

## Java Plum (*Syzygium cumini* (L.) Skeels) Leaf Extract Lowers Serum Urea Levels in Lead-Acetate-Induced Rats

### Ekstrak Daun Jamblang (*Syzygium cumini* (L.) Skeels) menurunkan Kadar Ureum Serum Tikus yang Diinduksi Timbal Asetat

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#### ABSTRACT

Lead is a hazardous metal to living things. Lead can cause oxidative stress in the body, inhibit enzyme activity, damage nucleic acids, and prevent DNA repair, resulting in cell death. Java plum (*Syzygium cumini* (L.) Skeels) leaves are rich with antioxidants. The research aimed to evaluate if Java plum leaf extract affected serum urea levels in lead acetate-induced rats. This study is an experimental study using 24 male rats with a randomized post-test only control group design. Rats were divided into three groups: the group that did not get any treatment, namely the negative control (NC); the group that was given lead acetate (40 mg/kg BW) was a positive control (PC); and the group that was given lead acetate (40 mg/kg BW) and Java plum leaf extract (150 mg/kg BW) was the treatment group (T), using an oral probe. The treatment was carried out for four weeks. After four weeks, the rats were killed, and their serum urea levels were examined using the urease-GLDH: enzymatic UV test method. The results showed the mean serum urea levels in the NC, PC, and T groups were  $17.87 \pm 2.18$ ,  $22.79 \pm 2.52$ , and  $18.12 \pm 2.19$  mg/dl, respectively. There were significant differences in all groups ( $p$ -value  $< 0.05$ ) in the one-way Anova. Additionally, the post hoc Tukey HSD test demonstrated a statistically significant difference between the positive control and treatment groups as well as between the negative and positive control groups ( $p$ -value  $< 0.05$ ). The conclusion was that Java plum leaf extract was able to reduce serum urea levels under lead intoxication conditions.

**Keywords:** Java plum leaf extract, lead acetate, rats, serum urea levels.

## ABSTRAK

Timbal merupakan kelompok logam yang berbahaya dan mematikan bagi organisme hidup. Timbal dapat memicu proses oksidasi dalam tubuh, menghambat kerja enzim, menghancurkan asam nukleat, dan menghambat perbaikan DNA, yang semuanya dapat menyebabkan kematian sel. Daun jambang mengandung antioksidan yang cukup tinggi. Penelitian ini memiliki tujuan untuk memeriksa apakah ekstrak daun jambang (*Syzygium cumini* (L.) Skeels) dapat mempengaruhi kadar ureum serum pada tikus yang telah diberikan timbal asetat. Penelitian ini merupakan penelitian eksperimental menggunakan 24 ekor tikus jantan yang dengan rancangan Randomized Post-Test Only Control Group Design. Tikus dibagi menjadi tiga kelompok: kelompok tanpa perlakuan yaitu kontrol negatif (KN), kelompok yang diberi timbal asetat (40 mg/kgBB) yaitu kontrol positif (KP), dan kelompok yang diberikan timbal asetat (40 mg/kgBB) dan ekstrak daun jambang (150 mg/kgBB) yaitu kelompok perlakuan (P), menggunakan sonde oral perlakuan. Perlakuan dilakukan selama 4 minggu. Setelah 4 minggu, tikus dimatikan dan diperiksa kadar ureum serum dengan metode Urease-GLDH: enzymatic UV test. Hasil menunjukkan rerata kadar ureum serum pada kelompok kontrol negatif, positif dan perlakuan secara berurutan yaitu  $17,87 \pm 2,18$ ;  $22,79 \pm 2,52$ ; dan  $18,12 \pm 2,19$  mg/dl. Terdapat perbedaan bermakna pada semua kelompok ( $p$ -value < 0.05) pada uji one-way Anova. Selanjutnya, uji post hoc Tukey HSD menunjukkan perbedaan bermakna antara kelompok KP dan P, dan antara KN dengan kelompok KP ( $p$ -value < 0.05). Kesimpulannya yaitu ekstrak daun jambang mampu menurunkan kadar ureum serum pada kondisi intoksikasi timbal.

**Kata kunci:** Ekstrak daun jambang, kadar ureum serum, tikus, timbal asetat.

## Introduction

One of the oldest poisons is lead, which has long been known (Rădulescu and Lundgren, 2019). Lead is commonly utilized in a variety of industrial and agricultural products, and it can also be found in nature as a byproduct of motor vehicle exhaust emissions (Tchounwou et al., 2012). Due to its widespread use, lead is a dangerous metal that pollutes the environment and endangers people's health all over the world (Jaishankar et al., 2014).

Lead is toxic and can cause problems with the liver, kidneys, and heart, among other organs. Lead causes

oxidative stress, which affects the body's organs. By producing too many Reactive Oxygen Species (ROS), lead can enhance lipid peroxidation, reduce saturated fatty acids, and increase the quantity of unsaturated fatty acids in membranes. Reactive oxygen species can interfere with metabolism by damaging cellular components. They are a by-product of numerous degenerative processes in many tissues. Lead also increases the production of reactive oxygen species (ROS) in many cells, leading to oxidative stress and a reduction in the body's natural antioxidant supply. As a result, nucleic acids will be damaged, and DNA

repair will be inhibited, leading to rapid cell destruction and even death (Ibrahim et al., 2012).

