3.5 g RWU and CWU in 350 ml of boiling water for 5 minutes and then filtered.

Experimental methods

Healthy male Wistar rats (*Rattus norvegicus*) with an average age of 3 months weighing 150-200 g, obtained from a local breeding company in Malang, East Java, Indonesia. The Ethical Committee has approved the ethical requirement of Brawijaya University. Rats were maintained in a controlled room (27.62 °C with a 12-h light and 12-h dark cycle), accessed freely to food and water, and acclimatized for one week. Wistar rats were divided into six groups- P0 (normal control), P1 (RWU), P2 (CWU), P3 (smoke control), P4 (smoke + RWU), P5 (smoke + CWU). The dose of wedang uwuh is 2.7 ml/kg BW, and the dose of exposure to cigarette smoke is one cigarette for 5 minutes for 30 days. The smoking process in this study uses a smoking chamber to ensure that the

smoke does not spread anywhere. On day 31, all rats were sacrificed, and blood was collected.

SOD

Serum was obtained by centrifuging the blood at a speed of 3500 rpm for 10 minutes. 200 µL of serum was put into a test tube. Added with EDTA 100 mM 200 µL, NBT 100 µL, Xanthine 100 µL, Xanthine oxidase 100 µL, then vortexed and centrifuged for 5 minutes at 3000 rpm then filtered if there is a colloid. The supernatant was taken, and distilled water was added to a volume of 3 mL, spectrophotometrically at a wavelength of 580 nm (modification of Bannister and Calabrese (Bannister And Calabrese, 1987)).

MDA

200 µL of serum was put into a test tube. Added 40% TBA as much as 2.5 times the volume (500 µL) and then vortexed. TBA is used for protein denaturation. After that, 1 N 200 µL of HCl was added to stabilize the pH, then 500 µL of distilled water and 100 µL of 1% TBA were added. Then heated in a heater at 100 °C for 20 minutes, cooled for 15 minutes, and centrifuged for 10 minutes at 3000 rpm. The supernatant was taken and transferred to another tube. Added distilled water to 3 mL and then spectrophotometrically at a wavelength of 520 nm (modification of Rael et al. (2004)).

Statistical analysis

Statistical analysis was carried out using one way ANOVA (Analysis of Variance) using Minitab 17. The results of the study were said to be significant if the results obtained were $p < 0.05$. If the results of the study were significant, further tests were conducted using Fisher Test.

Results and Discussion

Level of serum SOD

Superoxide Dismutase (SOD) is an antioxidant enzyme as a physiological defense against free radicals and reactive oxygen species (ROS) resulting from endogenous and exogenous stress (Stephenie et al., 2020). The level of Serum SOD observations was carried out on six treatment groups, and the results of the observations can be seen in Figure 1.

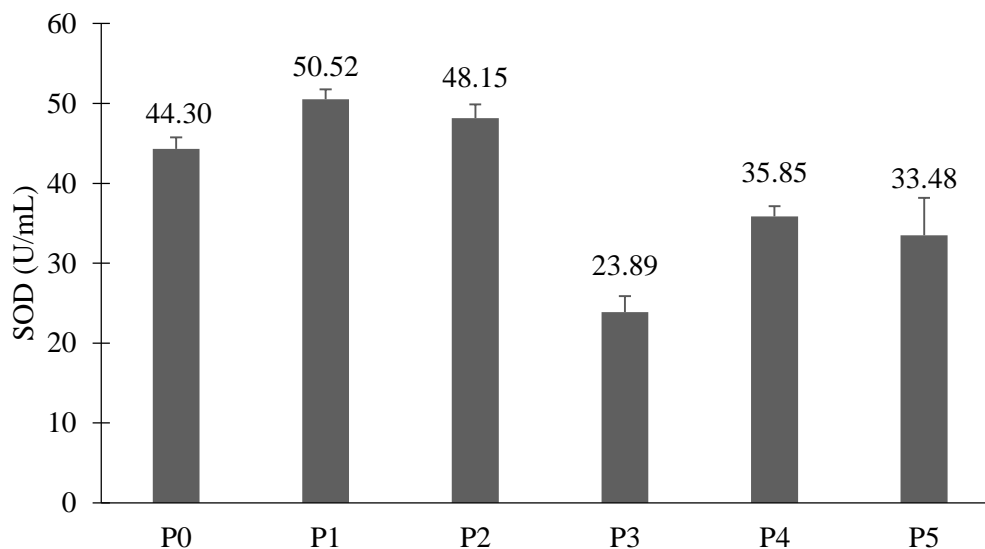


Figure 1. Level of serum SOD (Note: P0 = normal control, P1 = RWU, P2 = CWU, P3 = smoke control, P4 = smoke + RWU, P5 = smoke + CWU).

