

Rice brain administration reduces malondialdehyde (MDA) levels in the heart of carbon tetrachloride-induced rats

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World Journal of Biology Pharmacy and Health Sciences, 2024, 18(02), 403–409

Publication history: Received on 12 April 2024 revised on 20 May 2024; accepted on 22 May 2024

Article DOI: <https://doi.org/10.30574/wjbphs.2024.18.2.0307>

Abstract

Excessive production of free radicals proven associated with disease progression of coronary heart disease. Rice bran oil has a high content of antioxidants such as tocopherol, tocotrienol, and γ -oryzanol. This study aimed to determine the effect of rice bran oil administration on malondialdehyde (MDA) level as an oxidative stress biomarker in the heart of carbon tetrachloride (CCl_4)-induced rats. Thirty stored rat heart tissue samples that were divided into six groups, consisted of untreated control group, CCl_4 -treated group (CCI), rice bran oil-treated before CCl_4 induction groups with rice bran oil dosage of 0.5 ml (B1+CCI) and 1.5 ml (B2+CCI), and rice bran oil-treated after CCl_4 induction groups with rice bran oil dosage of 0.5 ml (CCI+B1) and 1.5 ml (CCI+B2), were measured for their MDA levels using Wills modified method. The highest heart's MDA levels were obtained in the group that only received carbon tetrachloride. The lowest heart's MDA levels were found in the group given 1.5 ml of rice bran oil before being given carbon tetrachloride. Heart MDA levels in all groups given rice bran either before or after administration of carbon tetrachloride were lower than those in the group given only carbon tetrachloride. The lowest heart MDA found in the group that was administered by 1.5 ml/day of rice bran oil before carbon tetrachloride induction.

Keywords: Malondialdehyde; Heart; Carbon Tetrachloride; Rice Bran

1. Introduction

Excessive production of free radicals has proven to be related to the development of coronary heart disease (CHD).^{1,2} Conditions where the production of free radicals is much higher than the antioxidant capacity is referred to as oxidative stress. Oxidative stress can be caused by various internal factors such as aging and external processes such as free radical exposure from the environment. Oxidative stress can cause damage to DNA, lipid, and protein that can eventually cause cell death.³

Oxidative stress causes heart damage through multiple mechanisms. First, oxidative stress causes macromoleptic damage such as lipids, proteins, and DNA. Lipid is primarily unsaturated fatty acid (polyunsaturated fatty acid, PUFA) is the main target of oxidative stress through lipid peroxidation process. ROS can also attack protein through protein carbonated processes. ROS can join a skeletal bond from nucleotide base so that it spoils nucleic acid structure. Second, oxidative stress can cause heart damage through inflammatory. Oxidative stress is an inflammatory initiator at once a result of inflammatory response. Oxidative stress can activate NF- κ B which is a transcription factor that plays a role in

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inflammatory regulation. Third, oxidative stress can also induce the death of the programmable cell through various mechanisms.⁴

Macromolecular damage due to oxidative stress can be characterized by an increase in malondialdehyde (MDA) levels which are products of lipid peroxidation.⁵ One way to cause oxidative stress on the heart of the mice is by providing carbon tetrachloride (CCl₄).⁶ CCl₄ is a compound that can form free radicals when entering into the body. This compound is commonly used in experimental research to induce oxidative stress in liver⁷ and also other organs such as heart.⁸

Antioxidants are compounds that can degrade and get rid of free radicals. In the body, antioxidants can be divided into enzymatic and non-enzymatic antioxidants. Examples of antioxidant enzymes namely superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). Examples of non-enzymatic antioxidants include glutathione (GSH), vitamin C, vitamin E, and carotenoids.³ Rice bran oil is the oil obtained from bekatul. Bekatul is a side product of rice grinding process. Rice bran oil (bekatul) is known to have a high non-enzymatic antioxidant content such as tocopherol, tocotrienol, and γ -oryzanol.⁹ Some studies on animals have shown that supplementation is known to be able to lower the level of oxidative stress by improving antioxidant activity and lowering the oxidative stress biomarker levels such as MDA.¹⁰⁻¹² Therefore, this research aims to know the antioxidant effects of rice bran oil at the heart of the mice induced carbon tetrachloride (CCl₄) by observing MDA levels as oxidative stress biomarker is the main antioxidant component of rice bran oil.¹³

