



# Combination of Jamblang Leaf Extract (*Syzygium cumini*) and Vitamin C Increased Albumin and Decreased Bilirubin Serum Level on Lead Acetate Induced Rats

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**Abstract.** Oxidative stress from lead acetate can impair organ function. Antioxidants, which can be obtained from plants like *Syzygium cumini*, are necessary to counter oxidative stress. This study aimed to analyze the effect of the combination of jamblang leaf extract and vitamin C on serum albumin and bilirubin levels in lead-acetate-induced rats. This study was conducted on 35 rats divided into five groups: a negative control group (only given food and drink), a positive control group induced by lead acetate 40 mg/kg body weight, and treatment groups induced by lead acetate with three combination doses of jamblang leaf extract and vitamin C (35 mg/kg BW and 15 mg/kg BW; 75 mg/kg BW and 35 mg/kg BW; and 150 mg/kg BW and 75 mg/kg BW). Serum albumin and bilirubin levels were read with a spectrophotometer. The results showed that combination therapy of jamblang leaf extract with vitamin C increased albumin levels and reduced serum bilirubin levels after lead acetate administration significantly ( $p < 0.05$ ). This study concludes that the combination therapy of jamblang leaf extract and vitamin C affects serum albumin and bilirubin levels.

**Keywords:** *Syzygium cumini*, vitamin C, albumin, bilirubin, lead acetate, rats

## 1 Introduction

Lead is a cause of oxidative stress found in combustion processes, gasoline used in vehicles, and even cosmetics. <sup>1</sup> Lead found in the body can increase oxidative stress by causing excess production of free radicals through inhibition of the delta-aminolevulinic acid dehydratase (ALAD), coproporphyrinogen oxidase, and ferrochelatase, but ALAD activity is the main impact. <sup>2</sup> Delta-aminolevulinic acid builds up when lead inhibits the function of the ALAD enzyme. Due to its ability to produce reactive oxygen species (ROS), such as hydroxyl radicals, superoxide, and hydrogen peroxide, ALA has been linked to oxidative damage. <sup>3</sup>

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I. P. Mahendra et al. (eds.), *Proceedings of Selected Papers of International Conference on Health, Science, and Environment (ICHSE 2023)*, Advances in Biological Sciences Research 39,

[https://doi.org/10.2991/978-94-6463-431-0\\_7](https://doi.org/10.2991/978-94-6463-431-0_7)

Oxidative stress due to lead can cause abnormalities in body organs such as brains, kidneys, and reproductive system, <sup>4,5</sup> and liver. <sup>6</sup> In the liver, lead can cause abnormalities in the liver's biochemical function, characterized by increased activity of glutamic-oxalacetic transaminase (AST) and glutamic-pyruvic transaminase (ALT). <sup>7-9</sup> Apart from increasing ALT and AST levels, lead is also reported to reduce albumin levels <sup>10</sup> and increase serum bilirubin levels. <sup>11</sup> Lead exposure damages liver cells by altering the permeability of cell membranes. <sup>10</sup>

Previous research found that jamblang leaf extract could overcome oxidative stress in lead intoxication, characterized by decreased serum malondialdehyde levels and increased catalase enzyme activity. <sup>12</sup> This is mediated by the antioxidant content of jamblang leaf extract, which contains high levels of antioxidants such as myricetin, acylated flavonol glycosides, octacosane, quercetin, triterpenoids, octadecane, tannins, eicosane, etc. <sup>12,13</sup> Apart from using herbal ingredients which tend to have low side effects, antioxidant vitamins such as vitamin C are also reported to be able to overcome oxidative stress due to lead intoxication. <sup>14</sup> Previous research examined the separate effects of jamblang leaf extract and vitamin C on lead intoxication. However, research on jamblang leaf extract's combined effect with vitamins is still limited. This study aims to analyze the effect of combining jamblang leaf extract with vitamin C on serum albumin and bilirubin levels induced by lead acetate.

