

# Analysis of Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) Levels in Rats Given Jamu *Cekok* Decoction

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## Abstract

Jamu *cekok* is a traditional medicine used by Indonesians, especially children to increase appetite. However, the safety test of jamu *cekok* on liver function using Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) as parameters has never been evaluated. This study aimed to determine the effect of jamu *cekok* on liver function by assessing SGOT and SGPT levels in rats subjected to acute toxicity. The study employed an analytical experimental design with the OECD 425 method. The test rats used were 12, divided into a control group, a 2000 mg/kg BW treatment group and a 5000 mg/kg BW treatment group. The treatment of jamu *cekok* decoction was given once on the first day of the acute toxicity test and was observed for 14 days. SGOT and SGPT levels were measured and analyzed using one-way ANOVA to assess differences between groups. Through this study, observations of behavior and body weight over the 14 days showed no signs of toxicity or mortality. The LD<sub>50</sub> value was determined to be greater than 5000 mg/kg BW, which is categorized as practically non-toxic. One-way ANOVA followed by Tukey's HSD post hoc tests revealed no significant differences in SGOT levels between the control and treatment groups ( $p > 0,05$ ). Similarly, SGPT levels also showed no significant difference ( $p > 0,05$ ) between groups. These findings indicate that the administration of jamu *cekok* at a dose of 2000 mg/kg BW and 5000 mg/kg BW does not induce toxic effects on liver function, as reflected by SGOT and SGPT measurements.

**Keywords:** Acute Toxicity Test; Jamu Cekok; Liver; SGOT; SGPT.

## INTRODUCTION

Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) are enzymes that can indicate damage or trauma to body tissues, especially the liver. These parameters are commonly used to evaluate liver function, although SGOT and SGPT are also found in other tissues, such as the brain, kidneys and heart (Lely et al., 2016). When liver cell damage occurs, the GOT and GPT enzymes in liver cells will enter and exit the bloodstream and cause SGOT and SGPT levels to increase (Yuneldi et al., 2018). An increase that exceeds normal levels indicates liver cell damage. The liver is a vital organ that plays a role in the synthesis, storage and metabolism of various compounds, including drugs and toxins (Rosida, 2016).

Jamu is one of the traditional medicines from Indonesia in the form of a type of herbal drink or herbal medicine made from natural ingredients that have properties to maintain health or to treat various diseases. Jamu *cekok* is one of the herbal medicines that is usually given to children to stimulate appetite (Handajani & Widhiastuti, 2018). The term *cekok* refers to a traditional

method of administering herbal medicine by directly forcing it into a child's mouth (Limananti & Triratnawati, 2010). In this study, the main ingredients of jamu *cekok* were *temulawak* (*Curcuma xanthorrhiza* Roxb.), ginger (*Zingiber officinale* Rosc.), turmeric (*Curcuma longa* Lin and kencur (*Kaempferia galanga* L.). A study by Marni and Ambarwati in 2015 reported that there was an increase in children's appetite and weight after receiving jamu *cekok* from their mothers. However, the safety of jamu *cekok* on liver function using SGOT and SGPT as parameters has never been carried out, even though this herbal medicine is consumed by children and even toddlers. This toxicity test is carried out with various dose variations to determine the relationship between the large dose variations and SGOT and SGPT levels. This safety test can be analyzed several groups of preparations with one dose per group and a parameter in the form of Lethal Dose (LD<sub>50</sub>) will be obtained.

Lethal Dose (LD<sub>50</sub>) is the dose of a test compound that can cause 50% death in test animals after a single dose (Ayun et al., 2021). This value is used as a

guideline for the ratio of efficacious dose and toxic dose as a determination of the therapeutic index of a drug. Acute toxicity tests are carried out based on the Organization for Economic Co-operation and Development (OECD) guidelines by looking at mortality, behavior, motor activity, body weight and organ index in test animals as well as quantitative LD<sub>50</sub> data (Organisation for Economic Co-operation and Development (OECD), 2022). Therefore, this study was conducted to conduct a toxicity test of jamu *cekok* and to determine the effect of jamu *cekok* on liver function in terms of SGOT and SGPT levels. Through this study, it is expected to be known whether jamu *cekok* is safe to consume and to know the correct dosage of jamu *cekok*.