2. Material and methods

2.1. Sample

The sample used in this research is a biological material stored in the form of a mice heart derived from the experimental research of Dr. drg. Retno Gunarti, M.S. with the title of "Bekatul Oil Potency In Inhibiting Stress Oxidative Induction Network Damages: Marker Antioxidant Review, Histopatology, Expression of Gen Inflammation and Apoptosis" that has escaped pickling with KET-674/UN2.F1/ETIK/PPM.00.02/2021. A total of 30 samples of the heart tissue are stored from the rat Sprague Dawley male rats with 8-12 weeks and a weight of 250-400 grams divided into six treatment groups. The first group is the Control group (without treatment, n=5), second is the CCl group (giving CCl₄ only, n=5), third is the B1+CCl group (treated with 0.5 ml rice bran oil before the induction of CCl₄, n=6), fourth is the B2+CCl group (treated with 1.5 ml rice bran oil before the CCl₄ induction, n=5), fifth is the CCl+B1 group (treated with 0.5 ml rice bran oil after the induction of CCl₄, n=5), and sixth is the CCl+B2 group (treated with 1.5 ml rice bran oil after the induction of CCl₄, n=5). Induction CCl₄ is done by providing CCl₄ through the continuation using a cervical needle with a single dose of 0.55 g/kg body weight. The rice bran oil is administered by the cervical needle through the continuation of daily for eight weeks before or after the CCl₄ induction with 48 hours feeding distance between CCl₄ and the rice bran oil.

2.2. Tissue homogenization

The sample of the freezed stored heart tissues were weighed 100 mg then homogenized using a PBS solution 0.01 M. After that centrifuged at 3.500 rpm for 10 minutes, then the formed supernatan were taken and stored in -20°C until used for the measurement of MDA levels. The MDA rate were measured using the modified Wills¹⁴ method.

2.3. Malondialdehyde (MDA) standard curve

MDA standard solution were made using MDA concentration of 0 nmol/mL; 0.3125 nmol/mL; 0.625 nmol/mL; 1.25 nmol/mL; 2.5 nmol/mL; 5 nmol/mL; as well as 10 nmol/mL. Sample solution were diluted by twice using aquadest with a final volume of 400 μ l. Sample solution were given TCA 20% solution then cooled using vortex. Then the sample solution were centrifugated at 5,000 rpm speed for 10 minutes, the formed supernatant were added into a new microtube then added by 400 μ l of 0.67% TBA solution. Next, the solution were synced with the temperature of 96-100°C for 10 minutes and then lift and disclaimed to reach the temperature of the room. Good standard solution and samples are made in duplo. The absorbance measurement is carried on the wavelength of 530 nm. The measurement of the MDA level of the sample is done by projecting the average absorbance of the sample against the standard curve with the formula $y = ax + b$ and multiplied by two as the dilution factor. After gaining MDA levels in nmol/mL, the value is divided by the weight of the balanced network so that the end unit of the MDA level is nmol/mg network. A sample that has a higher absorbance value than a standard solution is carried out measurements with greater dilution factors.

2.4. Statistical analysis

Data results of statistically analyzed research by using IBM SPSS Statistics software version 22. Shapiro-Wilk's normality test and Levene homogeneity is performed against MDA's heart level data. If the data is normal distributed and the

variant is homogeneous, then the ANOVA one-way parametric test is carried out. However, if the data is not normal distributed, it is done the Kruskal-Wallis non-parametric statistical test. The value of $p < 0.05$ is considered statistically significant.

3. Result and Discussion

3.1. Malondialdehyde (MDA) standard curve

MDA levels in the samples were calculated based on the equation obtained from the MDA standard curve. Based on the resulting standard curve (Figure 1), the equation $y = 0.0618x - 0.001$ was obtained with a coefficient of determination (R^2) = 0.9999 where y is the absorbance value and x is the MDA level. This equation was converted into the form $x = (y + 0.001)/0.0618$ to calculate the MDA content of the sample based on its absorbance value.