## 2 Material and Methods

### 2.1 Materials

Sigma Aldrich, a German company, provided the lead acetate. Jamblang leaf extract is created using ground Jamblang leaves, distilled water, and 96 percent ethanol. The leaves of *Syzygium cumini* were acquired from Universitas Andalas, Padang, Indonesia. The herbarium of Universitas Andalas has *Syzygium cumini* authenticated under the reference number 228/K-ID/ANDA/V/2022. Albumin FS kit and bilirubin Auto Total FS kit provided by Dyasis, Holzheim, Germany. L (+)-Ascorbic Acid EMSURE® ACS, Reag. Ph Eur was provided by Merck, Darmstadt, Germany.

### 2.2 Animals

This research was conducted on 35 male Wistar rats (150-240 grams), which met the inclusion criteria: healthy and active rats. Rats were acclimatized for one week before the study began. The rats were given unlimited access to food and water while maintaining a 12-hour cycle of light and darkness during the acclimation phase.

Animals were divided into 5 groups, namely the negative control group (C-), which was only given food and drink, the positive control group (C+) which was given lead acetate at a dose of 40 mg/kg BW, the treatment group received three doses of a combination of jamblang leaf extract and vitamin C, namely T1 (35 mg/kg BW and 15 mg/kg BW), T2 (75 mg/kg BW and 35 mg/kg BW), and T3 (150 mg/kg BW and 75 mg/kg BW). Lead acetate and vitamin C were administered for 30 days, as previously reported. <sup>12</sup> On the last day of the study, blood was taken to check serum albumin and

bilirubin. This research has passed the ethical test of the Faculty of Medicine, Universitas Andalas, with number: 870/UN.16.2/KEP-FK/2022

### 2.3 Preparation of *Syzygium cumini*'s leaves extracts

Jamblang leaves are thoroughly cleaned and free of other pollutants by being rinsed in water. After that, the jamblang leaves are divided into little pieces. The leaves of jamblang are then dried and ground into a powder by blending them until they are very smooth, and then macerate by using 96% ethanol to make jamblang leaf extract, as previously reported.<sup>12,15</sup>

### 2.4 Administration of Lead Acetate

For 30 days (4 weeks), rats received lead acetate orally at a dose of 40 mg/kg BW.<sup>9,12,15,16</sup> An ethanol extract from the Jamblang leaf was administered into the rat by oral gavage. The lead acetate administration took place in the morning.

### 2.5 Administration of *Syzygium cumini*'s leaves extracts and Vitamin C

Combination of *Syzygium cumini*'s leaves extracts and Vitamin C with three doses (35 mg/kg BW and 15 mg/kg BW); 75 mg/kg BW and 35 mg/kg BW; and 150 mg/kg BW and 75 mg/kg), were administered orally for four weeks following a four-hour lead acetate treatment.

### 2.6 Measurement of Albumin Serum Levels

Three tubes were prepared each for blank, standard, and sample. Pipette 10 µl of distilled water into a blank tube, 10 µl of standard into a standard tube, and 10 µl of serum into a sample tube. Albumin reagent (1000 µl) was put into blank, standard, and sample tubes, mixed until homogeneous, and then incubated for 10 minutes at 20 – 25 °C. After 10 minutes, the absorbance of the three solutions was read using a spectrophotometer (Microlab 300), at a wavelength of 546 nm. Serum albumin levels can be calculated using the formula: Sample Absorbance divided by Standard Absorbance times Standard Concentration (g/dl). Serum albumin examination was carried out in duplicate for each of the samples.

### 2.7 Measurement of Bilirubin Serum Levels

Three tubes were prepared each for blank, standard, and sample. In the first stage, 25 µl of blank, standard, and sample were put into each tube, then 1000 µl of reagent was added to each tube, mixed until homogeneous, incubated for 5 minutes at room temperature, then read as Absorbant 1 (A1) with a wavelength of 546 nm. In the second stage, blank tubes, standards, and samples were prepared, 250 µl of Reagent 2 was added, mixed and homogenized, incubated for 5 minutes at room temperature, and read as Absorbant 2 (A2), with a wavelength of 546 nm. Bilirubin levels (mg/dl) are determined by the formula: (A2-A1 sample)/(A2-A1 standard) x C standard.