## MATERIALS AND METHODS

### Study design

The study was conducted using 2–3-month-old female Wistar rats (*Rattus norvegicus* L.) weighing 100-200 grams in the Non-microscopic Laboratory of the Faculty of Medicine, Tanjungpura University, the Test Animal Laboratory of the Faculty of Medicine, Tanjungpura University in June-August 2024. Research tools include various laboratory equipment, such as rat cages, photometer analyzers and homogenizers, while the materials used include *temulawak* (*Curcuma xanthorrhiza* Roxb.), ginger (*Zingiber officinale* Rosc.), *kencur* (*Kaempferia galanga* L.), turmeric (*Curcuma longa* Lin.), SGOT and SGPT reagents and distilled water. The test rats used were 12, divided into a control group, a 2000 mg/kg BW treatment group, and a 5000 mg/kg BW treatment group. The treatment of jamu *cekok* decoction was given once on the first day of the acute toxicity test and was observed for 14 days. In this study, acute toxicity tests were conducted using LD<sub>50</sub> values with the OECD 425 method: Up and Down Procedure (UDP). Then, blood samples were taken from test animals to obtain SGOT and SGPT levels.

### Procedures

#### Test Materials Collection

The test materials used were *temulawak*, ginger, turmeric and *kencur* obtained from sellers at the Flamboyan market in Pontianak City, West Kalimantan. The sample was a single material.

#### Preparation of Decoction Extract Test Solution

Each ingredient (*temulawak* (*Curcuma xanthorrhiza* Roxb.), ginger (*Zingiber officinale* Rosc.), turmeric (*Curcuma longa* Lin.) and *kencur* (*Kaempferia galanga* L.)) was weighted at 250 grams, chopped into small pieces and finely blended with 1 liter of water. The resulting mixture was then placed in a decoction pan and heated in a water bath that had been preheated to 90°C. The pan was tightly covered and heated for 30 minutes with stirring performed 2-3 times during the process.

After heating, the decoction was allowed to cool and the residues were filtered out. The liquid extract was then thickened using a rotary evaporator to make it concentrated and suitable for long-term storage.

#### Preparation and Dose Calculation for The 2000 mg/kg BW Limit Test

In the limit test at dose of 2000 mg/kg BW, rats (*Rattus norvegicus* L.) weighing 100 grams received a corresponding dose of 200 mg. The administration volume was standardized at 1 ml per 100 grams of BW, requiring a test preparation with a concentration of 200 mg/ml. To achieve this concentration, a 100 ml stock solution was prepared by dissolving 20 grams of the extract in 100 ml of distilled water. The resulting solution was the administered orally to the test animals according to the predetermined dose and volume.

#### Preparation and Dose Calculation for The 5000 mg/kg BW Limit Test

Similar to the calculation of 2000 mg/kg BW limit test, in the limit test with a dose of 5000 mg/kg BW, rats weighing 100 grams received a corresponding dose of 500 mg. The administration volume was standardized at 1 ml per 100 grams of BW, requiring a test preparation with a concentration of 500 mg/ml. To achieve this concentration, a 100 ml stock solution was prepared by dissolving 50 grams of the extract in 100 ml of distilled water. The resulting solution was the administered orally to the test animals according to the predetermined dose and volume.

#### Preparation and Dose Calculation for Volume of Administration to Rats

The volume of test preparation administered to rats is set at 1 ml per 100 grams of body weight. For instance, if a rat weighs 250 grams, the required volume is calculated using a proportional formula, resulting in 2.5 ml. This volume is adjusted to ensure that the dose given is appropriate to the body weight of each test rat.

#### Preparation of Test Animals

Female rats (*Rattus norvegicus* L.) of the Wistar strain used in this study met the inclusion criteria (female, 2-3 months old, showed normal behavior and activity). Each rat was housed in a labelled cage which marked with a permanent marker for identification purposes. Before the experiment began, the rats were acclimatized for one week (at least five days) to minimize stress. During this period, their body weights were monitored daily. Each rat received 15–20 grams of feed per day and had free access to drinking water. The animal room was maintained at around 27°C, with a 12-hour light/dark cycle and humidity kept between 50–70%, following OECD guidelines (Organisation for Economic Co-operation and Development (OECD), 2022).

### Treatment Groups Procedure

In this study, three treatment groups were used, including the negative group (K-), the 2000 mg/kg BW dose group (J1) and the 5000 mg/kg BW dose group (J2). The doses of the 2000 mg/kg BW and 5000 mg/kg BW treatment groups are LD<sub>50</sub> groups determined based on the test results with the OECD 425 procedure.

### OECD 425 Acute Oral Toxicity Testing Stages

The acute oral toxicity test in this study followed the OECD 425 procedure to determine the LD<sub>50</sub> value in female Wistar rats (*Rattus norvegicus L.*). This procedure consists of six stages: weighing the rats, fasting them for 3-4 hours, administering distilled water to control rats, testing at a dose of 2000 mg/kg BW, conducting further testing at 5000 mg/kg BW, and performing a main test if any mortality occurred. Based on the OECD 425 procedure, the test was terminated if any of the following conditions were met: (1) three animals survived at the initial dose level, (2) five reversals occurred in six consecutively treated animals, or (3) at least four animals successfully completed the reversal test and the calculated likelihood ratio exceeded the critical threshold.