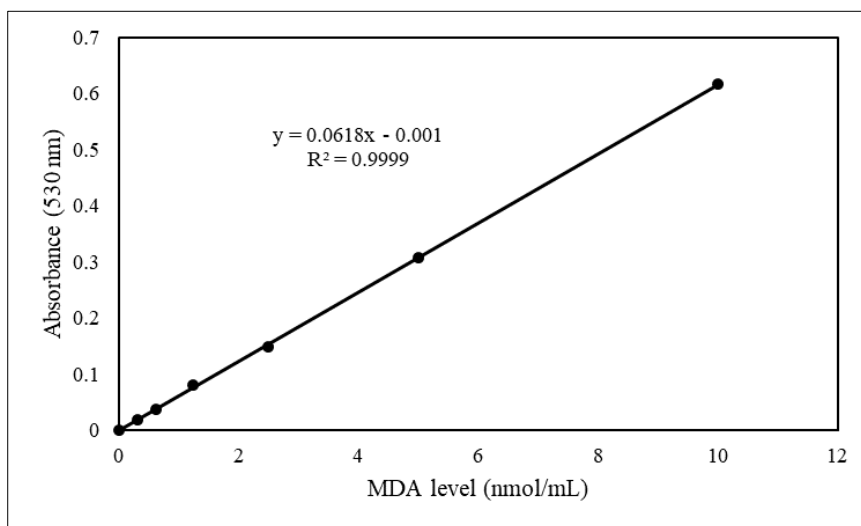


Figure 1 Malondialdehyde (MDA) level standard curve

3.2. Malondialdehyde (MDA) level

MDA levels of the samples were calculated using the formula of the MDA standard curve equation multiplied by the dilution factor and then divided by the weight of the heart tissue in milligrams used as homogenate.

The mean of heart MDA level of each group is presented in Figure 2. The mean of heart MDA level in the control group (without any treatment) is 0.128 nmol/mg tissue. The mean of heart MDA level in the group given carbon tetrachloride alone (CCl_4) is 0.194 nmol/mg tissue. The mean of heart MDA level in the group given 0.5 ml/day rice bran oil before carbon tetrachloride induction is 0.169 nmol/mg tissue. The mean of heart MDA level in the group given 1.5 ml/day rice bran oil before carbon tetrachloride induction is 0.117 nmol/mg tissue. The mean of heart MDA level in the group given 0.5 ml/day rice bran oil after carbon tetrachloride induction is 0.164 nmol/mg tissue. The mean of heart MDA level in the group given 1.5 ml/day rice bran oil after carbon tetrachloride induction is 0.167 nmol/mg tissue.

Based on the results, it can be seen that the highest heart MDA levels belonged to the group given carbon tetrachloride alone (0.194 nmol/mg tissue). The lowest heart MDA levels were found in the group given rice bran oil at a dose of 1.5 ml/day before being given tetrachloride ($\text{B2} + \text{CCl}_4$) (0.117 nmol/mg tissue).

Malondialdehyde (MDA) is a compound commonly used as a biomarker of oxidative stress in tissues because MDA is the end product of the lipid peroxidation process that occurs during oxidative stress.¹⁵ Oxidative stress conditions are conditions in which there is an excessive increase in the rate of production of free radicals, some of which are hydroxyl and superoxide radicals. Hydroxyl radicals can react with PUFAs in the plasma membrane to form lipid radicals. The diene conjugates formed from radical lipids will form peroxy radicals when oxidized. Peroxy radicals can then react with superoxide and other PUFAs to form hydroperoxides and new radical lipids. The resulting hydroperoxides will fragment into MDA and 4-HNE compounds due to their less stable nature.¹⁶ The higher the level of oxidative stress, the higher the expected production of MDA compounds and vice versa.

CCl_4 is a compound commonly used in research to induce oxidative stress in tissues.¹⁷ CCl_4 that enters the body will be converted by CYP enzymes into a reactive form, CCl_3^* . CCl_3^* will form a highly radicalized compound, CCl_3OO^* , if it reacts with oxygen so that high exposure to CCl_4 can cause oxidative stress. A lipid peroxidation chain reaction can be triggered by CCl_3OO^* when it meets PUFA.¹⁸ MDA is one of the compounds produced from the lipid peroxidation process so it can be used to measure oxidative stress in tissues.¹⁹ Based on the nature of this CCl_4 compound, treatment of rats with CCl_4 is expected to increase MDA levels in heart tissue.

