

# Acute Toxicity Test of Pigeon Pea Leaves Extract (*Cajanus cajan*) in Rats

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**Abstract.** Acute toxicity test was conducted to evaluate the safety level of pigeon pea (*Cajanus cajan*) leaves extract, using the Organisation for Economic Co-operation and Development (OECD 423) guideline method. The pigeon pea leaves were extracted using 96% ethanol as a solvent. A total of 9 male rats were used divided into 3 groups: 1 control group and 2 treatment groups. The rat in control group (group 1) received a single dose of distilled water while the rat in groups 2 and 3 received a single dose of pigeon pea leaves extract at doses of 300 mg/kg BW and 2000 mg/kg BW, respectively. The aquadest and extract suspension were administered orally using rat stomach tubedors. Mortality and clinical signs were examined in the first 4 hours (critical time), 24 hours, and 14 days after the treatments. The result revealed that the LD<sub>50</sub> values of the extract was estimated at more than 5000 mg/kg BW and classified as practically nontoxic.

Keywords: acute toxicity, *Cajanus cajan*, LD<sub>50</sub>, rat.

## 1 Introduction

Indian-*Cajanus cajan* leaves extract was reported having a hypoglycemic effect [1]. Indonesian- *Cajanus cajan* is well known as 'kacang gude' or 'undis'. Quan and Liang [2] reported that the bioactive compound of a certain plant could be different depending on the ecology condition where it planted. Wresdiyati *et al.*, [3] also reported that Indonesian *Cajanus cajan* leaves, especially from Lombok, West Nusa Tenggara, showed hypoglycemic activity in rats. So, for safety in using *Cajanus cajan* leaves as a candidate for antidiabetic, it's needed to test the toxicity of the leaves.

The acute toxicity test of a bioactive compound is generally carried out by determining the Lethal Dose value of 50% (LD<sub>50</sub>). LD<sub>50</sub> is a dose of a substance that can kill 50% of the experimental animal population [4].

Since the conventional acute toxicity test using many experimental animals and not following the concept of animal welfare, it has begun to be replaced with new methods that use experimental animals in smaller amounts or even without using experimental animals at all [5, 6]. One type of the new acute toxicity test is the OECD guideline no 423, which highlights the animal welfare side where the clinical symptoms of poisoning are endpoints of testing other than the death of experimental animals. In addition, this test also provides more information related to the target organ and the mechanism of poisoning [7].

This study aims to analyze the safety level of Indonesian *Cajanus cajan* leaves extract in *Sprague Dawley* rats through an acute toxicity test - LD<sub>50</sub> that refers to OECD423 and changes in liver and kidney tissues of experimental rats.

## 2 Materials and Methods

### 2.1 Materials

This study used *C. cajan* leaves that were obtained from Lombok, West Nusa Tenggara, Indonesia. After picking, the *C. cajan* leaves were air-dried and then oven-dried at 50°C, and the dried *C. cajan* leaves were ground and sifted using a 40 mm mesh [3]. A total of nine male *Sprague-Dawley* rats were used for the acute toxicity test in the present study.

### 2.2 Methods

#### 2.2.1 *Cajanus cajan* leaves extraction

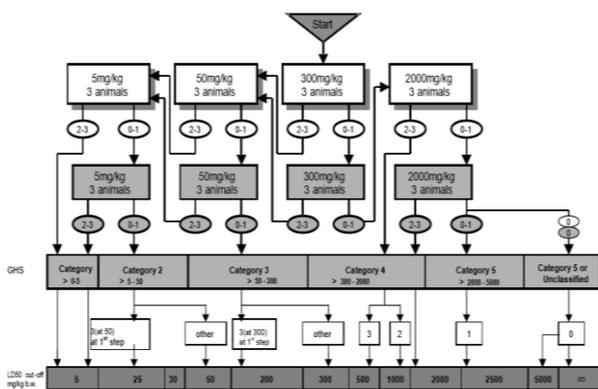
The extraction of *C. cajan* leaves was conducted by the maceration method using ethanol 96% as the solvents at a ratio of 1:5 for 24 hours then filtered. The maceration was repeated three times. The resulting filtrate was then dried using a vacuum evaporator to obtain dried extract [3].

