



# Amelioration of Cisplatin-induced Liver Injury by Extract Ethanol of *Pometia pinnata*

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## Abstract

**BACKGROUND:** Cisplatin use in clinical practice has been associated with an increase in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, and lactate dehydrogenase (LDH).

**AIM:** The aim of this study is to determine the hepatoprotective activity of extract ethanol *Pometia pinnata* on rats induced Cisplatin.

**MATERIALS AND METHODS:** Thirty rats were separated into six groups (five rats). Group I was received only carboxy methyl cellulose. Group II was received a 7 mg/kgbw Cisplatin injection on day 3. Group III-VI were extract groups (Vitamin C 1%, 100 mg/kgbw, 200 mg/kgbw, and 400 mg/kgbw) administered orally from day 1 to 7, followed by Cisplatin injection on day 3. On day 8, rats were injected with 1% ketamine, open the chest and draw blood directly from the heart and centrifugated 5000 RPM (10–15 min), take the supernatant layer for analysis AST, ALT, total protein, and LDH levels.

**RESULTS:** The effect of extract ethanol of *P. pinnata* on liver injury biochemical markers AST, ALT, LDH, and total protein. Group negative had a significant increase ( $p < 0.05$ ) in comparison to the normal that did not receive extract or Cisplatin. Meanwhile, there was a drop in biochemical parameters in the group given the extract in groups dose 100, 200, 400 mg/kgbw. Group VI of biochemical parameters statistically there is no significant different with group normal group ( $p > 0.05$ ) that showing *P. pinnata* extract has hepatoprotective activity.

**CONCLUSION:** In summary, extract ethanol of *P. pinnata* has hepatoprotective effect by reducing the level of AST, ALT, total protein, and LDH levels.

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## Introduction

Cisplatin is a chemotherapeutic medication that is frequently used to treat cancers of the bladder, lung, ovary, and testicle. In addition, it is known to be highly powerful against cancer. Cisplatin inhibits mitosis and induces apoptosis through oxidative stress and cross-linking with cancer DNA. Cisplatin kills cancer cells by generating DNA adducts that prevent cancer cells from entering the G2 cell cycle and inducing apoptosis. Although Cisplatin has been known effective in killing cancer cells clinically, it can be hazardous to the kidneys, liver, brain, and heart. The use of higher doses and recurrent administration raises the risk of organ damage in a variety of organs. Cisplatin can generate reactive oxygen species (ROS), which can result in liver apoptosis. ROS are extremely reactive molecules that can activate superoxide radicals, hydroxyl radicals, and hydrogen peroxide, which can damage lipids, proteins, and DNA in the body. Cisplatin may induce lipid peroxidation, which may contribute to liver damage. Cisplatin use in clinical practice has been associated with an increase in serum aspartate aminotransferase

(AST), alanine aminotransferase (ALT), alkaline phosphatase, and lactate dehydrogenase (LDH). The increase in serum indicators indicates the possibility of liver injury when Cisplatin is used [1], [2], [3], [4], [5].

Endogenous antioxidants such as superoxide dismutase (SOD), glutathione, and catalase play a critical role in neutralizing ROS generated by Cisplatin. When Cisplatin is used, an imbalance between endogenous antioxidants and ROS is created; when there is more ROS, the ROS are more capable of causing organ damage. As a result, when Cisplatin is used, extra supplements or chemicals that boost endogenous antioxidants are required. One method is to administer herbal treatment to individuals receiving Cisplatin. *Pometia pinnata* is a widely cultivated plant in Papua, Indonesia. The Papuan people have traditionally employed the bark, stems, fruit peels, fruits, and leaves of *P. pinnata* as medicine. However, research on *P. pinnata* is still in its infancy; few studies have been conducted on the *P. pinnata* plant. Numerous investigations have revealed that *P. pinnata* possesses a variety of pharmacological properties, including anti-diabetic, anti-inflammatory, and antihyperlipidemic properties. *P. pinnata* also includes flavonoids such

as quercetin, rutin, and myristate, which are known to have a variety of pharmacological properties, including hepatoprotective activity. In addition, previous study has showed that *P. pinnata* exhibits substantial antioxidant activity when measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, with an  $IC_{50} < 50$  ppm indicating that it exhibits strong antioxidant activity. Due to *P. pinnata* has high antioxidant activity, it is projected that this plant possesses a variety of pharmacological properties, including hepatoprotective properties [6], [7], [8], [9].

Thus, this study contributes novelty information about the hepatoprotective efficacy of an ethanol extract of *P. pinnata* in Cisplatin-induced mice.