### "AOT425" Software Analysis

This software analysis is used only during the main test and is not used if an LD<sub>50</sub> value of 2000 mg/kg BW or 5000 mg/kg BW has already been established during the limit test.

### Blood Sampling from Test Animals

Blood samples were collected after 14 days of testing. Blood was drawn from the tail vein of each rat as much as 1-2 ml. The blood samples were placed into blood tubes without anticoagulant and then centrifuged at 3000 rpm for 15 minutes. The resulting serum was collected using a pipette, transferred into Eppendorf tubes and immediately tested for SGOT and SGPT levels (BPOM RI, 2022).

### SGOT Testing

In this study, SGOT testing was conducted using the ProLine Kit through several steps. First, the working reagent was prepared by mixing 800 µL reagent A and 200 µL reagent B. Then, 1000 µL of the working reagent was placed into a test tube, followed by the addition of 100 µL of blood serum and the mixture was homogenized. Afterward, the mixture was sucked into the Semi-Auto Chemistry Analyzer. The absorbance was then measured at a wavelength of 340 nm.

### SGPT Testing

In this study, SGPT testing was conducted using the ProLine Kit through several steps. First, the working reagent was prepared by mixing 800 µL reagent A and 200 µL reagent B. Then, 1000 µL of the working reagent was placed into a test tube, followed by the addition of 100 µL of blood serum and the mixture was homogenized. Afterward, the mixture was sucked into the Semi-Auto Chemistry Analyzer. The absorbance was then measured at a wavelength of 340 nm.

### Data analysis

This study used a true experimental design with a post-test only control group design model to examine the causal relationship. To determine the differences in SGOT and SGPT activities in each treatment, an analysis was carried out using one-way ANOVA with SPSS software.

## RESULTS AND DISCUSSION

### Results

#### LD<sub>50</sub> Value Testing Using The OECD 425 Method

The LD<sub>50</sub> value is a parameter calculated based on the number of deaths in test animals. The results of LD<sub>50</sub> value testing using the OECD 425 method can be seen in Table 1.

**Table 1. Results of LD<sub>50</sub> Value Testing.**

No.	Treatment Group (Dose)	Status
1.	Control (0 mg/kg BW)	Negative Control 1
2.		Negative Control 2
3.		Negative Control 3
4.		Negative Control 4
5.	2000 mg/kg BW	T2000A
6.		T2000B
7.		T2000C
8.		T2000D
9.		T2000E
10.	5000 mg/kg BW	T5000A
11.		T5000B
12.		T5000C

LD<sub>50</sub> value is determined based on the number of deaths observed on test animals following the administration of a substance. In this study, based on the table above, the initial test was conducted using a dose of 2000 mg/kg BW administered to rats. After 48 hours of observation, no mortality was recorded. Subsequently, four additional rats were tested at the same dose, and no deaths were observed. The dose was then increased to 5000 mg/kg BW, starting with one rat. After 48 hours, no mortality occurred, prompting the addition of two more rats at the same dose. Following 14 days of observations, no deaths were observed in any of the test animals. The testing was concluded, having fulfilled the criteria

outlined in the OECD Guideline 425 for acute oral toxicity. Since no mortality was observed in the test animals at either the 2000 mg/kg BW or 5000 mg/kg BW doses, it can be concluded that the estimated LD<sub>50</sub> value of jamu *cekok* is greater than 5000 mg/kg BW.

Based on the OECD 425 method, clinical symptoms were also observed during the acute toxicity test according to predefined criteria. In this study, observations included behavior and body weight. The results of behavioral observations in test rats administered jamu *cekok* at doses of 2000 mg/kg BW and 5000 mg/kg BW during acute toxicity testing are presented in Table 2 and Table 3, respectively.

