

Jamblang (*Syzygium cumini*) leaf extract decreased hydrogen peroxide in lead acetate-induced rats



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ABSTRACT

Background: Free radicals are atoms or molecules with one or more unpaired electrons. Lead acetate has been reported to increase the presence of free radicals in the body.

Objective: This study aims to investigate the effect of oral administration of jamblang (*Syzygium cumini*) leaf extract on hydrogen peroxide (H₂O₂) serum levels in rats induced with lead acetate.

Methods: A total of 24 male rats were divided into three groups: the negative control, the positive control, and the treatment group. The negative control group received a standard diet, the positive control group received lead acetate at a dose of 40 mg/kg body weight, and the treatment group received lead acetate at the same dose along with jamblang leaf extract at a dose of 150 mg/kg body weight. Lead acetate and jamblang leaf extract administration was carried out for 30 days. Afterward, serum H₂O₂ levels were examined using the colorimetry method.

Results: Results revealed that H₂O₂ levels in the negative control, positive control, and treatment groups were 3.08±0.24, 4.94±0.75, and 3.44±0.65 nmol/L, respectively. Significant differences were observed between the groups, as well as between the negative control and positive control, and between the positive control and treatment group.

Conclusion: The study showed that jamblang leaf extract can reduce hydrogen peroxide levels in Wistar rats treated with lead acetate, indicating its ability to address oxidative stress.

Keywords: hydrogen peroxide, lead intoxication, *Syzygium cumini*

Introduction

Lead is a naturally occurring heavy metal that has been known to humans for a long time [1]. Exposure to lead can come from a variety of sources, including drinking water, food preservation, cigarettes, cosmetics, batteries, toys, home painting, plumbing, fuel, and industrial activities. The element is extremely dangerous and has been linked to a range of health issues and environmental damage worldwide [2,3].

Lead can form an insoluble complex with proteins and disrupts cell structure and function. Previous studies have shown that oxidative stress from heavy metals like lead can result from an imbalance between free radicals and antioxidants in the body [4,5]. Free radicals, such as O₂[•], OH, NO, RO, ONOO, and H₂O₂, can increase when

exposed to metals like lead. Antioxidants like glutathione protect cells from free radicals like H₂O₂ [3]. Human endothelium and vascular smooth muscle cells exposed to lead acetate produce more hydrogen peroxide. Intracellular exposure to H₂O₂ can become excessive due to both internal and external pathogenic factors that disrupt cellular redox metabolism and the immune system [6,7].

The antioxidant defense system consists of cellular enzymes responsible for the elimination of H₂O₂ (peroxiredoxins, glutathione peroxidases, and catalase), and reduced oxidized proteins (thioredoxins and glutaredoxins),

The cellular antioxidant defense system, which includes peroxiredoxins, glutathione peroxidases, catalase, thioredoxins, and glutaredoxins, is responsible for eliminating H₂O₂ and reduced

oxidized proteins. It is designed to counteract the harmful effects of oxidative stress caused by external environmental factors [8,9]. However, endogenous antioxidants alone may not be enough to fully protect against persistent oxidative stress. Therefore, exogenous antioxidants or extra antioxidants from outside the body are required. Jamblang (*Syzygium cumini*), or jamun leaves are a herbal plant that contains sufficient antioxidants. Jamblang leaves contain various antioxidants, including alkaloids, flavonoids, tannins, triterpenoids, and monoterpenoids [10]. In a previous study, it was found that jamblang leaf extract could lower malondialdehyde levels and increase catalase activity at a dose of 150 mg/kg body weight in rats [11]. The antioxidant concentration of jamblang leaf extract was also reported to be moderate to very active, with an IC₅₀ range of 8.85 ppm [12]. This study aims to investigate the effect of jamblang leaf extract supplementation on the levels of hydrogen peroxide produced by lead acetate in rats.

Methods

Animals

This study was approved by the Ethics Commission of the Andalas University Faculty of Medicine under authorization number 870/UN.16.2/KEP-FK/2022. Male Wistar rats weighing between 150 and 250 grams were obtained from the Immunology Laboratory, Faculty of Pharmacy, Andalas University.

Extract preparation

Jamblang leaf was extracted using 96% ethanol for three days in a location protected from sunlight. This was followed by a maceration operation for another three days to obtain the whole extract. The resulting macerate was then evaporated by vacuum distillation and a rotary evaporator at a temperature of 40°C, resulting in a thick jamblang leaf extract.

