Syzygium Cumini Leaves Extract from West Sumatra Indonesia Alleviate Oxidative Stress by Decreasing Malondialdehyde Level and Enhancing Catalase Activity in Rat Induced by Lead Acetate

Rauza Sukma Rita¹,*, Elmatris Sy²

¹Department of Biochemistry, Faculty of Medicine, Universitas Andalas, Padang, INDONESIA.
²Department of Chemistry, Faculty of Medicine, Universitas Andalas, Padang, INDONESIA.

Correspondence
Rauza Sukma Rita
Department of Biochemistry, Faculty of Medicine, Universitas Andalas, Padang, INDONESIA.
E-mail: rauzasukmarita@med.unand.ac.id

ABSTRACT

Introduction: Lead is one of the most dangerous heavy metals in the environment. Contaminated drinking water, battery manufacturing, lead paints, and industrial pollutants are all sources of lead exposure. Lead exposure can cause oxidative stress and is related to many health problems. To prevent oxidative stress caused by lead, the body needs additional antioxidants from the outside body. Syzygium cumini leaf is abundant in antioxidants, which help to minimize oxidative stress caused by lead.

Methods: The rats were divided into three groups: negative control, positive control (lead acetate 40 mg/kg BW, 30 days), and treatment (lead acetate 40 mg/kg BW and Syzygium cumini leaves extract 150 mg/kg BW, 30 days). At the end of the experiment, blood was collected and prepared to measure malondialdehyde and catalase activity.

Results: The leaf extract of Syzygium cumini reduced serum malondialdehyde levels while increasing catalase activity. Conclusion: Lead exposure induces oxidative stress, which can be reduced by Syzygium cumini’s leaves.

Key words: Lead acetate, Syzygium cumini’s leaves, Malondialdehyde, Catalase activity, Rat.

INTRODUCTION

Free radicals are molecules that contain unpaired electrons in a set of atoms. Excessive amounts of free radicals can cause damage to cells, DNA, and proteins. The long-term impact of free radicals is also associated with degenerative diseases, such as diabetes mellitus, coronary heart disease, etc. Reactive oxygen species (ROS) cause substantial damage to biological molecules such as DNA, enzymes, and proteins. These reactive oxygen species (ROS) can also cause cell membrane lipid peroxidation. Lead (Pb) is a persistent non-essential metal that can fight free radicals. Research related to the administration of Syzygium cumini leaf extract in overcoming oxidative stress due to lead exposure in West Sumatra, Indonesia, is still limited. Therefore, this study aims to analyze the effect of Syzygium cumini leaf extract on endogenous lipid peroxidation and antioxidant parameters due to lead exposure.

MATERIALS AND METHODS

Animals

This research was conducted on male Wistar rats (Rattus norvegicus). Before the study, rats were acclimatized for one week and given free access to food and drink. A total of 18 white male rats Wistar strain (Rattus norvegicus) were divided into three groups, namely the negative control group (normal saline), positive control (lead acetate 40 mg/kg BW for 30 days), and treatment (lead acetate 40 mg/kg BW and Syzygium cumini’ extracts 150 mg/kg BW for 30 days). After passing the ethical test performed...
by the Ethics Commission of the Faculty of Medicine, Universitas Andalas, No. 113/UN.16.2/KEP-FK/2020, this study was carried out.

**Chemicals and Reagents**

Tricarboxylic Acid and Thiobarbituric Acid were utilized to quantify Malondialdehyde serum using the Thiobarbituric acid reactive substance (TBARS) test. Catalase activity was measured using colorimetry methods utilizing potassium dichromate, acetic acid, and hydrogen peroxide. Lead acetate and all of the ingredients were provided by Sigma Aldrich in Germany.

**Preparation of Syzygium cumini’s leaves extracts**

Syzygium cumini leaves were obtained from West Sumatra Province, Indonesia, and confirmed from the Herbarium of Universitas Andalas, Padang, West Sumatra, Indonesia, No. 320/K-ID/ANDA/X/20. Syzygium cumini leaf extract was made at the Pharmacology Laboratory, Faculty of Pharmacy, Universitas Andalas. The leaves of Syzygium cumini, which are still fresh, weighing 2.5 kg, are cleaned and washed using running water, then cut into small pieces, and then dried by aerating in the open-air protected from sunlight. After drying, the leaves of Syzygium cumini are ground to form a coarse powder.

The extract of Syzygium cumini leaves used the maceration method with a 96% ethanol mixture. The maceration method is carried out for three days in a dark container protected from direct sunlight, then stirring regularly. The maceration process was carried out in the next three days to extract the entire extract. The collected macerate will be evaporated by vacuum distillation and then filtered using a tool called a rotary evaporator at a temperature of 40°C to then produce a pure extract of Syzygium cumini leaves with a thick texture.