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## 2.2.2 In vivo acute toxicity test in rats

Rat care and experimental procedures in this study were in accordance with the Ethical Approval Letter from Animal Care and Use Committee, Bogor Agricultural University, number 132-2018 IPB, 30 November 2018.

A total of 9 male *Sprague-Dawley* rats were used for this study. They were divided into 3 groups, consisting of 1 control group and 2 treatment groups. The control group (C) was given distilled water as much as 2 ml, while the treatment groups were given a solution of *Cajanus cajan* leaves extract with a dose referring to OECD423. The doses that were chosen in this study are 300 mg/kg body weight (T2) and 2000 mg/kg body weight (T3) (Figure 1). Intensive observations were conducted in the first 4 hours after administration of the extract solution, then continued with routine observations every 24 hours once for 14 days [8]. The main parameters observed to determine the Lethal Dose value of 50% (LD<sub>50</sub>) were clinical symptoms of moribund, severe pain, and rat mortality. Rats that exhibit clinical symptoms of moribund and severe pain must be put to sleep in a welfare manner and counted as rat mortality during the trial period.



**Figure 1.** Procedure for acute toxicity and LD<sub>50</sub> values test with a selected initial dose of 300 mg/kg body weight (OECD423 2001)

Determination of LD<sub>50</sub> values based on the OECD423 method (Figure 1). The initial dose of the extract that was chosen is 300 mg/kg BW. If at the dose there are 2-3 death, then the test is followed by extract with reduced dose to 50 mg/kg body weight. If at the dose of 50 mg/kg BW there is 0-1 death, then the test is followed by extract at a higher dose, 2000 mg/kg body weight. If at this dose 3 dead rats are found, the LD<sub>50</sub> value is 500 mg/kg body weight. If there are 2 dead rats, the LD<sub>50</sub> value is 1000 mg/kg body weight, while if the number of death found is 1 rat, the LD<sub>50</sub> value is 2500 mg/kg body weight. If the dead rat at this higher dose is not found at all, then there is an option to immediately determine the LD<sub>50</sub> value or carry out further testing at the maximum dose, which is 5000 mg/kg BW.

Testing at the maximum dose should only be done with several considerations including strong evidence that the LD<sub>50</sub> value is between doses of 5000 mg/kg BB to infinity (∞), there is information on the symptoms of

toxicity in humans, and death is found in testing doses of 2000 mg/kg body weight. If these requirements are not found, then testing with a maximum dose is not permitted to be carried out in order to protect the welfare of experimental animals. Without testing the maximum dose, LD<sub>50</sub> values can be taken in the range of doses of 5000 mg/kg body weight to ∞ or > 5000 mg/kg body weight.

## 2.2.3 SGOT, SGPT, Ureum, and Creatinin analysis

Serum GPT, GOT, ureum, and creatinine were measured using an Automatic chemical analyzer (Vital Scientific N.V., The Netherlands). Serum GPT and GOT were measured using IFCC Mod without pyridoxal method, while serum ureum and creatinine were measured using urease and Jaffe without deproteination

## 2.2.4 Histomorphological analysis of Liver and Kidney tissues

The liver and kidney tissues were fixed using paraformaldehyde, then the tissues were processed using the paraffin embedding standard method. The tissue slices were stained using hematoxylin and eosin. The histomorphological study was observed under a light microscope.