One of the organs that are negatively impacted by lead toxicity is the kidney. The mechanism through which lead causes kidney damage is an imbalance between antioxidant capacity and the generation of reactive oxygen species (ROS) in the kidneys. (Hussein et al., 2014) Recent studies have revealed that ROS, or free radicals, such as superoxide ion (O<sub>2</sub><sup>-</sup>), hydroxyl radicals (OH<sup>-</sup>), and nitrogen oxides (NO), contribute to lead-induced nephrotoxicity (Ghoniem et al., 2012). An increase in serum urea and creatinine levels is one sign of lead poisoning, which damages kidney cell proteins and causes a loss of function. The kidney's capacity to operate as an excretory organ will be impaired as a result of protracted cell death (Gargouri et al., 2020).

Antioxidant-rich natural products or herbal plants have been discovered to prevent tissue damage caused by free radicals. Herbal plants have advantages over conventional pharmaceuticals, which are very expensive and have a history of dangerous side effects when used to treat a variety of illnesses (Aziz et al., 2012). One of these is the usage of Java plum leaves, which have a high metabolite content and are supposed to act as natural antioxidants to combat free radicals (Artanti et al., 2019).

With values of 63.84 ppm, the ethanolic extract of Java plum leaves exhibits extremely significant antioxidant

activity. This is related to the content of active phytochemical compounds in the body that have free radical scavenging activity and can protect cell components from free radical damage, particularly DNA damage (Tamura et al., 2018). Rauza et al. discovered that giving rats Java plum leaf extract reduced malondialdehyde levels and increased catalase activity in rats that had been exposed to lead (Rita and Sy, 2021). The findings of Rahman's (2020) study, which revealed that Java plum leaf extract at a dose of 150 mg/kg BW played a role in preventing kidney cell damage caused by lead acetate injection, are also consistent with this research. This damage was demonstrated in renal histology preparations (Rahman et al., 2020). Therefore, the purpose of this study was to determine how the administration of Java plum leaf extract influenced the serum urea level in rats caused by lead acetate.

## **Research Method**

### **Materials**

Lead acetate was obtained from Sigma Aldrich, Germany. Ground Java plum leaves, distilled water, and 96 percent ethanol are used to make Java plum leaf extract. The FS kit from Diasys (Germany) was used to test serum urea levels using rat serum samples, control serum, and standard serum.

### **Animals**

Male Wistar rats weighing 150-250 g were obtained from Andalas

University's Laboratory of Immunology, Faculty of Pharmacy. Rats were given a 7-day acclimatization period before treatment. During the acclimatization period, the rats were fed and drank ad libitum and maintained a 12-hour light and dark cycle. The rats were divided into three groups: the negative control group (NC), which received only regular food Rat Bio (Citrafeed, Indonesia), consisting of water (maximum 12 %), protein (minimum 20 %), fat (maximum 4 %), fiber (maximum 4 %), calcium (12 %), and phosphor (0.7 %); the positive control group (PC), which received 40 mg/kg BW of lead acetate; and the treatment group (T), which received both the lead acetate and the Java plum leaf extract, at a dose of 150 mg/kg BW. (Rita and Sy, 2021) This study was granted approval by the Andalas University Faculty of Medicine Ethics Commission under the designation 516/UN.16.2/KEP-FK/2021.

#### *Experiments*

##### 1. Administration of lead acetate

Rats were administered 40 mg/kg BW of lead acetate orally every day for four weeks. The rat was maintained in place while being slowly pulled back up to the esophagus while being injected with an ethanol extract made from the Java plum leaf. The administration of lead acetate was carried out in the morning.

##### 2. Java plum leaf extract phytochemical test

Phytochemical tests on Java plum leaf extract were carried out to determine whether the Java plum leaf extract contained phenolic compounds, flavonoids, saponins, triterpenoids, alkaloids, and steroids.

##### 3. Administration of Java plum leaf extract

Making Java plum leaf extract needs a 96 percent ethanol mixture using the maceration process. With periodic stirring, the maceration process was carried out in a shady room and a dark container sheltered from direct sunlight for three days. The maceration process took place over the following three days in order to acquire the whole extract. The macerate will be evaporated by vacuum distillation and filtered using a rotary evaporator at a temperature of 40°C to produce a thick-textured, pure extract of Java plum leaves. A thick extract was diluted with distilled water. A dosage of 150 mg/kg BW of Java plum leaf extract was administered orally for four weeks following a four-hour lead acetate treatment.

