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**Submission date:** 05-May-2023 07:30PM (UTC+0700)

**Submission ID:** 2085032587

**File name:** C-2\_2022\_BMJ\_artikel\_MDA-Beta\_vulgaris.pdf (402.17K)

**Word count:** 4909

**Character count:** 26177



# The Effect of *Beta vulgaris* L. on the Malondialdehyde Levels in Male Wistar Rats Exposed to Cigarette Smoke

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Received: 2022-08-10  
Accepted: 2022-10-02  
Published: 2022-11-09

## ABSTRACT

**Introduction:** Oxidative damage can be brought on by inhaling free radicals from cigarette smoke. Malondialdehyde (MDA), a byproduct of lipid peroxidation, is a biomarker for oxidative stress. *Beta vulgaris* L., sometimes known as beetroot, is a root vegetable with phenolic and betalain chemicals that have antioxidant qualities and can reduce oxidative stress in the body. Determine how beetroot juice affects male Wistar rats exposed to cigarette smoke regarding their MDA levels.

**Methods:** 24 samples of male Wistar rats, separated into 4 groups, were utilized in this genuine experimental investigation using a posttest-only control group design. Traditional food and beverages were served to Group N. Two cigarettes were smoked each day by the cigarette smoking group, BV1, and BV2. *Beta vulgaris* L. juice was also administered to group BV1 at a dose of 8 ml/kg BW per day and to group BV2 at 16 ml/kg BW per day. MDA serum levels were measured using the TBARS technique after 28 days of therapy. The one-way ANOVA and the Games-Howell test were used to evaluate the data.

**Result:** The group control's mean MDA level was 1,539 ppm; the cigarette smoking group was 3,167 ppm; BV1's was 2,452 ppm, and BV2's was 2,0527 ppm. Between-group control and cigarette smoking group BV1 and BV2, as well as between BV1 and BV2, there were significant differences in MDA levels (p 0,05).

**Conclusion:** The MDA levels of the groups exposed to cigarette smoke and given beetroot juice were lower than those of the groups exposed to cigarette smoke alone, with the MDA levels of the group given a dosage of 8 ml/kg BW being lower than the group given a dose of 16 ml/kg BW.

**Keywords:** *Beta vulgaris* L. juice, malondialdehyde, and cigarette smoke.

**Cite This Article:** Suharso, A.B., Dewi, P.K., Limijadi, E.K.S., Setyawati, A.N. 2022. The Effect of *Beta vulgaris* L. on the Malondialdehyde Levels in Male Wistar Rats Exposed to Cigarette Smoke. *Bali Medical Journal* 11(3): 1509-1514. DOI: 10.15562/bmj.v11i3.3806

## INTRODUCTION

Smoking is one of the health problems in Indonesia. The 2018 Basic Health Research (Riskesdas) data of the Indonesia Ministry of Health, the prevalence of smoking in the Indonesian population aged 10-18 years increased to 9.1% from 7.2% in 2013.<sup>1</sup> Cigarettes contain addictive, harmful substances that can cause health issues. The main toxins in cigarettes include nicotine, tar, carbon monoxide (CO), and various heavy metals.<sup>2</sup> More than 4000 chemicals make up cigarettes, of which 250 are toxic to human health, and another 50 can cause cancer. One cigarette puff contains between 1014 and 1015 free radicals. Smoking can cause oxidative damage through several mechanisms, including direct damage by radical species, inflammatory responses, and lipid peroxidation.<sup>3</sup>

Free radicals damage lipids with molecules that have double-bonded carbon chains, which results in a phenomenon known as lipid peroxidation.<sup>4</sup> This process has three steps: start, propagation, and termination. Lipids primarily form lipids' cell membrane structure, and cellular permeability will be disrupted by oxidative damage to lipids, which might result in cell death. Malondialdehyde is a reaction product of lipid peroxidation (MDA). MDA levels in the blood or tissue can be measured to identify the degree of oxidative stress. Due to its high reactivity and toxicity, MDA is a reliable biomarker of lipid peroxidation and has been used in several types of research.<sup>5</sup> According to earlier studies, smoking up to two cigarettes a day for 28 days could raise rats' MDA levels.<sup>6</sup> The body naturally produces

antioxidants such as catalase, glutathione (GSH), and superoxide dismutase (SOD) to combat free radicals. The body also receives exogenous antioxidants, which aid in the fight against free radicals, from dietary consumption or dietary supplements.<sup>7</sup>