Treatment

The rats were allowed to acclimate for seven days prior to treatment. Three groups of rats

were used: the negative control group (K-), which received only regular food, the positive control group (K+), which received 40 mg/kg body weight of lead acetate, and the treatment group (P), which received the same dose of lead acetate and 150 mg/kg body weight of jamblang leaf extract. The rats were given 40 mg/kg BW of lead acetate (Sigma Aldrich, Germany) orally in the morning for four weeks. The jamblang leaf extract at a dose of 150 mg/kg body weight was administered using oral gavage four hours after the lead acetate was given [11]. After 30 days of lead acetate and jamblang leaf extract administration, serum samples were collected to measure H₂O₂ levels. The rats were then euthanized by cervical dislocation.

Hydrogen peroxide level measurement

The H₂O₂ level was measured using the colorimetric method (Elabscience, USA). Buffer solution, ammonium molybdate reagent, 1 mol/L H₂O₂ standard, distilled water, normal saline (0.9% NaCl), and PBS (0.01 M, pH 7.4) were prepared. One milliliter of buffer solution was added to each blank tube, standard tube, and sample tube, and they were then incubated at 37°C for 10 minutes. Next, 0.1 ml of distilled water was added to the blank tube, 0.1 ml of H₂O₂ standard was added to the standard tube, and 0.1 ml of the sample was added to the sample tube. Then, 1 ml of ammonium molybdate reagent was added to each tube, and the contents were mixed gently. Finally, the H₂O₂ level was measured using a spectrophotometer with a 405 nm wavelength.

Data analysis

The data were analyzed using One Way Anova test followed by Post Hoc Test Tukey HSD. The results were presented as mean ± SD.

Results

The average hydrogen peroxide levels were 3.08±0.24 nmol/L in the negative control group, 4.94±0.75 nmol/L in the positive control group, and 3.44±0.65 nmol/L in the treatment group. The Tukey

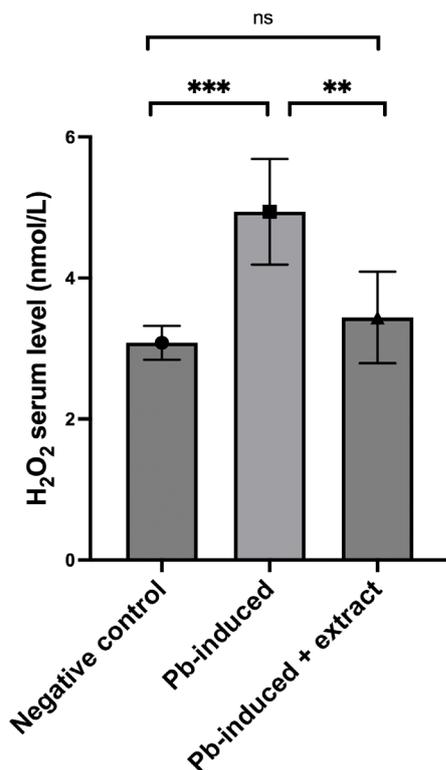


Figure 1. Comparison of H₂O₂ serum level between groups (**p < 0.01, ***p < 0.001, ns : not significant)

HSD Post Hoc Test revealed significant differences between the positive control group and the treatment group, as well as between the positive control group and the negative control group. However, no significant difference was found between the negative control group and the treatment group.

Discussion

This study found that the levels of hydrogen peroxide increased after administering lead acetate, but that the administration of jamblang leaf extract at a concentration of 150 mg/kg body weight significantly reduced this increase. Lead acetate has been shown to increase free radicals, including malondialdehyde and hydrogen peroxide [7,11]. This causes oxidative stress due to the formation of reactive oxygen species (such as H₂O₂, hydroperoxides (HO₂•), single oxygen, and oxygen), and the depletion of antioxidant reserves [13].

Due to the antioxidant content of jamblang leaves, the administration of the extract (150 mg/kg BW) considerably lowered hydrogen peroxide

levels following the administration of lead acetate. Jamblang leaf extract contains antioxidants such as flavonoids and phenolics, which can neutralize free radicals and convert them into non-radical chemicals [14]. These compounds can also lower lipid peroxide formation in membranes, reduce hydrogen peroxide levels, and increase the expression of protein enzymes like catalase and SOD [15]. Therefore, the chelating effect of flavonoids and phenolics in this extract could block lead from disrupting various proteins, including catalase, as evidenced by the decrease in H₂O₂ levels [16].

Conclusion

The results of this study demonstrate that the administration of jamblang leaf extract at a concentration of 150 mg/kg body weight is effective in reducing hydrogen peroxide levels in Wistar rats treated with lead acetate, thereby indicating its potential to alleviate oxidative stress.

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Author contributions

RSR, E Sy, and E designed research methods and wrote the manuscript. RSR obtained funding, conducted experiments, analyzed data, and reviewed the manuscript. RSR, E Sy, and E finalized the manuscript.

Declaration of interest

There was no competing interest.

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