**Table 2.** Results of Observations on Rats' Behavior During the Acute Toxicity Test (Dose: 2000 mg/kg BW).

Observed Effects		Dose 2000 mg/kg BW					
		0 H	0.5 H	1 H	2 H	24 H	T
<b>Platform</b>		7.4	6.4	5.8	6	6.8	6.6
<b>Motor Activity</b>	Increased (%)	-	-	-	-	-	-
	Normal (%)	100	-	-	-	100	100
	Decreased (%)	-	80	80	100	-	-
	Immobile (%)	-	20	20	-	-	-
<b>Straub (%)</b>		-	-	-	-	-	-
<b>Piloerection (%)</b>		-	-	-	-	-	-
<b>Ptosis (%)</b>		-	-	-	-	-	-
<b>Pinna Reflex (%)</b>		100	100	100	100	100	100
<b>Corneal Reflex (%)</b>		100	100	100	100	100	100
<b>Lakrimation (%)</b>		-	-	-	-	-	-
<b>Ctalysis (%)</b>		-	-	-	-	-	-
<b>Body Posture</b>	Normal (%)	100	100	100	100	100	100
	Abnormal (%)	-	-	-	-	-	-
<b>Hanging Posture (%)</b>		100	80	80	100	100	100
<b>Retablismen (%)</b>		100	80	80	100	100	100
<b>Flexion Reflex (%)</b>		-	-	-	-	-	-
<b>Haffner's Reflex (%)</b>		100	100	100	100	100	100
<b>Mortality (%)</b>		-	-	-	-	-	-
<b>Grooming (%)</b>		100	100	100	60	80	100
<b>Defecation (%)</b>		100	80	20	-	100	-
<b>Urination (%)</b>		100	20	20	40	100	40
<b>Respiration</b>	Tachypnea (%)	-	-	-	-	-	-
	Normal (%)	100	100	100	100	100	100
	Dyspnea (%)	-	-	-	-	-	-
<b>Salivation (%)</b>		-	-	-	-	-	-
<b>Vokalization (%)</b>		-	-	-	-	-	-
<b>Tremor (%)</b>		-	-	-	-	-	-
<b>Seizure (%)</b>		-	-	-	-	-	-
<b>Writhing (%)</b>		-	-	-	-	-	-

Notes:

(-) = not observed

T = immediately before blood collection

**Table 3.** Results of Observations on Rats' Behavior During the Acute Toxicity Test (Dose: 5000 mg/kg BW).

Observed Effects	Dose 5000 mg/kg BW					
	0 H	0.5 H	1 H	2 H	24 H	T
<b>Platform</b>	7.3	5.6	6.6	6	6.3	6.6
<b>Motor Activity</b>	Increased (%)	-	-	-	-	-
	Normal (%)	100	-	-	-	100
	Decreased (%)	-	66.6	100	100	-
	Immobile (%)	-	33.3	-	-	-
<b>Straub (%)</b>	-	-	-	-	-	-
<b>Piloerection (%)</b>	-	-	-	-	-	-
<b>Ptosis (%)</b>	-	-	-	-	-	-
<b>Pinna Reflex (%)</b>	100	100	100	100	100	100
<b>Corneal Reflex (%)</b>	100	100	100	100	100	100
<b>Lakrimation (%)</b>	-	-	-	-	-	-
<b>Ctalepsy (%)</b>	-	-	-	-	-	-
<b>Body Posture</b>	Normal (%)	100	100	100	100	100
	Abnormal (%)	-	-	-	-	-
<b>Hanging Posture (%)</b>	100	66.6	66.6	100	100	100
<b>Retablismen (%)</b>	100	66.6	66.6	100	100	100
<b>Flexion Reflex (%)</b>	-	-	-	-	-	-
<b>Haffner's Reflex (%)</b>	100	100	100	100	100	100
<b>Mortality (%)</b>	-	-	-	-	-	-
<b>Grooming (%)</b>	80	66.6	33.3	100	100	33.3
<b>Defecation (%)</b>	33.3	66.6	33.3	-	-	100
<b>Urination (%)</b>	66.6	100	33.3	33.3	-	100
<b>Respiration</b>	Tachypnea (%)	-	-	-	-	-
	Normal (%)	100	100	100	100	100
	Dyspnea (%)	-	-	-	-	-
<b>Salivation (%)</b>	-	-	-	-	-	-
<b>Vokalization (%)</b>	-	-	-	-	-	-
<b>Tremor (%)</b>	-	-	-	-	-	-
<b>Seizure (%)</b>	-	-	-	-	-	-
<b>Writhing (%)</b>	-	-	-	-	-	-

Notes:

(-) = not observed

T = immediately before blood collection

Table 2 and Table 3 show that platform observations decreased at the 30-minute, 1-hour, and 2-hour intervals after administration of the test preparation, then increased again after 24 hours. Similarly, motor activity decreased at the same intervals and returned to normal after 24 hours. Other parameters remained within normal ranges. Aside from observations on behavior, alertness, and motor activity, all other results were considered normal. No mortality was observed during the 14-day observation period. Overall, no abnormalities were

detected, as the rats' behavior returned to normal within 24 hours.

The OECD 425 method also includes body weight observation. In this study, body weight observations were carried out for 14 days before and 14 day after administration of the test preparation. A total of 12 test rats were divided into three groups: 4 rats in the control group, 5 rats in the 2000 mg/kg BW dose group, and 3 rats in the 5000 mg/kg BW dose group. Body weight was recorded daily, and the results are presented in the graphs shown in Figure 1 and Figure 2.