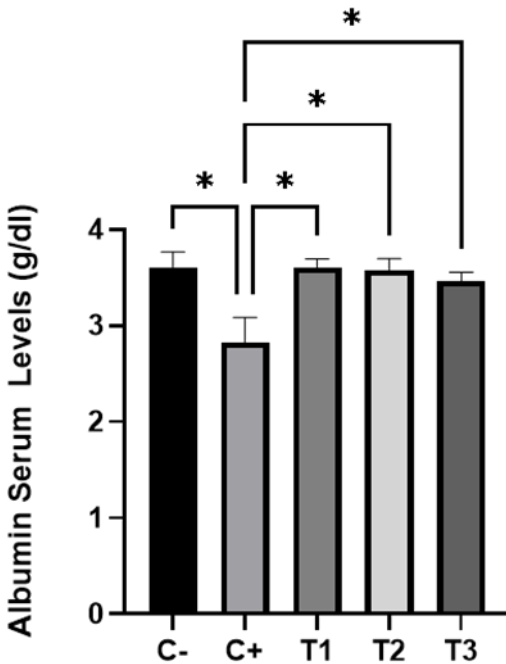
## 2.8 Data Analysis

Data are displayed as mean  $\pm$  SEM, analyzed with One way Anova, followed by Post Hoc Tukey HSD. Data is significant if the p-value  $<0.05$ .

## 3 Results

### 3.1 Combination of Vitamin C with Jamblang Leaf Extract (*Syzigium cumini*) Can Increase Serum Albumin Levels in Lead Intoxication

The negative control group had higher serum albumin levels than the positive control group induced by lead acetate ( $3,605 \pm 0.159$  vs  $2,826 \pm 0.260$  g/dl). Administration of a combination of jamblang leaf extract with vitamin C (T1: 35 mg/kg BW and 15 mg/kg BW; T2: 75 mg/kg BW and 35 mg/kg BW; and T3: 150 mg/kg BW and 75 mg/kg BW) was able to increase serum albumin levels significantly compared to the positive control group ( $3.605 \pm 0.094$ ;  $3.577 \pm 0.130$ ;  $3.463 \pm 0.094$  g/dl vs.  $2.826 \pm 0.260$  g/dl).

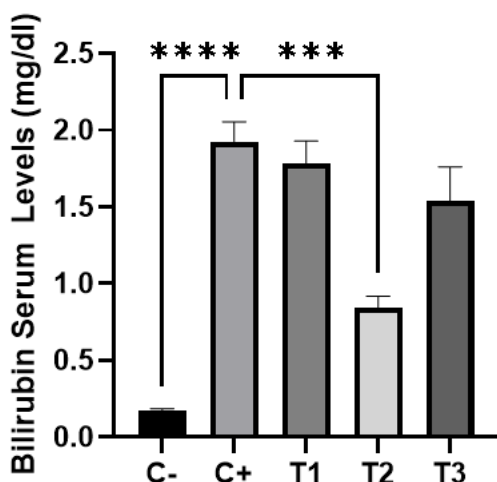


**Fig.1.** Effect of Vitamin C Combination with Jamblang Leaf Extract on Lead Acetate-Induced Serum Albumin Levels. Lead acetate induction led to a significant decrease in serum albumin levels in the positive control group (C+) compared to the negative control group (C-). The combination of vitamin C with jamblang leaf extract with three

different doses (T1, T2, and T3) significantly increased serum albumin levels. \*p value < 0.05

### 3.2 Combination of Vitamin C with Jamblang Leaf Extract (*Syzygium cumini*) Can Reduce Serum Bilirubin Levels in Lead Intoxication

Administration of lead acetate caused an increase in serum bilirubin levels in the positive control group compared to the negative control group ( $1.926 \pm 0.300$  vs  $0.170 \pm 0.016$  mg/dl). Combining jamblang leaf extract with vitamin C reduced serum bilirubin levels compared to the positive control group ( $1.783 \pm 0.147$ ;  $0.847 \pm 0.074$ ;  $1.539 \pm 0.223$  vs  $1.926 \pm 0.300$  mg/dl). A significant reduction was shown by the combined dose of T2 (75 mg/kg BW and 35 mg/kg BW)