## Method

### Materials

Cisplatin purchased from Kimia Farma (Indonesia), Ethanol purchased from Bratachem (Indonesia), DPPH purchased from Sigma Aldrich (Germany), Water pro injection purchased from Bratachem (Indonesia), Vitamin C purchased from Sigma Aldrich (Germany), Gallic Acid purchased from Sigma Aldrich (Germany), Folin-Ciocalteu purchased from Sigma Aldrich (Germany), Quercetin purchased from Sigma Aldrich (Germany), Cisplatin purchased from Kimia Farma (Indonesia).

### Animal

The experimental study used thirty rats (*Rattus norvegicus*) in good health and weighing between 150 and 200 g. Rats are housed in plastic cages with a humidity level of 40–60% and a 12-h dark/light cycle. In addition, rats were given cratachem producing pellet diet and water *ad libitum*. The University of North Sumatra had granted ethics clearance for this project.

### Plant

*Pometia pinata* was collected in Papua, cleaned, dried, and then blended till a dry powder was formed. In addition, the powder obtained was stored at room temperature and opened as needed.

### Extract preparation

Seven hundred grams of dried powder *P. pinnata* was dissolved in 96% ethanol and then steered occasionally; the solution was then macerated for 7 days and steered occasionally each day; the solution was then filtered using Whatman paper no 1, and the results were filtered (EEPE). Following that,

phytochemical screening (alkaloids, flavonoids, tannins, saponins, glycosides, and steroids/triterpenoids) is performed [10], [11].

### Experimental design

Thirty rats were separated into six groups of five each. Group I was a control group in which rats received only carboxy methyl cellulose. Group II was a negative control group in which rats received a 7 mg/kgbw Cisplatin injection on day 3. Group III was a positive group in which rats were administered 1% Vitamin C for 7 days and then injected with Cisplatin on day 3. Groups IV-VI were extract groups (100 mg/kgbw, 200 mg/kgbw, 400 mg/kgbw) in which rats were administered extract orally from day 1 to day 7, followed by Cisplatin injection on day 3. On day 8, rats were injected with 1% ketamine, which draws blood directly from the heart.

### Biochemical parameters measurement

Three milliliters of blood from each rat was centrifuged at 4000 RPM (50°C) for 10 min, and then 0.5 mL of the supernatant was extracted and directly injected into a Cobas 6000 (Swiss company Roche Diagnostic) for the determination of AST, ALT, total protein, and LDH.

### Data analysis

Statistical package for social science program 21 was used to analysis of the data. Data are expressed as Mean SEM. Comparison for more than 2 groups using one-way ANOVA followed by *post-hoc* tukey. Statistical significance was set at  $p < 0.05$ .

## Results

### Analysis of AST and ALT levels

AST and ALT levels are the key indicators of liver injury; higher levels of AST and ALT are directly proportional to liver damage; hence, when AST and ALT levels rise, liver damage will also worsen. Cisplatin produced liver injury in this investigation, as indicated by the AST and ALT levels in Table 1 below.

**Table 1: Data values for AST and ALT (U/L)**

Groups	Mean AST (U/L)	ALT (U/L)
Group 1	35.36 ± 1.521	40.51 ± 2.731
Group 2	125.12 ± 10.873*	105.67 ± 10.484*
Group 3	32.67 ± 1.73	38.45 ± 1.937
Group 4	86.38 ± 3.83	78.62 ± 5.873
Group 5	65.44 ± 2.44	58.12 ± 3.88
Group 6	42.21 ± 2.01#	38.43 ± 1.89#

Information: group 1 (normal), group 2 (Cisplatin only), group 3 (Cisplatin + Vit C), group 4 (Cisplatin + 100 mg/kgbw), group 5 (Cisplatin + 200 mg/kgbw), group 6 (Cisplatin + 400 mg/kgbw). \* ( $p < 0.05$ ) significant different from normal group. # ( $p < 0.05$ ) significant different from group 2. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase.

The effect of extract ethanol of *P. pinnata* on liver biochemical markers such as AST and ALT is shown in Table 1. Group 2 had a significant increase ( $p < 0.05$ ) in comparison to the group 1 that did not receive extract or Cisplatin. Meanwhile, there was a drop in biochemical parameters in the group given the extract in groups 4, 5, and 6. In group 6 statistically, there is no significant difference with group 1 ( $p > 0.05$ ) owing to the *P. pinnata* leaf extract's high antioxidant activity, which conferred hepatoprotective action.

### Analysis of LDH and total protein levels

LDH is a critical enzyme in the anaerobic metabolic pathway that belongs to the oxidoreductase class; an increase in LDH levels indicates an increase in lactate levels, particularly in muscles; an increase in LDH levels in an organ also signals organ damage. Total protein is also used to determine the extent of liver damage. Data are included in Table 2 below.