## 3 Results and Discussion

### 3.1 Lethal Dose 50% (LD<sub>50</sub>)

Acute toxicity testing aims to determine the level of toxicity of a compound by one of the ways to identify the LD<sub>50</sub> value and the degree of toxicity from the LD<sub>50</sub> value. LD<sub>50</sub> values were obtained from the number of the rat with clinical symptoms of moribund, extreme pain, and death after treatment. Rat mortality due to administration of *Cajanus cajan* leaves extract was not found either in the observation 24 hours or 14 days after administration of the extract.

Death is not found at a dose of 2000 mg/kg body weight. The results of the acute toxicity test of *C. cajan* leaves extract ever conducted by Kevin et al (2018) using aquades and hydroethanolic solvents in *Wistar* strain rats, obtained LD<sub>50</sub> values of each solvent are 3715.35 mg/kg BW and 1174.90 mg/kg BW, respectively. In addition, no information related to the symptoms of toxicity in humans or deaths were found at a dose of 2000 mg/kg body weight, so testing at the maximum dose is not permitted and LD<sub>50</sub> values are categorized in the range of dose of 5000 mg/kg body weight to infinity (∞) is based on the Globally Harmonized System [8] and is practically non-toxic according to Hodge and Sterner [9].

### 3.2 The profile of SGOT, SGPT, Ureum, and Creatinin

The profile of serum glutamate oxalate transaminase (SGOT), serum glutamate piruvate transaminase (SGPT), ureum, and creatinin of the control group (C), and treated

groups; 300 mg/kg body weight (T1) and 2000 mg/kg body weight (T2) was showed in Table 1.

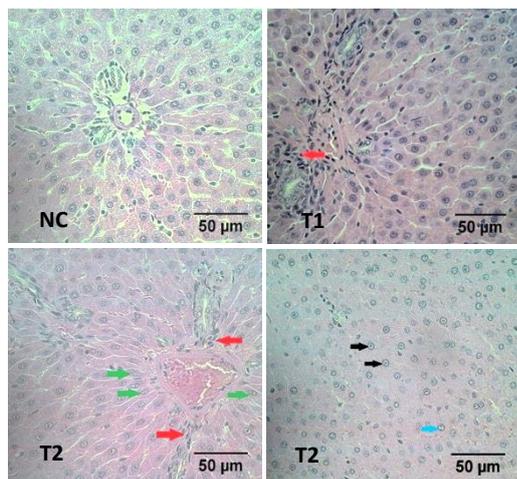
**Table 1.** The profile of SGOT, SGPT, ureum, and creatinin of treated rats

Group	SGOT (U/l)	SGPT (U/l)	Ureum (mg/dl)	Creatinin (mg/dl)
C	116.67±30.89	29.33±3.21	47.67±1.15	0.58±0.02
T1	100.67±34.96	31.00±2.00	52.33±7.23	0.47±0.24
T2	102.00±25.24	30.67±6.11	50.33±9.07	0.59±0.04

All parameters for liver and kidney tissue injury indicators showed no significant difference among treated groups. All parameters of the control and treated group are in a normal range of rats [10,11]. It means there is no tissue damage in both liver and kidney. In another way, the *C. cajan* extract is not a toxic agent.

### 3.3 Histomorphological observation of liver and kidney tissues

The liver tissues of treated rats were shown in Figures 2. Administration of *C. cajan* leaves extract showed changes in the Kiernan triangle area. Changes were found in the form of inflammatory cell infiltration and denaturation of hepatocyte cell nucleus proteins, while necrose (cell death) was not found.



**Figure 2.** Micrograph of rats liver tissues in the Kiernan area showed a few numbers in inflammatory cell proliferation (red arrow), bile duct proliferation (blue arrow) and protein denaturation in the nuclei (black arrow). Staining with hematoxylin & eosin

Acute inflammatory cell infiltration is generally caused by an acute hepatitis virus infection, autoimmune diseases [12], or side effects from drugs [13] including herbal medicines [14]. This inflammation is common in oral drugs that are metabolized by the liver [15].

