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Research Article

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

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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.

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INTRODUCTION

Free radicals are molecules that contain unpaired electrons in a set of atoms.[1–3] 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.[4–6] The source of free radicals can come from inside the body and from outside the body.[5,7] One of the sources of free radicals from outside the body comes from heavy metal pollution, such as Cadmium, Chromium, Copper, Mercury, Lead, and Zinc.[8–11] Among these elements, lead is a heavy metal that is the most essential toxic in the environment.[12,13]

Lead (Pb) is a persistent non-essential metal that is colorless, odorless, and tasteless.[14] Lead tends to catalyze oxidation reactions and lead to the formation of reactive oxygen species or ROS.[1,15] Reactive oxygen species (ROS) cause substantial damage to biological molecules such as DNA, enzymes, and proteins.[16] These reactive oxygen species (ROS) can also cause cell membrane lipid peroxidation.[17–19] Lead is absorbed, conjugated in the liver, and transported to the kidneys, where small quantities are eliminated through urine; nevertheless, most lead accumulates in numerous organs.[14]

Endogenous antioxidants are insufficient to counteract oxidative stress in the body induced by lead acetate poisoning. As a consequence, antioxidants from the outer body are also required. One plant that is rich in antioxidants is Jamun or Syzygium cumini. It is an important indigenous plant from Indonesia and India[20] which belongs to the Myrtaceae family.[20,21] Syzygium cumini has been reported to have effects in overcoming diseases, such as antibacterial, antimicrobial, anti-HIV, anti-diarrhea, anti-inflammatory, antifungal, gastroprotective and anti-ulcerogenic, anorexigenic, and others.[22,23]

Various parts of Syzygium cumini, namely root, stem, leaves, flower, fruit pulp, and seed, have been used for medicinal purposes. Among the components of Syzygium cumini, the leaves are known to contain many active phytochemical compounds such as crategolic acid, mycaminose, betulinic acid, ß-sitosterol, n-nonacosane, n-hepatcosane, n-hentriacontane, n-dotricontanol, n-triacontanol, noctacosanol, myricetin, quercetin, octacosane, octadecane, eicosane, tannins, triterpenoids, acylated flavonol glycosides, and rhamnopyranosides.[22,24,25] Most of these compounds are rich in antioxidants 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

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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 Malondyadehide

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 H_2O_2 (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 H_2O_2 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).

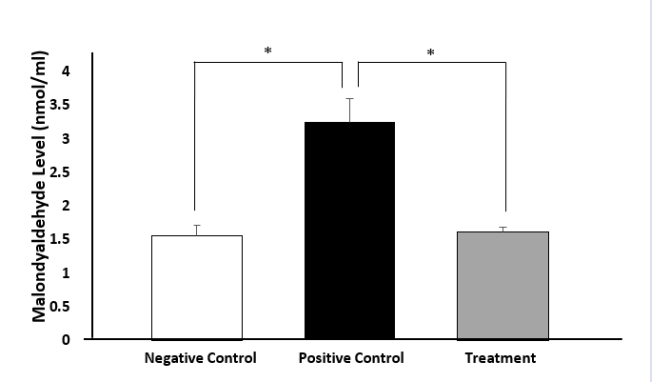

Figure 1: Syzygium cumini's extract decreased malondialdehyde serum levels. Lead acetate increased malondialdehyde serum level compared to negative control group (3.25 nmol/ml vs 1.56 nmol/ml, *p-value < 0.05). Administration of Syzygium cumini's extract counteracts Pb acetate increased malondialdehyde levels (1.62 nmol/ml vs 3.25 nmol/ml, *p-value < 0.05).