Based on Figure 1, the average level of serum SOD was different between the treatment groups. The highest level of serum SOD level that is shown in the P1 group (healthy rat with roselle-based wedang uwuh), which was 50.52 U/mL was significantly different ($p < 0.05$) compared to the P0, P3, P4 and P5 groups but not significantly different with the P2 (healthy rat with commercial wedang uwuh). Meanwhile, the lowest serum SOD level was shown in the P3 group (smoke control), which was 23.89 U/mL and significantly different ($p < 0.05$) compared to the other groups. Figure 1. shows that the cigarette smoke exposure group (P3, P4 and P5) had significantly lower serum SOD levels than the healthy group (P0, P1 and P2). The group of exposed rats given roselle-based wedang uwuh (P4) showed an increase in serum SOD levels of 50.08% when compared to smoke control (P3), which was higher than the group of exposed rats given commercial wedang uwuh (P5) of 40.16%.

Serum SOD levels in the smoke control group (P3) was lower than controls (P0), indicating the occurrence of oxidative stress due to the high free radicals caused by exposure to cigarette smoke for 30 days. Tobacco smoke contains free radicals in both particulate and gas phases and can cause biological oxidative damage (Goel et al., 2018). The significant increase in ROS production due to exposure to cigarette smoke will cause an imbalance between free radicals and body antioxidants (Ayala et al., 2014). SOD is an antioxidant enzyme that plays a role in protecting tissues from damage caused by

ROS. The large production of ROS from cigarette smoke causes a decrease in systemic endogenous antioxidant capacity in the form of enzymes such as SOD and glutathione peroxidase (GPx) (Szczeklik et al., 2016).

Rat exposed to cigarette smoke for 30 days with the administration of roselle-based wedang uwuh (P4) and commercial wedang uwuh (P5) had higher SOD levels than the smoke control group (P3) due to compounds that have antioxidant activity, such as phenolic in wedang uwuh. Antioxidants can inhibit the increase in free radicals through scavenging and indirectly inhibiting the activity/expression of free radical-producing enzymes or increasing the activity/expression of intracellular antioxidant enzymes (Crascì et al., 2018). The increase in serum SOD levels in the group given roselle-based wedang uwuh (P1 and P4) and commercial wedang uwuh (P2 and P5) was in line with the results reported by Gad et al. (2021), Izquierdo-Vega et al. (2020) and Hoseini et al. (2021), compounds that have an antioxidant activity such as anthocyanins, phenols, flavonoids and organic acids significantly increase SOD levels and suppress oxidative stress.

Level of serum MDA

Malonaldehyde (MDA) is the end product of enzyme and free radical-catalyzed lipid peroxidation of polyunsaturated fatty acids that is toxic to cells and is a dialdehyde compound with three carbon chains and low molecular weight. MDA levels were used as an indicator of

oxidative stress in unsaturated fats and an indicator of the presence of free radicals (Tsikas et al., 2016). Serum MDA observations were carried

out on six treatment groups. The results of the observation of serum MDA levels can be seen in Figure 2.