*Beta vulgaris* L. is high in well-identified antioxidant phytochemical compounds, including flavonoids, phenolic compounds, carotenoids, epicatechin, caffeic acid, a lipoic acid, and rutin. Plants include a variety of chemical substances that are beneficial as antioxidants, including flavonoids, polyphenols, vitamin C, vitamin E, and beta-carotene.<sup>8</sup> The beetroot tubers (*Beta vulgaris* L.) contain substances with antioxidant characteristics such as phenolic compounds and betalains. Free

radicals can obtain hydrogen atoms from the antioxidants in beetroot through their structure.<sup>9</sup> Antioxidants contribute hydrogen atoms at the lipid peroxidation process's last stage. This process stops the peroxidation of lipids from creating a more stable, non-radical product.<sup>10</sup>

Beetroot juice provides a significant amount of antioxidant power. This finding was demonstrated by a test on beetroot juice using 2,2-diphenyl-1-picrylhydrazyl (DPPH), which demonstrated that the juice might prevent the production of free radicals.<sup>11</sup> The group that received 8 ml/kg BW/day of beetroot juice for 28 days had lower MDA levels than the group that did not get beetroot juice, according to studies on rats in which liver damage was induced by carbon tetrachloride (CCl<sub>4</sub>).<sup>12</sup> A study was conducted to determine the impact of consuming beetroot juice on MDA levels. This study was done in light of the history of the potential of cigarettes to act as exogenous free radicals in raising MDA levels and the effectiveness of beetroot as an antioxidant. We also aimed to identify the Effect of *Beta vulgaris* L. on the MDA in male Wistar rats with cigarette smoke treatment.

## METHODS

### Sample

Beetroots (*Beta vulgaris* L.) were purchased from a fresh vegetable market in Semarang. We kept the *Beta vulgaris* L. at room temperature until processed. The medical chemistry laboratory identified the beetroots.

### Preparation of samples

We washed the fruits and peeled them to remove sand, dirt, debris, and debris. The peeled beetroots were then processed.

### Preparation of *Beta vulgaris* L. juice

*Beta vulgaris* L. is washed with clean water and cut into several pieces. Ten grams (10gms) of the peeled beetroot were blended with 50ml of distilled water in an electronic blender (Miyako BL51-G1 electric blender) to get a constant consistency mix. The mixture was poured into a measuring cylinder and added with distilled water to make a total volume of 100 ml, 10g/100ml (10%). The solution was centrifuged using a low-speed centrifuge

with 12 holes 80-2 for 10mins at 500rpm. The supernatant was collected and stored in plastic tubes. All the supernatants were stored in a refrigerator at 0°C until utilized within twenty-four hours.<sup>13</sup>

### Treatment of Experimental Animals

24 male Wistar rats were adapted to be given standard food and drink for 7 days. Randomly divided 24 rats into 4 groups and housed them based on the group so that each cage contained 1 group of 6 rats. Rats were treated according to their groups for 28 days. Cigarette smoke exposure was carried out using a smoking chamber, and *Beta vulgaris* L. juice was administered through a gastric probe. The following details of each group's treatment: Group control: 6 rats were given standard food and drink for 28 days. Cigarette smoking group: 6 rats were given standard food and drink and exposure to cigarette smoke at a dose of 2 sticks/day every morning for 28 days. The male Wistar rats (*Rattus norvegicus* L.), separated into 4 groups, are the experimental subjects in this true experiment with a post-test-only group design. N: Normal group. Rats were given a standard diet without exposure to cigarette smoke and *Beta vulgaris* L. juice for 28 days. Cigarette smoking group: Negative control group. Rats were given a standard diet and exposure to cigarette smoke without *Beta vulgaris* L. juice for 28 days. BV1: Treatment group 1. BV2: Treatment group 2. X1: Rats were given a standard diet, exposure to cigarette smoke, and *Beta vulgaris* L. juice at a dose of 8 mL/kg BW/day for 28 days.