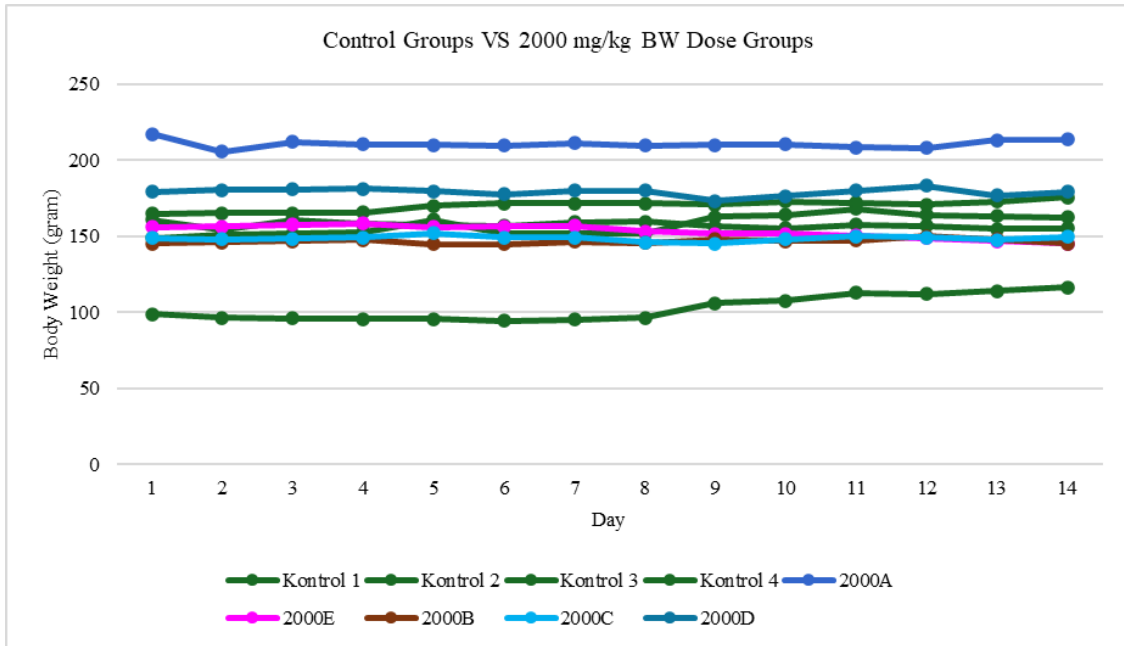


Figure 1. Body Weight Changes in Rats During The Treatment Period: Comparison Between Control and 2000 mg/kg BW Group.

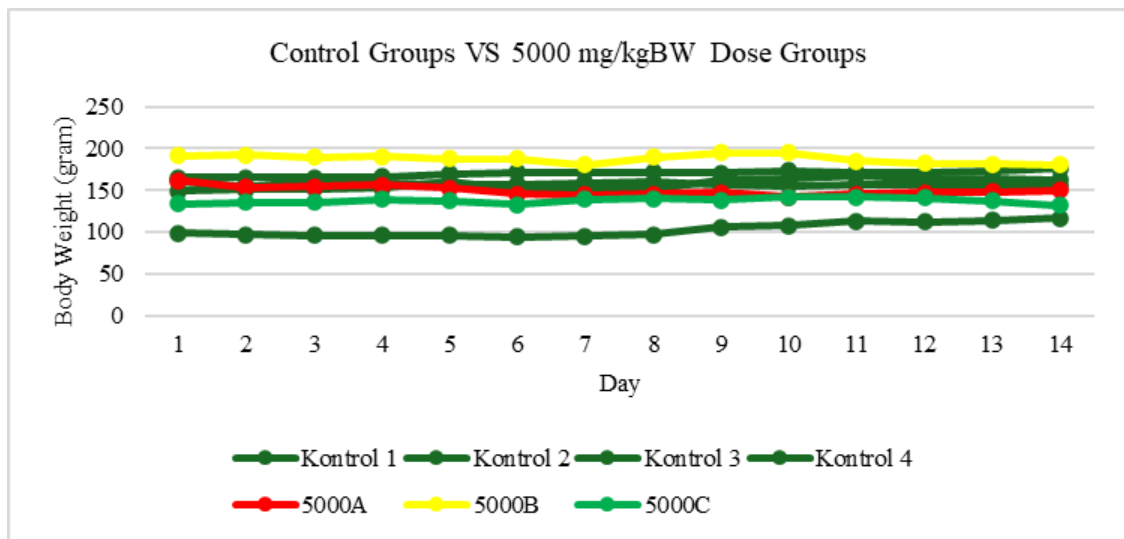


Figure 2. Body Weight Changes in Rats During The Treatment Period: Comparison Between Control and 5000 mg/kg BW Group.