##### 4. Measurement of rat serum urea level

Following anesthesia, blood (2 ml) was collected from the retroorbital vein. After centrifuging blood for 10 minutes at 1500 rpm, serum was then placed in a microtube. The Diasys FS urea kit was used to measure the level of serum urea (Germany). Reagen 1,

Reagan 2, and Standard are included in the Diasys FS urea kit. The components of Reagan 1 are TRIS (pH 7.8), 2-Oxoglutarate, ADP, urease, and glutamate dehydrogenase. Reagan 2 is composed of NADH. Prepare three tubes (blank, standard, and sample), then put 10 µl aquades into a blank tube, 10 µl standard into a standard tube, and 10 µl sample into a sample tube. To each tube, add 1000 µl of Reagent 1, then mix and incubate for 5 minutes. Each tube should contain 250 µl of Reagent 2, which should then be incubated for either 30–40 seconds at a temperature of 37°C or 60 seconds at a temperature of 20–25°C. Then use a spectrophotometer with a 340 nm wavelength to read the serum urea level.

#### Data Analysis

A mean and the standard error of the mean are used to present the data. Using one-way ANOVA and the Tukey's multiple comparisons test, the data were statistically assessed. A p-value of 0.05 or lower indicates that the data is significant.

#### Results and Discussion

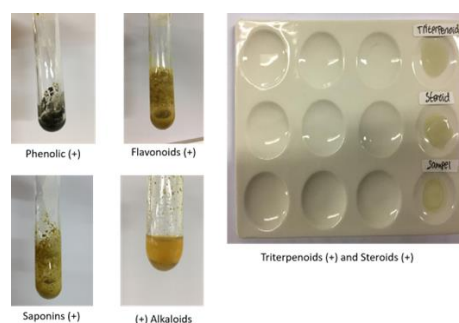
According to phytochemical analysis, the chemicals listed in Table 1 and Figure 1 are present in Java plum leaf extract, including flavonoids, phenolics, triterpenoids, saponins, alkaloids, and steroids. Oxidative stress caused by lead induction can be

countered by the phytochemical composition of Java plum leaf extract, especially its antioxidant content.

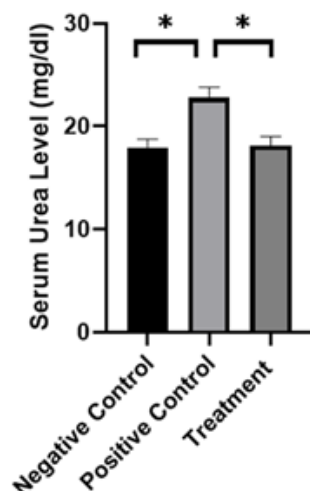
It was statistically significant (p-value < 0.05) that the positive control group rats had a higher mean serum urea level ( $22.79 \pm 2.52$  mg/dl) than the negative control group rats ( $17.87 \pm 2.18$  mg/dl). The administration of 150 mg/kg BW of Java plum leaf extract reduced lead acetate's mean serum urea level ( $18.12 \pm 2.19$  mg/dl, p-value < 0.05) (Figure 2). According to this study, rats' serum urea levels were altered by Java plum leaf extract. Serum urea levels were lower in the group of treated rats (T) subjected to lead acetate at a dose of 40 mg/kg BW along with an ethanol extract of Java plum leaves at a dose of 150 mg/kg BW.

**Table 1.** Java plum leaf extract phytochemical content

Phytochemical group	Result
Flavonoids	Positive
Phenolic	Positive
Triterpenoids	Positive
Alkaloids	Positive
Saponins	Positive
Steroids	Positive



**Figure 1.** Phytochemical test results of java plum leaf extract



**Figure 2.** Mean serum urea levels of rats in the negative control, positive control, and treatment groups.

\*p-value<0.05

This research aligns with the findings of Rahman et al. (2020), who employed *Syzygium cumini* leaf extract to repair histological damage in the kidneys of lead-exposed rats (Rahman et al., 2020). The results of this study corroborate those of Manu (2022), who discovered that rats fed an ethanol extract of Java plum leaves at a dose of 200 mg/kg BW had lower serum urea levels than rats given ethanol alone (Manu et al., 2022).

Antioxidants are chemicals that have the power to prevent other molecules from oxidizing, which is primarily caused by free radicals. Antioxidants are classified as either synthetic or natural, and both are present in the formulation (Sharifi-Rad et al., 2020). Exogenous antioxidants obtained from Java plum leaf extract include flavonoids and phenolic

substances. These antioxidants are polar, meaning they can bind to free radicals and convert them to non-radicals for a short period (Ahmed et al., 2019). Java plum leaves have also been shown to lower hydrogen peroxide levels by inhibiting lipid peroxidase reactions in membranes and restoring proteins that aid in the stabilization of physiologically active cell membranes. Java plum leaves also enhance enzymes that make cells stronger in the face of free radical damage and other negative consequences (Chagas et al., 2015). As a result, lead-induced cellular damage is diminished, including structural and functional kidney damage indicated by a decrease in serum urea levels.

## Conclusion

Rats exposed to lead acetate had lower serum urea levels after receiving Java plum leaf extract. In the next study, it should be examined how administering Java plum leaf extract affects several organ function parameters connected to lead poisoning.

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