**Table 2: Data values for LDH (U/L) and total protein (g/dL)**

Groups	Mean LDH (U/L)	Total protein (g/dL)
Group 1	186.4 ± 12.873	4.62 ± 0.218
Group 2	856.76 ± 21.98*	8.56 ± 0.183*
Group 3	201.32 ± 12.83	4.65 ± 0.213
Group 4	455.4 ± 10.31	5.66 ± 0.381
Group 5	324.6 ± 12.57	5.21 ± 0.478
Group 6	188.24 ± 9.38#	4.82 ± 0.229#

Information: group 1 (normal), group 2 (Cisplatin only), group 3 (Cisplatin + Vit C), group 4 (Cisplatin + 100 mg/kgbw), group 5 (Cisplatin + 200 mg/kgbw), group 6 (Cisplatin + 400 mg/kgbw). \*( $p < 0.05$ ) significant different from normal group. #( $p < 0.05$ ) significant different from control group 2. LDH: Lactate dehydrogenase.

The effect of extract ethanol of *P. pinnata* on liver biochemical markers such as LDH and total protein is shown in Table 2. Group 2 had a significant increase ( $p < 0.05$ ) in comparison to the Group 1 that did not receive extract or Cisplatin. Meanwhile, there was a drop in biochemical parameters in the group given the extract in groups 4, 5, and 6. In Group 6 statistically, there is no significant difference with Group 1 ( $p > 0.05$ ) owing to the *P. pinnata* leaf extract's high antioxidant activity, which conferred nephroprotective action.

## Discussion

The highest level of AST and ALT can be found in the Group 2 that only injected cisplatin on day 3 which are  $125.12 \pm 10.873$  U/L and  $105.67 \pm 10.484$  U/L, while in the Group 6 that given *P. pinnata* extract dose 400 mg/kgbw showed the reduction of the AST and ALT level which are  $42.21 \pm 2.01$  U/L and  $38.43 \pm 1.89$  U/L. The significant antioxidant activity of extract ethanol of *P. pinnata* demonstrates the extract's ability to diminish the ROS produced by Cisplatin. Antioxidants are found in abundance in plants; therefore, if the antioxidant capacity of

the extract is high, the extract may have additional pharmacological activities such as anticancer, as the inhibitory pathway against cancer cells is generally to increase the expression of endogenous antioxidants such as SOD, GSH, CAT, and GPX. In addition, the antioxidants in the extract may enhance the ability of endogenous antioxidants in the body to neutralize ROS such as OH<sup>-</sup>, H<sub>2</sub>O<sub>2</sub>, and O<sup>-</sup>. Of course, antioxidants can also protect cells from free radicals. Flavonoids are secondary metabolites found in plants that exhibit antioxidant action. Their activity is largely reliant on the group's location of free OH. Earlier research indicated that the ethanolic extract of matoa leaves contained 1.9% flavonoids. Differences in total flavonoid output may be a result of genetic diversity and seasonality in the environment, both of which affect flavonoids in these plants [12], [13].

The highest level of LDH and total protein can be seen in the Group 2 that only injected cisplatin which is  $856.76 \pm 21.98$  U/L and  $856.76 \pm 21.98$  g/dL while in the group given extract dose 400 mg/kgbw showed significant reduction of LDH and total protein level which are  $188.24 \pm 9.38$  U/L and  $4.82 \pm 0.229$  g/dL. Cisplatin buildup in the liver and drug metabolic abnormalities can result in liver damage. Genetic or hereditary liver illness, such as hemochromatosis, a condition of iron metabolism characterized by excessive iron deposition in the organs. Immune disorders, such as autoimmune hepatitis, are diseases caused by the body's own tissues developing resistance. In general, in autoimmune hepatitis, the liver cells are at odds, resulting in persistent inflammation [14], [15], [16], [17]. AST and ALT are two transaminases that are mostly produced by liver cells. When liver cells are destroyed, as in hepatitis or cirrhosis, these two enzymes typically increase in concentration. Both are believed to provide a picture of a liver illness based on the findings of laboratory tests. Liver illnesses characterized by a marked increase in AST and ALT levels are hepatocellular in origin. ALT is more sensitive than AST at detecting viral hepatitis in general. In alcoholic liver disease, the AST level is two or more times that of the ALT [18], [19], [20], [21]. In many studies shows that cisplatin increases the level of liver biochemical injury such as blood urea nitrogen, AST, MDA, and creatinine while reduce the level of endogenous antioxidant such as SOD, glutathione, and catalase. Cisplatin also increases hepatic inflammatory such as tumor necrosis factor  $\alpha$  and nuclear factor erythroid 2-related factor 2 (NRF-2). This study was in line with previously study that stated Cisplatin can damage the liver by increasing apoptosis which causes increasing levels of biochemical parameters [22], [23]. This study still has limitations because of lack of parameters analysis but the primer information regarding the ability of *P. pinnata* extract can be valuable for further research.

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