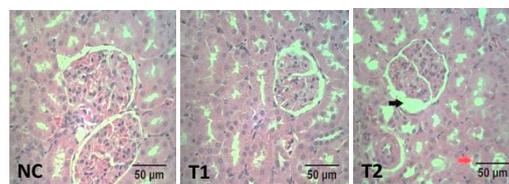
The most common cause of denaturation is extreme temperatures, but some chemicals can also cause denaturation of this protein. Protein denaturation can be reversible or irreversible depending on how much damage is caused and how long the exposure of the material

causing the denaturation. In denaturation that is reversible, the cell will be able to return to normal function if the exposure to the material causing the denaturation is stopped. In irreversible denaturation, the liver can regenerate as long as the number of healthy hepatocyte cells is not less than 25% [16].

According to Kleiner [17], acute damage is generally characterized by lobular changes, while changes in the Kiernan triangle area are generally characteristic of chronic damage. However, in the case of drugs that induce damage to the liver, symptoms of acute hepatitis make it possible to bring up histopathological features with patterns of inflammation such as chronic damage. Overall damage that occurs to the liver both at a dose of 300 mg/kg body weight and 2000 mg/kg body weight can be categorized as mild or mild damage due to changes that occur only in a small area in the Kiernan triangle.

The kidney tissues of treated rats were shown in Figure 3. Administration of *C. cajan* leaves extract showed changes in the renal corpuscles and distal renal tubules. Changes were found in the form of Bowman's space dilatation and protein deposition in the distal renal tubules, while cell necrose was also not found.

Bowman's space expansion is generally caused by glomerular problems both atrophy [18] and an increased infiltration rate [19]. According to Radhakrishnan and Perazella [20], glomerular damage is quite common as a side effect of drugs in which endothelial cells are the cells most often damaged due to the influence of these medications. The damage to endothelial cells and the regeneration process is thought to cause atrophic glomerular appearance. On the other hand, the presence of certain drugs in the body can increase the process of creatinine removal which causes primary urine storage to increase in the Bowman room so that the Bowman room has expanded [21].



**Figure 3.** Photomicrograph of rats kidney tissues showed a few numbers in dilatation of Bowman space (black arrow), protein precipitation in distal tubule (red arrow). S Staining with hematoxylin & eosin staining with hematoxylin & eosin

Other changes include protein deposition in the distal renal tubular lumen which can be caused by a disruption in glomerular filtration, failure of the proximal tubules to reabsorb protein from the primary urine, or a combination of both [22, 23]. Glomerular endothelial cell damage as mentioned previously is thought to be the cause of leakage of protein into the urine on observations of the T3 group micrograph, while the proximal tubules do not appear to be altered.

According to the Acute Dialysis Quality Initiative (ADQI), acute damage to the kidneys is classified into risk, injury, kidney failure, loss of kidney function, and end-stage kidney disease or commonly referred to as

classification RIFLE. While the Acute Kidney Injury Network (AKIN) adapted from the RIFLE classification with several updates, obtaining the results of the acute division of kidney damage into 3 categories, namely stage 1 for mild damage conditions, stage 2, and stage 3 for severe damage conditions. Both of these methods use serum creatinine levels, glomerular filtration rate, and urine output as a standard for determining the degree of damage so that determining the degree of damage only by looking at micrographs without accompanying laboratory results from urine is difficult to use to determine the degree of damage to the kidneys of these experimental rats [24]. In the present study, the value of SGOT, SGPT, ureum, and creatinin of ethanolic *C. cajan* extract-treated rats at a dose of 300 mg/kg BW and 2000 mg/kg BW supported that there is no any alteration in the liver and kidney function.

## 4 Conclusion

The present study concluded that the LD<sub>50</sub> value of *C. cajan* leaves extract is greater than 5000 mg/kg BW and is classified as practically non-toxic. Histopathological evidence in liver and kidney tissues are categorized as mild. The extract did not affect the function of rats liver and kidney.

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