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# SYNERGISTIC COMBINATION OF JAVA PLUM (SYZYGIUMCUMINI(L.) SKEELS) LEAF EXTRACT AND VITAMIN C ON ASPARTATE AMINOTRANSFERASE AND ALANINE AMINOTRANSFERASE LEVELS IN LEAD ACETATE-INDUCED RAT

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**ABSTRACT**

*Lead (Pb) is a toxic metal that is used to trigger oxidative stress. Oxidative stress causes damage to organs, especially the liver increasing aspartate aminotransferase (AST) and alanine aminotransferase (ALT). This study aims to determine the synergistic effect of antioxidant as Java plum leaf extract and vitamin C together on lead-induced levels of AST and ALT in rats.This type of research is true experimental research with a randomized posttest-only-control group design. The study used 20 male white rats which were divided into 4 groups. The treatment was carried out for 30 days. The examination for AST and ALT levels using the Micro Lab 300 with the Sample Star method. Data analysis was performed by using One-way Anova and Post Hoc-Turkey HSD.In this study, The results were obtained in the form of a decrease in AST and ALT levels in rats that had been induced lead acetate 40 mg/KgBW by administration of Java plum leaf extract dose 75 mg/KgBW and vitamin C dose 35 mg/KgBW as well as the administration of Java plum leaf extract at a dose of 150 mg / KgBB and vitamin C dose of 75 mg / KgBB with significant influence at the lower dose (p=0,01).It can be concluded that administrating of Java plum leaf extract dose 75 mg/KgBB and vitamin C dose 35 mg/KgBB was effective to protect the liver based on the decrease of AST and ALT levels in rats that had been induced lead acetate dose 40 mg/KgBB.*

**ABSTRAK**

Timbal (Pb) merupakan logam toksik yang digunakan untuk memicu stres oksidatif. Stres oksidatif menyebabkan kerusakan organ, terutama hati, dengan meningkatkan kadar aspartat aminotransferase (AST) dan alanin aminotransferase (ALT). Penelitian ini bertujuan untuk mengetahui efek sinergis antioksidan ekstrak daun sirih dan vitamin C secara bersamaan terhadap kadar AST dan ALT yang diinduksi timbal pada tikus. Jenis penelitian ini merupakan penelitian eksperimental murni dengan rancangan acak kelompok kontrol pasca-uji saja. Penelitian ini menggunakan 20 ekor tikus putih jantan yang dibagi menjadi 4 kelompok. Perlakuan dilakukan selama 30 hari. Pemeriksaan kadar AST dan ALT menggunakan Micro Lab 300 dengan metode Sample Star. Analisis data dilakukan dengan menggunakan Anova satu arah dan uji Post Hoc-Turkey HSD. Dalam penelitian ini, Hasil yang diperoleh berupa penurunan kadar AST dan ALT pada tikus yang diinduksi timbal asetat 40 mg/KgBB dengan pemberian ekstrak daun sirih dosis 75 mg/KgBB dan vitamin C dosis 35 mg/KgBB serta pemberian ekstrak daun sirih dosis 150 mg/KgBB dan vitamin C dosis 75 mg/KgBB dengan pengaruh yang signifikan pada dosis yang lebih rendah (p=0,01). Dapat disimpulkan bahwa pemberian ekstrak daun sirih dosis 75 mg/KgBB dan vitamin C dosis 35 mg/KgBB efektif melindungi hati berdasarkan penurunan kadar AST dan ALT pada tikus yang diinduksi timbal asetat dosis 40 mg/KgBB.

## INTRODUCTION

Lead is a type of heavy metal that is widely distributed in the environment and has a variety of uses such as in various agricultural products, iron coatings, paints, and as a mixture for motor vehicle fuels.(Tchounwou et al., 2012) In the bodies of living things, this compound is a pure toxic that can affect the pathological changes of organs. This effect then causes damage to the cardiovascular system, liver, bones, and kidney.(Martines et al., 2018) Based on data from WHO, it was found that one in three children has blood lead levels of more than 50 mg/L which affects cognitive abilities.(WHO, 2020)