**Fig.2.** Effect of Vitamin C Combination with Jamblang Leaf Extract on Lead Acetate-Induced Serum Bilirubin Levels. Lead acetate induction led to a significant increase in serum bilirubin levels in the positive control group (C+) compared to the negative control group (C-). The combination of vitamin C with jamblang leaf extract with three different doses (T1, T2, and T3) reduced serum bilirubin levels compared to the positive control group. A significant decrease was obtained in the T2 group. p-value < 0.001, \*\*\*\*p value < 0.0001

## 4 Discussion

This study analyzed the effect of a combination of jamblang leaf extract with vitamin C on serum albumin and bilirubin levels in mice given lead acetate. The results showed that combination therapy increased albumin levels and reduced serum bilirubin levels.

This shows improvements in liver cell function by administering a combination of jamblang leaf extract and vitamin C.

Lead acetate is a pollutant that is often found in the environment. Prolonged administration of lead acetate can cause abnormalities in the body's organs, one of which is the liver. In this study, oral administration of lead acetate caused a decrease in serum albumin levels. A decrease in albumin levels caused by lead acetate can cause damage to liver cells by increasing the permeability of liver cells.<sup>10,17</sup> The combination of jamblang leaf extract with vitamin C was able to increase serum albumin levels significantly compared to the positive control group. This increasing effect was not significantly different between the groups combining jamblang leaf extract with vitamin C. The increase in serum albumin was not significantly different between the negative control group and the combination therapy group, indicating that combination therapy was able to normalize serum albumin levels. This effect is caused by the content of jamblang leaf extract which contains high antioxidants from the flavonoid group, which can restore liver function to normal. Previous research has shown the effect of overcoming oxidative stress from jamblang leaves due to the various active substances contained in jamblang leaves, such as betulinic acid, myricetin, noctacosanol, quercetin, etc.<sup>12</sup> Vitamin C has also been reported able to overcome oxidative stress when administering lead acetate.<sup>14</sup> Therefore, the combination of jamblang leaf extract with vitamin C can restore liver function to normal.

Serum bilirubin levels in the positive control group increased significantly compared to the positive control group. This is because lead acetate can cause liver cell damage, increasing bilirubin levels. Lead acetate caused hepatocellular lesions and the destruction of the bill duct, which resulted in damaged cell membranes and increased liver enzymes. Furthermore, bilirubin was released into the bloodstream due to hepatocyte destruction. This causes bilirubin levels to increase in the blood circulation. Increased bilirubin levels due to lead acetate induction can be overcome with combination therapy of jamblang leaf extract with vitamin C. Significant results were shown in the T2 combination. In the combination group T1 and T3, bilirubin levels decreased, although it was not statistically significant. Reducing serum bilirubin levels with combination therapy of jamblang leaf extract and vitamin C can improve liver cell function due to the active components of jamblang leaf extract and vitamin C. The active components contained in jamblang leaf extract, such as myricetin, kaempferol, and quercetin, which are included in the flavonoid group, are able to improve liver function.<sup>12,15</sup> Vitamin C is also a powerful water-soluble antioxidant and has also been reported to increase catalase activity so that oxidative stress is reduced.<sup>14</sup> Vitamin C is also reported to be able to improve liver function after exposure to lead acetate.<sup>18</sup> Taking together, the combination of vitamin C and jamblang leaf extract can improve liver function when exposed to lead acetate.

In conclusion, the combination of jamblang leaf extract therapy with vitamin C was able to improve liver function induced by lead acetate, characterized by a decrease in bilirubin levels and an increase in serum albumin levels.

## 5 Acknowledgment

Under contract number 01 /UN.16.02/Fd/PT.01.03/2023, the Faculty of Medicine, Universitas Andalas provided financial assistance for this work under the Fundamental Scheme Research Grant

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