### Study procedure

*Beta vulgaris* L. juice was given to rats using a gastric probe at 8 mL/kg BW and 16 mL/kg BW before cigarette smoke exposure.

### Measurement of Antioxidant Activity

#### *Beta vulgaris* L. Juice

Antioxidant activity was measured using the DPPH method (2,2-diphenyl-1-picrylhydrazyl) on the first day of *Beta vulgaris* L. juice preparation. The DPPH test was utilized to forecast antioxidant activity because antioxidants prevent lipid oxidation by scavenging DPPH radicals.<sup>14</sup>

### MDA assay

The examination was carried out using TBARS with the colorimetric method and a spectrophotometer at a wavelength of 532 nm in triplicate.<sup>15</sup>

### Cigarette exposure

The inhalation area was developed using a custom smoking chamber to produce the sidestream smoke. The cigarettes produced smoke after they were lighted and allowed to glow in the lower level. They were placed on a higher level to expose the fumes. An airflow-maintaining vacuum suction device was attached to the outflow airway.<sup>16, 17</sup>

## RESULT

This study used 24 male Wistar rats (*Rattus norvegicus* L.) aged 2-3 months with a body weight of 150-250 grams. The adaptation course was 7 days. During the study, there were no research samples that

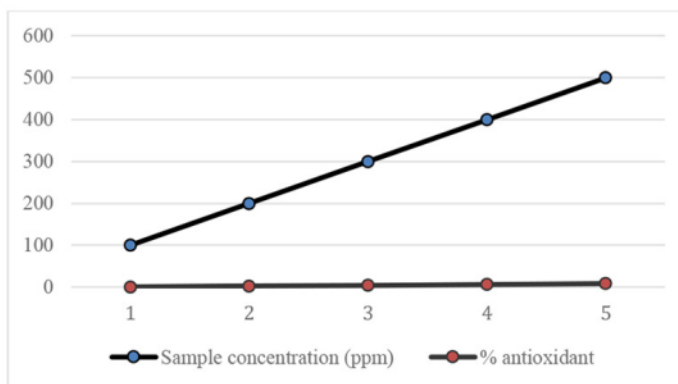


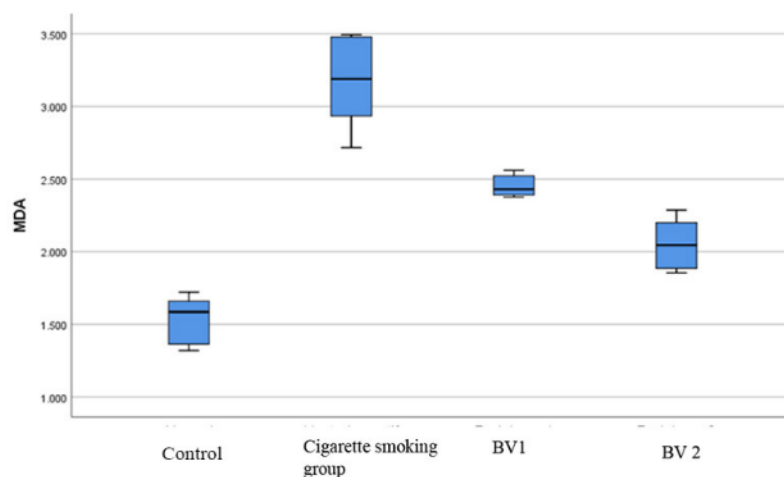
Figure 1. DPPH measurement results of *Beta vulgaris* L. juice

**Table 1.** Normality test of MDA level

Group	n	Normality test (p value)
Control		0,396*
Cigarette smoking group	6	0,546*
BV1	6	0,385*
BV2	6	0,675*

Note: \*: Normally distributed data ( $p > 0,05$ ), juice for 28 days.