One of the organs that has most impact on lead poisoning is the liver. The liver it's self is an important organ that covers various physiological processes of the body including macronutrient metabolism, blood regulation, immune system, gluconeogenesis, endocrine-regulated growth regulation, lipid homeostasis, cholesterol, and metabolism of poisons and drugs.(Trefts et al., 2018) When lead enters into the body, lead will trigger bonds with endogenous antioxidants and act as free radicals.(Ghanwat et al., 2016) cells damage and changes in permeability causes increasing on Aspartate aminotransferase (AST) and alanine aminotransferase serum known as liver injury indicators.(Haouas et al., 2014; Rita &Sy, 2021)

The discovery of plants rich in antioxidants is known to be able to prevent damage to cells and tissues caused by free radicals. One of the herbal plants that are used because it has high antioxidant activity is Java plum leaves. Java plum leaves are known to contain rich polyphenols, especially flavonoids, and phenoliv which act as exogenous antioxidant.(Amriza et al., 2023) The ability of Java plum leaves is categorized as very strong with an inhibitor concentration IC 50 value of 8.85 ppm.(Ayu Nirmala Sari, 2017) Research by Rauza et al found Java plum leaves extracted with 96% ethanol at a dose of 150 mg/KgBW were able to reduce the effects of oxidative stress based on MDA values which had significant value in lead acetate-induced rats.(Rita &Sy, 2021) In the liver, administration The ethanol extract of Java plum leaves is known to be able to protect the liver from oxidative stress damage based on the significant differences between ALT in lead acetate-induced rats where AST and ALT are known indicators of liver injury.(Zarwin& Rita, 2020)

Vitamin C is a water-soluble vitamin known as a stable antioxidant that is able to bind to free radicals to form stable radical compounds that do not harm the body due to cell damage. The protection mechanism provided by vitamin C as an antioxidant occurs through the transfer of hydrogen atoms to free radical compounds to form free radicals-ascorbyl which is stable, causing reduced oxidative damage to cells.(Pehlivan, 2018) Vitamin C is known to have very strong antioxidant power with an IC 50 value of 6.98 ppm.(Nurhalisa et al., 2021) Based on research by Arifuddin et al, it is known that giving vitamins C dose of 75 mg/KgBB is known to play a role in preventing liver cell damage in mice induced by lead acetate.(Arifuddin et al., 2016) Thus, the purpose of this study was to see how the effect of giving two types of fat-soluble and water-soluble antioxidants in the form of ethanol extract of Java plum leaves and vitamin C on AST and ALT levels in lead acetate-induced rats.

## METHOD

The research in the form of experiments (true-experimental research) using a randomized posttest only-control group design on Wistar rats (Rattusnorvegicus) as the object of research and has been declared to have passed the ethical test based on the decision of the Research Ethics Commission of the Faculty of Medicine, Andalas University number 870/UN.16.2/KEP-FK-2022.

1. Preparation of Java plum leaf ethanol extract

   Java plum leaves obtained are cleaned of various kinds of pests and other impurities. The Java plum leaves are then dried at room temperature without being exposed to sunlight because of the risk of damaging the compounds contained in the Java plum leaves. After the Java plum leaves are dry, the process of crushing the leaves into fine powder is carried out. The fine powder of Java plum leaves is then macerated using 96% ethanol in a room without exposure to sunlight and stirred regularly. After three days, the filtering process is carried out so that the juice from Java plum leaves is obtained. Java plum leaf extract was then extracted using a rotary evaporator at 40°C to obtain a concentrated extract.

2. Administration of lead acetate

   The dose calculation was carried out on the mass of mice with a dose of 40 mg/KgBW. After the calculation of the dose was carried out, it was diluted with a concentration of 5 mg/ml. Lead acetate solution was administered orally for 4

weeks to rats at 02.00 GMT +7. Lead acetate induction was carried out by positioning the rat properly, then slowly inject the solution into the esophagus using an oral tubel.