Body weight was monitored to assess the impact of the jamu *cekok* on the test animals. The results, shown in Figure 1 and Figure 2, display changes in the weights of rats from the control group and those given doses of 2000 mg/kg BW and 5000 mg/kg BW. Overall, the rats maintained stable body weights, indicating they remained healthy throughout the study. In the control group, most rats gained weight, except for obe (control 2) which showed a slight decrease by day 14. Based on both graphs, it can be seen that one of the rats had a lower weight than the others but was still included due to the limited animal availability. In the 2000 mg/kg BW group, three out of five rats (A, B, and E) lost weight, while the other two gained. In the 5000 mg/kg BW

group, all rats experienced some weight loss by day 14. Although there were variations, the change in weight did not exceed 20%, so it was still considered acceptable. The overall weight stability suggests that the rats' metabolism and vital organ functions remained normal during the study.

**SGOT and SGPT Examination in Rats Blood Following Acute Toxicity Acute**

Blood analysis in rats involved the determination of SGOT and SGPT levels. These parameters serve as indicators of liver function and are used to evaluate potential hepatic damage. The corresponding results are shown in Table 4 and Table 5, respectively.

**Table 4.** Results of SGOT Level Measurements in Rat Blood.

No.	Treatment Group (Dose)	SGOT (U/l)	Average U/l)
1.	Control (0 mg/kg BW)	Negative Control 1	114.33
2.		Negative Control 2	135
3.		Negative Control 3	123.33
4.		Negative Control 4	113.33
5.	2000 mg/kg BW	T2000A	112.33
6.		T2000B	166.67
7.		T2000C	126.67
8.		T2000D	141.67
9.		T2000E	157.33
10.	5000 mg/kg BW	T5000A	98.67
11.		T5000B	92.33
12.		T5000C	111.67

**Table 4.** Results of SGPT Level Measurements in Rat Blood.

No.	Treatment Group (Dose)	SGPT (U/l)	Average U/l)
1.	Control (0 mg/kg BW)	Negative Control 1	33.67
2.		Negative Control 2	46.33
3.		Negative Control 3	68
4.		Negative Control 4	56.67
5.	2000 mg/kg BW	T2000A	45.33
6.		T2000B	56.67
7.		T2000C	45.33
8.		T2000D	58.67
9.		T2000E	53.33
10.	5000 mg/kg BW	T5000A	54
11.		T5000B	61.33
12.		T5000C	67.33

SGOT levels in rat samples were measured three times. The data obtained were analyzed using a one-way ANOVA statistical test with the assistance of SPSS software. Normality was tested using the Shapiro-Wilk test, which showed that all groups: the control group (Sig. = 0.358), the 2000 mg/kg BW dose group (Sig. = 0.871), and the 5000 mg/kg BW dose group (Sig. = 0.625) had Sig. values greater than 0.05, indicating that the data were normally distributed. Although the ANOVA test produced a significance value of 0.026, indicating a statistically significant difference, further analysis using the Tukey HSD Post Hoc test revealed that the differences were not biologically significant between the control group and the 2000 mg/kg BW group (Sig. = 0.238), nor between the control group and the 5000 mg/kg BW group (Sig. = 0.281). This indicates that there were no significant differences in SGOT levels among the treatment groups based on serum analysis, suggesting that the cekok herbal extract did not cause liver dysfunction.

Measurements of SGPT levels showed a similar pattern. The data were analyzed using the Shapiro-Wilk normality test, which yielded Sig. values of 0.987

(control), 0.220 (2000 mg/kg BW), and 0.890 (5000 mg/kg BW), all of which were greater than 0.05, indicating that the data were normally distributed. The one-way ANOVA test for SGPT levels showed a significance value of 0.406 ( $> 0.05$ ), indicating that there were no significant differences in SGPT levels among the groups. Therefore, it can be concluded that administration of the cekok herbal extract at both 2000 mg/kg BW and 5000 mg/kg BW did not cause liver toxicity. Overall, although there were slight fluctuations in SGOT and SGPT levels among the treatment groups, these variations did not indicate any significant toxic effects. In fact, at the 5000 mg/kg BW dose, the SGOT level was lower than in the control group, and the SGPT levels remained within the normal range.

## Discussion

### Determination of the LD<sub>50</sub> of acute toxicity of jamu cekok decoction

Toxicity testing plays a crucial role in assessing the potential harm a substance can cause to both living organisms and non-living materials. Typically, these tests are conducted on two types of animals: rodents and non-

rodents. In this study, an acute toxicity test was performed following the OECD 425 method: Up and Down Procedure (UDP). The testing was done using two dose limits: 2000 mg/kg body weight and 5000 mg/kg body weight (Sasmitho et al., 2015); Nurmala, 2017; Marni & Ambarwati, 2015).