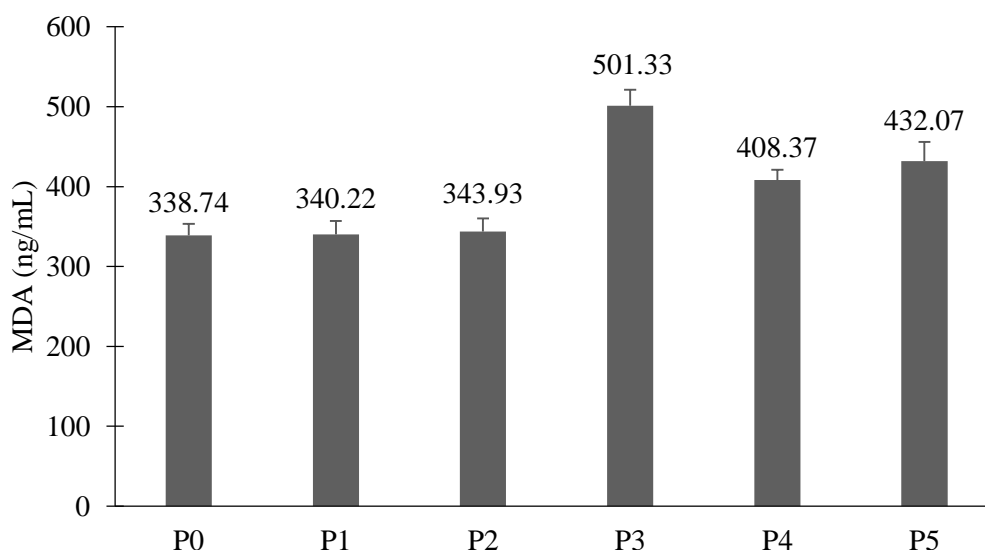


Figure 2. Level of serum MDA (Note: P0 = normal control, P1 = RWU, P2 = CWU, P3 = smoke control, P4 = smoke + RWU, P5 = smoke + CWU).

The average level of serum MDA (Figure 2.) shows differences between the treatment groups. The highest serum MDA level was shown in the P3 group (smoke control), which was 501.33 ng/mL and significantly different ($p < 0.05$) compared to the other groups. Meanwhile, the lowest serum MDA level was shown in the P0 group (normal control), was 338.74 ng/mL and significantly different ($p < 0.05$) compared to the P3, P4 and P5 groups, but not significantly different from the roselle-based wedang uwuh (P1) and commercial wedang uwuh (P2). Figure 2. shows that the cigarette smoke exposure group (P3, P4 and P5) had significantly higher serum MDA levels than the healthy group (P0, P1 and P2). The group of exposed rats given roselle-based wedang uwuh (P4) showed a decrease in serum MDA levels of 18.54% when compared to smoke control (P3), which was higher than the group of exposed rats given commercial roselle-based wedang uwuh (P5) by 13.82%.

Free radicals can be formed in the body as a result of metabolic processes. If the amount is low, these compounds can be neutralized by natural antioxidants in the body. However, the entry of excess free radicals from outside can cause an imbalance in free radicals with the body's natural antioxidants, thus triggering oxidative stress (Wojtunik-Kulesza et al., 2016). Serum MDA levels in the positive control group (P3)

were higher than the negative control (P0), indicating oxidative stress due to the high free radicals caused by exposure to cigarette smoke for 30 days. Tobacco cigarette smoke contains many toxic, carcinogenic and mutagenic chemicals and free radicals in the particulate and gas phases and can potentially cause biological oxidative damage (Goel et al., 2018). High ROS (reactive oxygen species) can cause direct damage to lipids. The primary sources of endogenous ROS production are mitochondria, plasma membranes, endoplasmic reticulum, and peroxisomes. At the same time, the source of exogenous ROS comes from stimuli such as ionizing radiation, ultraviolet light, infectious pathogens, environmental toxins and cigarette smoke. The entry of free radicals from the outside in large quantities will cause an imbalance between free radicals and the body's antioxidants and trigger oxidative stress, characterized by increased MDA levels resulting from lipid peroxide (Ayala et al., 2014).

The group exposed to cigarette smoke for 30 days with the administration of wedang uwuh roselle (P4) and commercial wedang uwuh (P5) had lower MDA levels than the positive control group (P3) due to compounds that have antioxidant activity, such as phenolic in wedang uwuh. Antioxidant activity can inhibit the increase in MDA levels by several mechanisms; directly by binding (scavenging) free radicals through

direct scavenging or by preventing the formation of free radicals through chelation of free metal ions or converting H_2O_2 into other harmless compounds and indirectly by inhibiting the activity/expression of free radical-producing enzymes or increasing the activity/expression of intracellular antioxidant enzymes (Aldini et al., 2021). Gad et al. (2021) reported that polyphenolic compounds (anthocyanins, phenols and flavonoids and glycoside derivatives) in roselle have antioxidant activity that can scavenge free radicals, singlet and triplet oxygen quench, as well as peroxide decomposition.

Conclusion

The administration of roselle-based wedang uwuh to the group of rats exposed to cigarette smoke significantly reduced MDA levels and increased SOD levels compared to the smoked control group and was better than commercial wedang uwuh.

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