3. Administration Java plum leaf extract
   Calculation of the dose given to the mass of rats with a dose of 75 and 150 mg/KgBW. After the dose calculation was carried out, it was diluted with a concentration of 15 mg/ml. Java plum leaf extract solution was administered orally for 4 weeks to rats at 06.00 GMT +7.
4. Administration of vitamin C
   Calculation of the dose given to the mass of rats with a dose of 35 and 75 mg/KgBW. After the dose calculation was carried out, it was diluted with a concentration of 10.5 mg/ml. Vitamin solution was administered orally for 4 weeks to rats at 06.00 GMT +7.
5. Measurement of serum AST and ALT levels
   After anesthesia, blood (2 ml) was collected from the retroorbital venous plexus. After that, it was centrifuged for 10 minutes at 1500 rpm. The serum obtained was then placed in a microtube and labeled according to the sample. The ASAT (GOT) FS kit and ALAT (GPT) FS kit from Diasys (Germany) was used to measure serum levels of AST and ALT.

In this study, parametric statistical tests were used after normalizing and homogenizing tests. The mean and standard error of the data is used as the basis for data processing using one-way ANOVA and Turkey's multiple comparison test so that the statistical value of the data is known. A p-value of 0.05 or smaller indicates the data has a significant value.

## RESULT AND DISCUSSION

### Result

Based on statistical tests, it was found that significant values (p-value <0.05) increased levels of AST (25.08 ± 4.99) and ALT (27.2 ± 3.69) in the positive control group compared to AST (16.04 ± 1 .46) and ALT (17.80 ± 1.46) in the negative control group. The administration of Java plum leaf extract at a dose of 75 mg/KgBW and vitamin C at a dose of 35 mg/KgBW had AST (17.88 ± 1.26) and ALT (20.22 ± 2.84) levels with lower values for AST and ALT in the positive control group and significant difference (p-value <0.05). The administration of Java plum leaf extract at a dose of 150 mg/KgBW and vitamin C at a dose of 75 mg/KgBW had levels of serum AST (19,30±2,15) and serum ALT (21,6±4,72) with lower means than AST and ALT in the positive control group and the difference was significant on level of serum AST (p-value < 0.05).

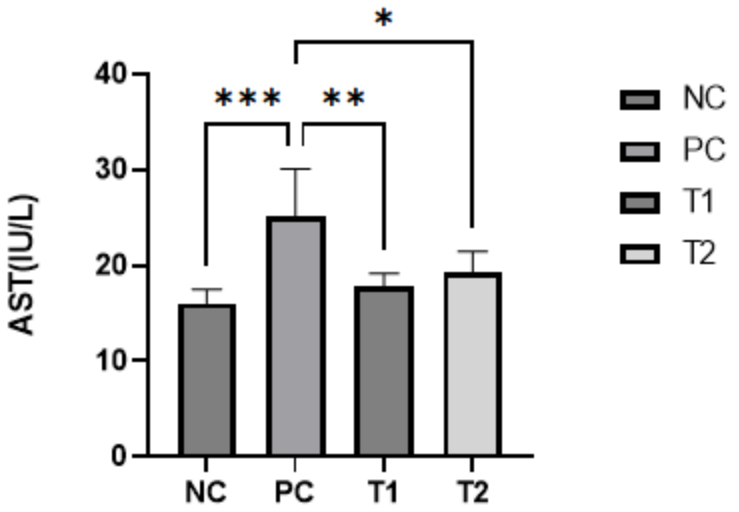


**Figure 1** Mean serum AST levels of rats in the negative control, positive control, and treatment groups. *p-value < 0,05

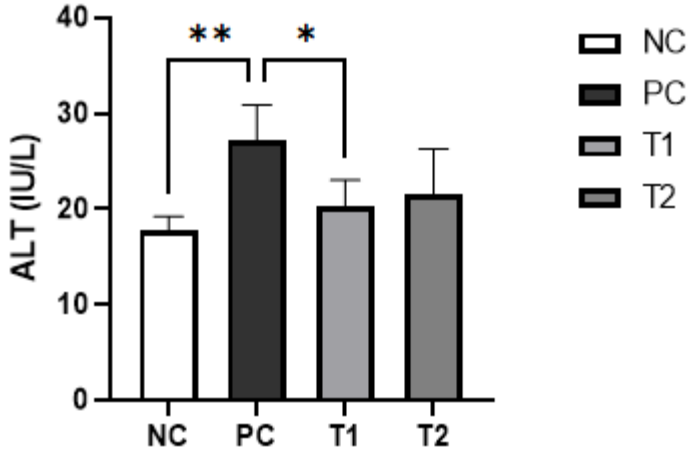


**Figure 2** Mean serum AST levels of rats in the negative control, positive control, and treatment groups. *p-value < 0,05