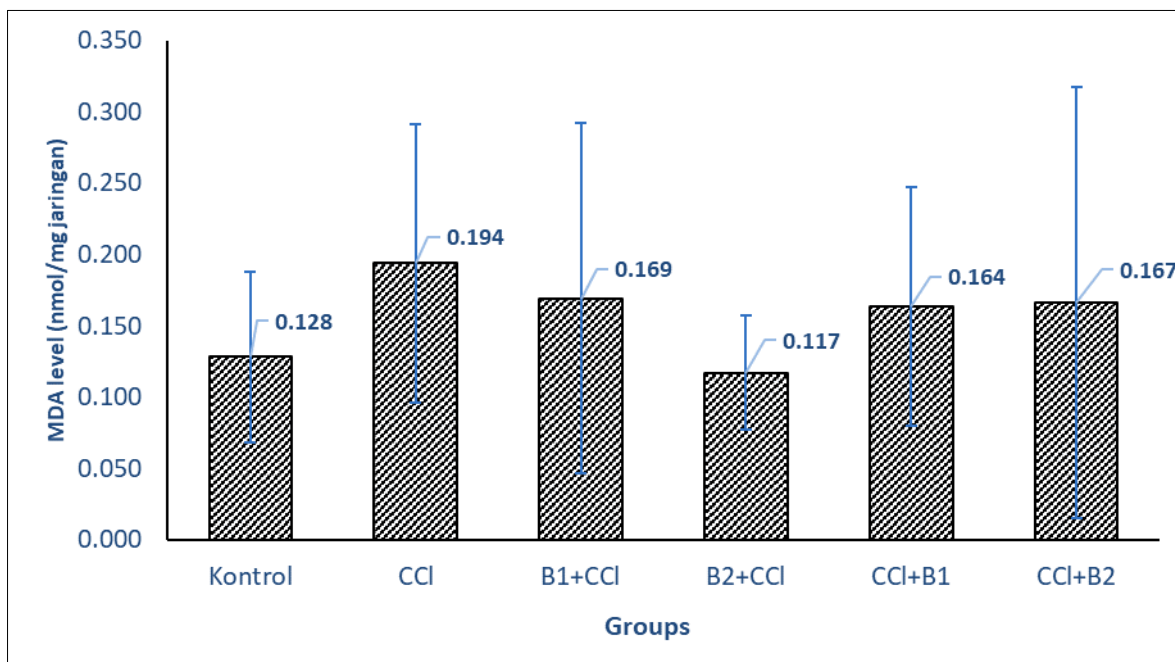


Figure 2 Malondialdehyde (MDA) levels in each group. Control= no treatment, CCl₄= CCl₄ treatment only, B1+CCl₄= rice bran oil treatment 0.5 ml before CCl₄, B2+CCl₄= rice bran oil treatment 1.5 ml before CCl₄, CCl₄+B1= rice bran oil treatment 0.5 ml after CCl₄, CCl₄+B2= rice bran oil treatment 1.5 ml after CCl₄. Error bars indicate standard deviation. One-way ANOVA test results showed non-significant differences ($p = 0.550$). Notes: MDA= malondialdehyde; CCl₄ = carbon tetrachloride; B = rice bran.

The results in this study showed that the group of rats given CCl₄ at a dose of 0.55 g/kg body weight had higher average heart MDA levels than the control group. This is in line with research conducted by Eshaghi et al.²⁰ on male Wistar rats which showed that the administration of CCl₄ at a dose of 1 ml/kg body weight significantly increased the average MDA levels in the hearts of rats. Research by Zdravković et al.²¹ on male Wistar rats also showed that the heart of rats in the CCl₄ treatment group at a dose of 1 ml/kg body weight had significantly higher MDA levels than the control. The study also measured antioxidant activities such as SOD, peroxidase (POD), GPx, GSH, and glutathion S-transferase (GST) in the heart. The activities of the five antioxidants were found to be significantly lower in the CCl₄-treated group compared to the control. The decrease in antioxidant activity in the tissue indicates that there is an increase in oxidative stress in the tissue. Similar results were also obtained by other studies conducted by Jayakumar et al.²² and Thirupathi et al.²³ which showed an increase in cardiac MDA levels and a decrease in antioxidant activity due to CCl₄ treatment. This indicates that CCl₄ is proven to increase oxidative stress in the rat heart which can be observed from the increase in MDA levels and decreased activity of antioxidants.

A balance between antioxidants and free radicals is necessary to prevent oxidative stress. Antioxidants are compounds that can degrade or remove free radicals.²⁴ There are two types of antioxidants, enzymatic and non-enzymatic antioxidants. One example of an enzymatic antioxidant is vitamin E which consists of tocopherols and tocotrienols. Vitamin E works as an antioxidant by donating electrons to free radicals thereby eliminating their radical properties.³ Vitamin E is a fat-soluble vitamin that plays an important role in fighting oxidative stress on cell membranes such as lipid peroxidation.²⁵ Bran oil is known to be rich in vitamin E content such as tocopherols and tocotrienols. In addition, bran oil also has a high content of another non-enzymatic antioxidant compound, γ -oryzanol, which is a mixture of various ferulic acid ester compounds of triterpene alcohols and sterols. Just like vitamin E, γ -oryzanol works as a non-enzymatic antioxidant by donating its electrons to free radicals. Tocopherol, tocotrienol, and γ -oryzanol are the three main antioxidant compounds in rice bran oil.^{13,26} The administration of rice bran oil to rats is expected to reduce the

level of oxidative stress in the CCl₄-induced heart and is observed by a decrease in MDA levels as a biomarker of oxidative stress.