Control: Negative control group. Cigarette smoking group: Rats were given a standard diet and exposure to cigarette smoke without *Beta vulgaris* L. juice for 28 days. BV1: Treatment group 1. BV2: Treatment group 2. X1: Rats were given a standard diet, exposure to cigarette smoke, and *Beta vulgaris* L. juice at a dose of 8 mL/kg BW/day for 28 days.



**Figure 2.** Result of blood MDA analysis. BV1: Rats were given a standard diet, exposure to cigarette smoke, and *Beta vulgaris* L. juice at a dose of 8 mL/kg BW/day for 28 days. BV2: Rats were given a standard diet, exposure to cigarette smoke, and *Beta vulgaris* L. juice at a dose of 16 mL/kg BW/day for 28 days.

**Table 2.** Results of the Games-Howell post hoc test for rat MDA levels

Group	Average difference	p-value
Control – Cigarette smoking group	-1,628	<0,001*
Control – BV1	-0,913	<0,001*
Control – BV2	-0,514	0,002*
Cigarette smoking group – BV1	0,716	0,008*
Cigarette smoking group – BV2	1,115	<0,001*
BV1 – BV2	0,398	0,005*

Note: \*Significant ( $p < 0,05$ ), BV1: Rats were given a standard diet, exposure to cigarette smoke, and *Beta vulgaris* L. juice at a dose of 8 mL/kg BW/day for 28 days. BV2: Rats were given a standard diet, exposure to cigarette smoke, and *Beta vulgaris* L. juice at a dose of 16 mL/kg BW/day for 28 days.

dropped out. Five milliliters of blood were drawn from a retro-orbital vein on day 29.

### Analysis of Antioxidant Levels of *Beta vulgaris* L. Juice

The antioxidant power of *Beta vulgaris* L. juice was measured using the DPPH method (2,2-diphenyl-1-picrylhydrazyl). DPPH measurement results and IC are presented in Table 1. We calculated the value of antioxidants using formula  $y = 0,02x - 1,7062,50 = 0,02x - 1,7062$ .  $x = 2585 \text{ ppm}$  (see Figure 1). The IC50 result of *Beta vulgaris* L. juice is 2585 ppm, classified as low because the IC50 is more than 200 ppm.

The results of assessing the MDA levels in rats were used to obtain the primary data. The Shapiro-Wilk test was utilized to check for data normality and identify the data distribution. Table 1 displays the analysis's findings.

The Shapiro-Wilk normality test revealed that the group's data had a normal distribution with a p-value of 0.05 or more. Figure 2 displays the rats' MDA levels.

The cigarette smoking group had the data on the highest average MDA levels, with an average value of 3.167 0.313 ppm. Group control had the lowest average MDA level, with an average value of 1.539 0.162 ppm. Levene's test was used to assess the data variations. It was determined using Levene's Test that the data variant was not equal to  $p = 0.009$  ( $p < 0.05$ ).

The research data were normally distributed; hence the one-way ANOVA test was performed to verify the hypothesis. Based on The one-way ANOVA test, the results of  $p < 0.001$  ( $p < 0.05$ ) were obtained, which showed that at least two groups had significantly different levels of MDA. Because The one-way ANOVA test results are meaningful and the data variants are different, a post hoc analysis of Games-Howell was conducted to determine which inter groups have differences. The results of the Games-Howell post hoc test can be seen in Table 2.

According to the post hoc analysis findings, there were statistically significant differences in the MDA levels of group control with cigarette smoking group, BV1, and BV2, as well as between the BV1 and BV2 groups the cigarette smoking group with BV1 and BV2.