This study used a mixture of four rhizomes: temulawak (*Curcuma xanthorrhiza* Roxb.), turmeric (*Curcuma longa* Lin.), ginger (*Zingiber officinale* Rosc.), and kencur (*Kaempferia galanga* L.). All four types of rhizomes were obtained from the same supplier (Flamboyan Market, Pontianak City) to ensure that there was no variability in raw materials. The extraction method used was decoction. Decoction extract is the result of the plant extraction process using boiling water to obtain active substances from the plant material. In this process, the plant mixture was placed in a specialized decoction vessel and heated in a water bath at 90–100°C for 30 minutes. In this study, the decoction pan used as the container was preheated to 90°C and stirred 2-3 times during the 30-minute heating period. After heating, the mixture was filtered to separate the liquid extract from the plant residues. The remaining dregs were then concentrated using a rotary evaporator to extend shelf life. Water was chosen as the solvent because, compared to other options, it effectively dissolves polar compounds, is inexpensive, non-flammable, and non-toxic thereby reducing the risk of introducing bias into the research findings (BPOM RI, 2023).

The test animals used in this study were female rats (*Rattus norvegicus* L.) of the Wistar strain. Before testing, they were acclimatized for 14 days to allow them to adjust to the laboratory environment. During this period, the rats had free access to food and water (ad libitum). The initial test was conducted on rats administered a dose of 2000 mg/kg BW, following a 3-hour fasting period. This fasting ensured that the test substance could interact directly with the digestive system without interference from recent food intake (Makiyah & Tresnayanti, 2017). After 14 days of monitoring, no deaths were recorded among the five rats in the 2000 mg/kg BW group. Based on these results, a second test was conducted using a higher dose of 5000 mg/kg BW, following the same procedure. Observations continued for another 14 days, and again, no mortality was observed in either dose group.

Based on observations of behavior, motor activity, and changes in body weight, the decoction extract of jamu cekok at both 2000 mg/kg BW and 5000 mg/kg BW doses was found to be practically non-toxic. No mortality was observed in any of the test animals, indicating that the LD<sub>50</sub> value is greater than 5000 mg/kgBW. This suggests that the jamu cekok extract does not produce toxic effects in the test animals. (Lukito et al., 2022). Although no deaths occurred in the treatment groups, a serological analysis was also

conducted to evaluate liver function. This included testing the levels of SGOT and SGPT to assess any potential effects of the jamu cekok on the liver.

#### **Assessment of SGOT and SGPT levels in the blood of rats after acute toxicity testing**

The parameters used to assess liver function in this study included the activity of the enzymes Glutamic Oxaloacetic Transaminase (SGOT) and Glutamic Pyruvic Transaminase (SGPT). These two enzymes serve as indicators of liver damage (Saraswati, 2016), where an increase in their level in the blood reflects impaired liver function due to the release of enzymes from damaged liver cells or as a result of oxidative stress (Widarti & Nurqaidah, 2019). The results of the statistical analysis indicate that administering jamu cekok to rats, at either higher or lower doses, did not significantly affect SGOT and SGPT levels. This suggests that the natural ingredients contained in jamu cekok (temulawak (*Curcuma xanthorrhiza* Roxb.), ginger (*Zingiber officinale* Rosc.), turmeric (*Curcuma longa* Lin.) and kencur (*Kaempferia galanga* L.)) don't have toxic effects on the liver at the given doses. This further supports the conclusion that the jamu cekok extract, containing temulawak (*Curcuma xanthorrhiza* Roxb.), turmeric (*Curcuma longa* Lin.), ginger (*Zingiber officinale* Rosc.) and kencur (*Kaempferia galanga* L.), has non-toxic properties and is safe for liver function (Nuari et al., 2023). This study also demonstrates that these four herbal ingredients contain bioactive compounds such as curcumin, gingerol, flavonoids, and terpenoids, which exhibit antioxidant, anti-inflammatory, and hepatoprotective properties, and may even help protect the liver from oxidative stress-related damage (Arief et al., n.d.). These findings are consistent with previous research showing that plants from the *Zingiberaceae* family, including temulawak, turmeric, ginger, and kencur, do not exhibit toxic effects, even at high doses (up to >5000 mg/kg BW), as confirmed by both LD<sub>50</sub> testing and serological evaluations. In conclusion, the cekok herbal extract can be classified as non-toxic and safe for oral consumption, and does not cause liver dysfunction, based on SGOT and SGPT level parameters.