**Measurement of Malondialdehyde**

Rat serum (500 μl) is added with 2.5 ml of TCA 5%, then mixed with a vortex mixer. Once mixed, centrifuge for 10 minutes at a speed of 2000 RPM. The centrifuge is repeated for 10 minutes, the same treatment on standard and blank solutions. According to the label, each tube is taken 1.5 ml filtrat using a pipette, then inserted into the tube. Furthermore, on each tube added 1.5 ml Na Thio Barbituric Acid, mixed using a vortex mixer. After that, it is heated in the water bath for 30 minutes, then cooled and ready to read using Spectrophotometer (Spectronic 20) at λ 550 nm.26

**Measurement of Catalase Activity**

In the tube, 4 ml H₂O₂ (hydrogen peroxide, 0.2 M) solution was added, followed by 5 ml buffer phosphate. After that, slowly add 1 ml of serum and homogenize. This reaction is measured in milliliters and then added to 2 milliliters of glacial acetate. This procedure is repeated 60 seconds apart on various tubes. To eliminate blue precipitation and produce green precipitation, the tube is heated in boiling water for 10 minutes. At a wavelength of 570 nm, the absorbant is measured. When the process is stopped by acetic acid, the standard curve is used to determine how much H₂O₂ remains. The amount of protein consumed is determined by the activity of enzymes.26

**Statistical Analysis**

The data is presented as a mean s.e.m. One-way ANOVA was used in the statistical analysis, followed by multiple comparison tests. Statistical significance was defined as p values < 0.05.

**RESULTS**

**Effect of Syzygium cumini’s extract on malondialdehyde serum levels**

Lead acetate administration (40 mg/kg BW, 30 days) increased malondialdehyde serum level in the rat. This increase is significant compared to the negative control group (p<0.05). Syzygium cumini’s extract (150 mg/kg BW, 30 days) counteracts lead acetate-induced malondialdehyde enhancement, and this level no different with a negative control group (Figure 1).

**Effect of Syzygium cumini’s extract on catalase’s activity**

In figure 2, catalase activity decreased by administration of lead acetate (40 mg/kg BW for 30 days), and this was significant compared to either the negative control group and treatment group (p-value < 0.05). The treatment group showed an increase in catalase activity significantly compared to a positive control group (p-value < 0.05).

**DISCUSSION**

In this study, the administration of lead acetate (40 mg/kg BW) led to increased Malondialdehyde levels and decreased activity of serum catalase in rats. Lead acetate is a heavy metal that is widely found in everyday life.16,27 Exposure to lead acetate can increase free radicals in the body;16,28–30 Increased free radicals lead to a rise in lipid peroxidation,31–33 one of the remaining lipid peroxidations often found...
is Malondialdehyde. Previous research has shown that exposure to lead acetate leads to elevated levels of blood Malondialdehyde. Furthermore, increased free radicals due to lead acetate also lead to decreased levels of endogenous antioxidant activity, such as catalase, SOD, and Glutathione peroxidase. Antioxidant enzyme activity is lowered because lead has a strong affinity for SH groups or metal cofactors in antioxidant enzymes and substances.35

In rats given lead acetate (40 mg/kg BW), administering Syzygium cumini leaf extract (150 mg/kg BW) resulted in lower serum malondialdehyde levels and increased catalase activity. Tannins, flavonoids, phenols, triterpenoids, saponins, alkaloids, glycosides, steroids, fatty acids, proteins, and other substances are found in Syzygium cumini leaf extract. Phenol and flavonoid components are reported to have high antioxidant activity. According to another study, Syzygium cumini leaf extract contains quercetin, myricetin, kaempferol, and glycosides. Because of the high antioxidant content of Syzygium cumini leaf extract, Malondialdehyde levels are reduced, and catalase activity was raised, thus diminishing oxidative stress. Reactive oxygen species can be neutralized by exogenous antioxidants acquired from medicinal plants.

CONCLUSION

Lead acetate exposure can raise free radical levels in the body, causing oxidative stress. Because of its potent antioxidant content, this study reveals that Syzygium cumini leaf extract has the potential to overcome oxidative stress caused by lead exposure.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENT

Research financially supported this study by Grant Fundamental Scheme No.23/UN.16.02/Fd/PT.01.03/2020 from the Faculty of Medicine, Universitas Andalas, Padang, Indonesia.

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Rita RS, et al.: Syzygium Cumini Leaves Extract from West Sumatra Indonesia Alleviate Oxidative Stress by Decreasing Malondialdehyde Level and Enhancing Catalase Activity in Rat Induced by Lead Acetate

Cite this article: Rita RS, Sy E. Syzygium Cumini Leaves Extract from West Sumatra Indonesia Alleviate Oxidative Stress by Decreasing Malondialdehyde Level and Enhancing Catalase Activity in Rat Induced by Lead Acetate. Pharmacogn J. 2021;13(6):1408-1412.