## Discussion

This research is in line with research conducted by Adilla et al (2020) where the administration of Java plum leaf extract was able to reduce ALT levels in lead acetate-induced rats.(Zarwin& Rita, 2020) Another study by Arifuddin et al found that giving vitamin C was effective at protecting the liver based on minimal damage to the liver histopathology compared to only being given lead acetate.(Arifuddin et al., 2016)

Oxidative stress is a condition that often results in degenerative diseases in the body. The pathology that appears cannot be separated from the formation of reactive oxygen species (ROS) which is a normal physiological response (i.e redox biology) to trigger cellular dysfunction and inhibit cell processes, especially inefficient oxidative phosphorylation in mitochondria.(Zhang et al., 2018) Oxidative stress arises due to an imbalance in

physiological processes in the body, causing ROS and antioxidant values in the body to be unbalanced.(Kisaoglu et al., 2013)

Lead is known to be capable of causing liver histopathological damage and is capable of disrupting normal biochemical processes in the body resulting in increased levels of liver enzymes in the form of AST and ALT. Based on autopsy studies of people exposed to lead, it was found that the liver as the largest repository much as 33% of lead among soft tissues was stored in the liver, followed by the renal cortex and medulla. The damage caused by lead to cells is caused by an increase in ROS which results in damage to cell membranes and DNA.(Haouas et al., 2014)

Antioxidants are compounds that can break free radical chains to prevent damage related to oxidative stress. Antioxidants are divided into water soluble and fat soluble based on their solubility. Water-soluble antioxidants can be absorbed easily by the body due to the water content in the body, but cause them to be easily eliminated from the body through urine, such as vitamin C. Fat-soluble antioxidants are antioxidants that can only be absorbed if fat is present. This type of antioxidant will be difficult for the body to eliminate and will accumulate in the body such as flavonoids and vitamin E.(Sharifi-Rad et al., 2020)

Exogenous antioxidants obtained from Java plum leaf extract include flavonoids and phenolic substances. This antioxidant is fat-soluble and polar, so it can bind free radicals and turn them into non-radicals in a short period.(Amriza et al., 2023) Java plum leaf extract is also known to be able to reduce MDA levels and increase the ability of enzymes to face free radicals.(Rita &Sy, 2021). Java plum leaves extract contain flavonoids in the form of quercetin and myricetin which function as free radical scavengers.(Ayyanar&Subash-Babu, 2012). The ability of Java plum leaves is categorized as very strong with an inhibitor concentration IC 50 value of 8.85 ppm.(Ayu Nirmala Sari, 2017)

Vitamin C is a water-soluble antioxidant with a strong reducing ability through the transfer of hydrogen atoms to the hydroxyl-enediol groups on C2 and C3 atoms to form free radicals ascorbyl stable.(Pehlivan, 2018). Vitamin C is known to have very strong antioxidant power with an IC 50 value of 6.98 ppm.(Nurhalisa et al., 2021). Giving Java plum leaf extract as fat-soluble antioxidants and vitamin C as water-soluble antioxidants with strong IC 50 power can reduce liver damage including structural and functional in lead acetate-induced rats based on serum levels of AST and ALT. It can be seen from the differences between T1 and T2 that closed to normal control meant it can normalize liver function after inducing lead acetate

## CONCLUSION

This study demonstrated that lead acetate induction at a dose of 40 mg/KgBW resulted in an increase in AST and ALT levels in rats. Administration of *Syzygium cumini* (jamblang) leaf extract at both 75 mg/KgBW and 150 mg/KgBW, as well as vitamin C at 75 mg/KgBW, significantly reduced AST and ALT levels in lead acetate–induced rats. Furthermore, the combined administration of jamblang leaf extract (75 mg/KgBW) with vitamin C (35 mg/KgBW), or jamblang leaf extract (150 mg/KgBW) with vitamin C (75 mg/KgBW), also effectively lowered AST and ALT levels. Notably, the combination of jamblang leaf extract (75 mg/KgBW) and vitamin C (35 mg/KgBW) showed a statistically significant effect ($p=0.01$). These findings suggest that jamblang leaf extract and vitamin C,

either alone or in combination, possess hepatoprotective potential against lead acetate–induced liver injury.

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