## DISCUSSION

*Beta vulgaris* L., commonly known as beetroot, has been used to treat various diseases, such as constipation, fever, diabetes, cancer, constipation, cancer, diabetes, liver and heart diseases. *Beta vulgaris* L. extract can prevent and repair histological and biochemical transformations in the liver following a hepatotoxic agent.<sup>18</sup> Inflammation is a complex and adaptive physiological reaction toward harmful stimulants and tissue insults associated with various antigens. The antioxidant action of betanin in *Beta vulgaris* L., with its ability to donate electrons, has been identified as the leading cause of its enormous antioxidant capacity. Moreover, its efficacy in radical scavenging activity also improves the activation of Nrf2 signaling and the defense of oxidants.<sup>12</sup> Betaine is a vital methyl group donor that dampens inflammation through repressing nuclear factor-kappa light chain enhancer of activated B cells (NF- $\kappa$ B) and Akt activation.<sup>19</sup> Exposure to cigarette smoke works as an exogenous free radical in this study. When ROS levels up, the detachment of Nrf2 from the original Kelch-like ECH-associated protein 1 (Keap1) occurs when ROS increases.<sup>19</sup> After that, it will migrate into the nucleus and adhere to the antioxidant-responsive element (ARE), stimulating the gene expression of antioxidant and defensive genes. The Nrf2 regulates the adaptive mechanism of cells toward various oxidants and electrophiles. Nrf2 could reduce excessive ROS through the antioxidant activity of *Beta vulgaris* L. and activation of Nrf2.<sup>10</sup>

*Beta vulgaris* L. and its juice possess significant antioxidant and radical scavenging abilities, positively correlated with the natural antioxidants.<sup>12</sup> The combined use of alcohol and cigarette smoke on the liver or kidneys induces liver, and kidney injuries, in both morphology and biochemically chronically cigarette smokes treated rats. This damage is related to biochemical changes in rats.<sup>13</sup>

Administration of *Beta vulgaris* L. juice can help endogenous antioxidants maintain and prevent oxidative stress or damage to cellular components. Antioxidants can neutralize free radicals by donating electrons to free radicals not

to react/damage cells or DNA. Betanin in *Beta vulgaris* L. may act as an antioxidant because of its high ability to donate electrons to prevent free radicals from damaging cell membranes. Based on an in vitro study, betalain metabolites in betanin and betanidine can reduce the occurrence of lipid membrane oxidation in *Beta vulgaris* L. and be the most effective in inhibiting lipid peroxidation.<sup>24</sup> This vital mechanism is due to the inactivation of the enzymes involved in the biochemical response (lipoxigenase and cyclooxygenase).<sup>21</sup>

The two primary components of cigarette smoke, tar and gas have been shown to contain high concentrations of oxidants that lead to lipid peroxidation.<sup>22</sup> When unsaturated fatty acids are converted to lipid peroxide in the phospholipids of the cell membrane, MDA is the result. MDA can contribute to DNA mutation and cell damage brought on by lipid oxidation, which takes place gradually. Compared to the control group, the MDA levels in the cigarette smoking group were considerably higher ( $p < 0.05$ ). The result indicates that the increased free radicals in the tissues due to chemical components inhaled from cigarette smoke induce the lipid peroxidation process, which causes an increase in MDA levels.<sup>23</sup>

Cigarette smoke is a source of exogenous free radicals. Inhaling cigarette smoke can cause the formation of free radicals, such as hydrogen peroxide, hydroxyl ions, superoxide, and peroxy radicals, in large quantities, which then cause oxidative stress in the body. Oxidative stress conditions caused by smoking occur through several mechanisms: direct damage by inhaled free radicals and the inflammatory response induced by smoking. Direct cell damage due to free radicals is caused by their unstable structure and is highly reactive so that they can bind electrons from other molecules.<sup>24</sup> Inflammatory response due to smoking. This occurs as a result of inhaled cigarette smoke causing damage to lung epithelial cells and triggering the release of inflammatory mediators.<sup>23</sup>

Inflammatory reactions that continue to occur produce endogenous free radicals that cause oxidative stress conditions. A lipid peroxidation process occurs when

free radicals attack lipids that have double bonds between carbon and carbon in their structure. Malondialdehyde (MDA) is a product of lipid peroxidation whose levels will increase.<sup>25</sup> As free radicals increase in the body, MDA levels are often used as a biomarker to measure oxidative stress in the body. In this study, group BV2 participants who received beet juice at a dose of 16 ml/kg BW had lower measured MDA levels than group BV1 participants who received juice at a rate of 8 ml/kg BW.