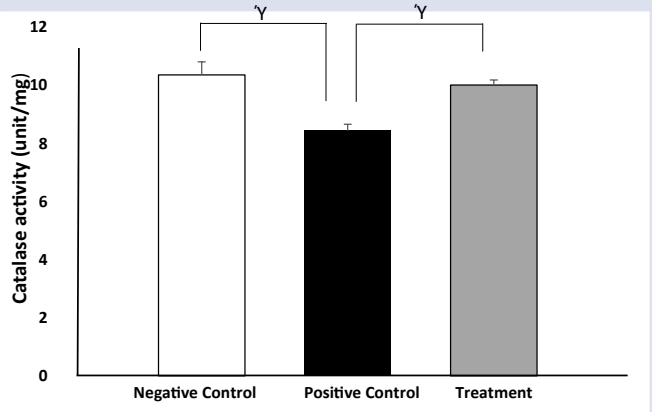

Figure 2: Catalase activity enhanced by Syzygium cumini's extract. Administration of Pb acetate decreased catalase activity compared to negative control group significantly (8.45 unit/mg vs 10.37 unit/mg, *p-value < 0.05). Syzygium cumini's extract enhanced catalase activity compared to positive control group significantly (10.01 unit/mg vs 8.45 unit/mg, *p-value < 0.05).

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.[12,27] 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.[17–19] Previous research has shown that exposure to lead acetate leads to elevated levels of blood Malondialdehyde.[34] Furthermore, increased free radicals due to lead acetate also lead to decreased levels of endogenous antioxidant activity, such as catalase, SOD, and Glutathione peroxidase.[34,35] 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.[20] Phenol and flanovoid components are reported to have high antioxidant activity.[20,36] According to another study, Syzygium cumini leaf extract contains quercetin, myricetin, kaempferol, and glycosides.[37] 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.[37]

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.

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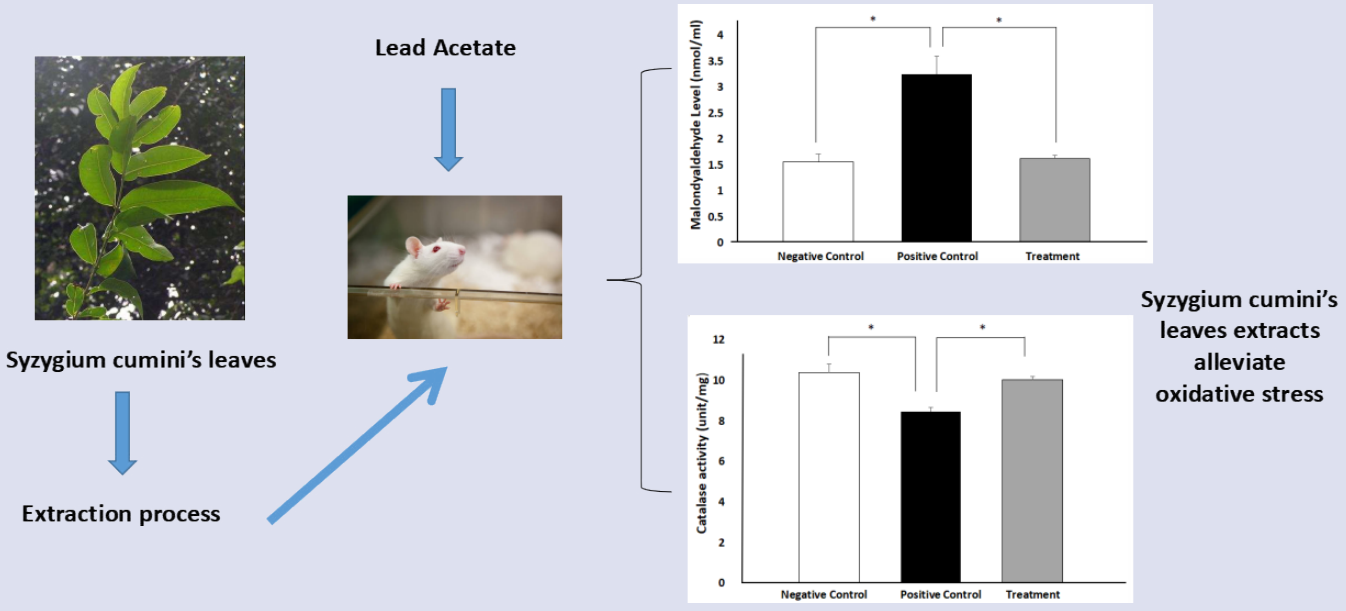
GRAPHICAL ABSTRACT



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