Munkong *et al.*¹⁰ through their research on male Sprague Dawley rats showed that the administration of bran extract at 2,205 mg/kg body weight/day orally for four weeks significantly reduced heart MDA levels induced by high fat diet (HFD) as a trigger of oxidative stress. HFD in the study was given simultaneously with the administration of rice bran extract. Based on these results, Munkong *et al.*¹⁰ concluded that rice bran extract provides a preventive effect against oxidative damage in HFD-induced heart tissue. Similar results were also provided by the research of Garcia *et al.*¹¹ on male Wistar rats induced by high sugar fat (HSF) diet to trigger oxidative stress. The study showed that the group given HSF diet induction along with bran supplementation had significantly lower cardiac MDA levels than the HSF diet-induced group. Based on their results, Garcia *et al.*¹¹ concluded that bran supplementation can prevent heart damage by modulating oxidative stress in the myocardium of HFD rats.

Administration of rice bran oil before oxidative stress induction is expected to increase the antioxidant capacity of the heart before oxidative stress occurs due to the high content of non-enzymatic antioxidants in the form of vitamin E and γ -oryzanol in rice bran oil. This increased antioxidant capacity can get rid of the radicals formed during oxidative stress later. Therefore, the administration of rice bran oil before CCl₄ induction in rats is expected to lead to lower cardiac MDA levels than without rice bran oil administration.

In this study, the average heart MDA levels in the group of rats given rice bran oil at a dose of 0.5 ml/day and 1.5 ml/day for eight weeks before CCl₄ induction were lower than the group of rats that were only induced by CCl₄ although not significantly different. These results are in line with research by Bardhan *et al.*¹² on male Charles Foster rats where the administration of rice bran oil at doses of 0.5 mg/kg body weight, 1 mg/kg body weight, 2 mg/kg body weight, and 4 mg/kg body weight daily for 12 days before induction of oxidative stress reduced the average heart MDA levels due to isoproterenol-induced oxidative stress although only the dose of 2 mg/kg body weight made a significant difference. This proves that the administration of rice bran oil before the induction of oxidative stress can prevent the increase in MDA levels in rat hearts.

Rice bran oil given after oxidative stress induction is expected to increase antioxidant activity in the heart. This increase in antioxidant activity can get rid of free radicals that have been formed due to oxidative stress induction, thus reducing the rate of free radical production. The decrease in the production rate of free radicals will also reduce the production rate of MDA. In the cell, MDA that is formed can be metabolized enzymatically into non-radical compounds.²⁷ Therefore, the administration of rice bran oil after CCl₄ induction in rats is expected to provide lower heart MDA levels than without the administration of rice bran oil due to a decrease in the ratio between the rate of production and elimination of MDA in the heart.

The results of this study showed that the group of rats given rice bran oil at a dose of 0.5 ml/day and 1.5 ml/day for eight weeks after CCl₄ induction had lower average heart MDA levels than the group of rats induced by CCl₄ alone although the difference was not statistically significant. These results are in line with the research of Sethi *et al.*²⁸ who analyzed the benefits of vitamin E administration in Sprague Dawley rats induced myocardial infarction. The study conducted on the group of rats induced myocardial infarction was treated with vitamin E, resulting in significantly lower average cardiac MDA levels than the group of rats induced myocardial infarction that was not treated with vitamin E. The group of rats treated with vitamin E in the study also showed smaller infarct size although not statistically significant. The results prove that the administration of rice bran oil after induction of oxidative stress can reduce MDA levels in rats' hearts.

4. Conclusion

The highest heart malondialdehyde (MDA) level found in the group that was only given carbon tetrachloride alone without being given rice bran intake. Rice bran oil administration at a dose of 0.5 ml/day and 1.5 ml/day for eight weeks both before and after carbon tetrachloride administration are lowering the heart MDA levels of rats. The heart MDA level in groups administered with rice bran oil at a dose of 1.5 ml/day for eight weeks are lower than 0.5 ml/day both before or after carbon tetrachloride induction. The lowest heart MDA found in the group that was administered by 1.5 ml/day of rice bran oil before carbon tetrachloride induction.

Compliance with ethical standards

Acknowledgments

Authors convey their appreciation to laboratory staff in the Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia for technical assistance during the experiments.

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

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