In this study, the measurement of MDA levels in group BV2 who were given beetroot juice at a dose of 16 ml/kg BW were lower than the MDA levels in group BV1, given beetroot juice at a dose of 8 ml/kg BW. In this study, the measurement of MDA levels in group BV2 who were given beetroot juice at a dose of 16 ml/kg BW were lower than the MDA levels in group BV1, given beetroot juice at a dose of 8 ml/kg BW. Under average body metabolic conditions, there is a balance between antioxidant components and pro-oxidant agents, such as free radicals, to prevent oxidative stress. The content of compounds that act as antioxidants in beetroot juice can strengthen endogenous antioxidant defenses against oxidative damage caused by free radicals.<sup>26</sup> In the lipid peroxidation process, antioxidants play a role in the termination stage of biosynthesis; these include condensing the beta-chromatin chromophore, balsamic acid, with cyclo-dopa and amino acids or amino acids alone or those involved in the construction of the related aldimine from the red-purple beta and yellow betaxanthins.<sup>9</sup>

Effect of Exposure to Cigarette Smoke Against MDA Levels of Male Wistar Rats. In this study, the levels of MDA in the cigarette smoking group were significantly significant ( $p < 0.05$ ) compared to the control group. This shows an increase in free radicals in tissues due to chemical components inhaled from smoke. Cigarettes induce lipid peroxidation processes which cause an increase in MDA levels.

One of the sources of exogenous free radicals is cigarette smoke. When free radicals such as hydrogen peroxide, ions hydroxyl, superoxide, and peroxy radicals are formed from inhaling cigarette smoke, this can lead to oxidative stress

in the body.<sup>27</sup> Oxidative stress conditions due to smoking occur through several mechanisms: direct damage by inhaled free radicals and the inflammatory response.<sup>28</sup>

The demographic location and cultivating process influence the phytochemical components and antioxidant activity of *Beta vulgaris* L.<sup>29</sup> Free radicals can oxidatively damage mitochondria and regulate gene expression, contributing to fibrosis and the chronic inflammatory process.<sup>30</sup> Betalains have a consistent structural characteristic derived from balsamic acid and a radical R1 or R2, where the substituents can be *Beta vulgaris* L. holds approximately 75–95% betacyanins and 5–25% betanin. Falling levels of cytokines, superoxide anion, and nuclear factor kappa B (NF-κB) after initiation of lipopolysaccharide (LPS) occur after betanin treatment.<sup>31</sup> Pigments from betanin. The number of betalains in processed beetroot juice was examined in prior research. Betanin was shown to be the most prevalent component at 300–600 mg/kg BW, as observed by violaxanthin and betanin. Betanins hinder ROS production by neutrophils and decrease the number of penetrating neutrophils.<sup>32</sup> The MDA levels of rats exposed to cigarette smoke were measured as part of research to determine the impact of administering beetroot in alternative dosage forms.

## CONCLUSION

Cigarette smoke exposure increased the blood levels of malondialdehyde (MDA) in Wistar rats (Cigarette smoking group) compared to the control group. Blood MDA levels of Wistar rats were lower in Groups BV1 and BV2, which received beetroot juice in addition to kretek cigarette smoke, than in the cigarette smoking group, which received exposure to kretek cigarette smoke. Wistar rats exposed to beetroot juice and cigarette smoke had lower MDA levels in the group that received 16 ml/kg BW of juice than in the group that received 8 ml/kg BW of juice.

## ACKNOWLEDGMENT

We thank the technician and laboratory staff who helped carry out this study.

## FUNDING

The authors are responsible for the study funding without a grant or any external funding source.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ETHICAL CONSIDERATION

The Health Research Ethics Commission (KEPK.) Faculty of Medicine, Diponegoro University, approved this study with register no. 99/EC/H/FK-UNDIP/VIII/2021.

## AUTHOR CONTRIBUTION

Conceptualization, ABS, ANS, and PKD; methodology, PKD, and ANS validation, PKD, EKSL, and ANS; formal analysis, ANS; investigation, PKD, and ANS; data curation, ANS; writing—original draft preparation, ANS; writing—review and editing, ANS; and funding acquisition, ABS. The final text has been reviewed and approved by all authors.

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