The decoction extract of the cekok herbal mixture, composed of four types of rhizomes — temulawak (*Curcuma xanthorrhiza* Roxb.), ginger (*Zingiber officinale* Rosc.), turmeric (*Curcuma longa* Lin.), and kencur (*Kaempferia galanga* L.) — contains compounds that may protect against liver damage (Saraswati, 2016). Turmeric (*Curcuma longa* Lin.) contains various chemical compounds, including curcuminoids, essential oils, protein, phosphorus, potassium, iron and vitamin C. Curcumin, a polyphenolic compound, offers numerous benefits, such as antioxidant, anti-inflammatory, antibacterial, antiviral, antifungal, antitumor, antispasmodic, and hepatoprotective properties (Riset et al., n.d.). In this study, turmeric (*Curcuma longa* Lin.)

was found to have no toxic effects on liver function. This finding is consistent with the research conducted by Nuari et al., which showed that administering turmeric (*Curcuma longa* Lin.) at a dose of 10 g/BW did not produce toxic effects, possibly due to the presence of flavonoids that protect cells from damage.

Curcuma (*Curcuma xanthorrhiza* Roxb.) contains several active compounds, including terpenoids, curcuminoids, and phenolics. Like turmeric, which also contains curcumin, curcuma has been reported to exhibit antimicrobial, antioxidant, antifungal, hepatoprotective, anti-inflammatory, anticancer, antitumor, and lipid-lowering properties (Fadila et al., 2024). In this study, curcuma (*Curcuma xanthorrhiza* Roxb.) did not show any toxic effects on liver function at the tested doses, indicating its safety for use. This finding aligns with research by Nuari et al., which showed that ethanol extracts of curcuma (*Curcuma xanthorrhiza* Roxb.) administered at doses ranging from 50 to 6400 mg/BW caused no toxic effects or mortality in test animals at both 2000 mg/kg BW and 5000 mg/kg BW doses during 14 days of observation.

Ginger (*Zingiber officinale* Rosc.) contains a variety of important phytochemicals and phytonutrients, including essential oils, starch, oleoresin, resin, organic acids (such as malic and oxalic acids), gingerin, gingerone, resin oil, flavonoids, polyphenols, alkaloids, and mucilage. Among these, gingerol is a key bioactive compound with antioxidant properties that help protect cell membranes from oxidation, inhibit cholesterol oxidation, and enhance the immune system (Erinda, 2012). In this study, ginger (*Zingiber officinale* Rosc.) did not exhibit toxic effects on liver function, supporting its safety at the tested doses. This finding is consistent with the acute toxicity study by Nuari, et al., which reported that ginger (*Zingiber officinale* Rosc.) administered at doses of 1000 mg/BW, 3000 mg/BW, and 5000 mg/BW caused no mortality and showed no significant toxicity, even at the highest dose.

*Kencur* (*Kaempferia galanga* L.) plays a role in supporting liver metabolism, particularly in detoxification processes that help remove toxins from the liver. Its main bioactive compound, *ethyl p-methoxycinnamate*, has anti-inflammatory effects, while its flavonoid content provides antioxidant activity (Erindyah et al., 2024; Rezki et al., 2024). In this study, *kencur* (*Kaempferia galanga* L.) did not exhibit toxic effects on liver function, indicating its safety at the tested doses. This finding is consistent with the acute toxicity study conducted by Astuti female Swiss Webster mice using the OECD 425 Up-and-Down Procedure. The results showed that the LD<sub>50</sub> value of *ethyl p-methoxycinnamate* exceeded 5000 mg/kg BW, classifying it as practically non-toxic. Furthermore, histopathological examinations revealed that administration of *ethyl p-methoxycinnamate* had no adverse effects on the liver, stomach, or kidneys in mice (Astuti, 2019).

These findings further support the conclusion that the *jamu cekok* extract, which contains *temulawak* (*Curcuma xanthorrhiza* Roxb.), ginger (*Zingiber officinale* Rosc.), turmeric (*Curcuma longa* Lin.), and *kencur* (*Kaempferia galanga* L.), is non-toxic and safe for liver function. This study also demonstrates that these four herbal ingredients contain bioactive compounds such as curcumin, gingerol, flavonoids, and terpenoids, which possess antioxidant, anti-inflammatory, and hepatoprotective properties, and may help protect the liver from damage caused by oxidative stress. These results are consistent with previous studies showing that plants from the Zingiberaceae family—including *temulawak* (*Curcuma xanthorrhiza* Roxb.), ginger (*Zingiber officinale* Rosc.), turmeric (*Curcuma longa* Lin.), and *kencur* (*Kaempferia galanga* L.)—do not exhibit toxic effects, even at high doses (exceeding 5000 mg/kg BW), as demonstrated through LD<sub>50</sub> testing and serological evaluations. In conclusion, the *cekok* herbal extract can be classified as non-toxic and safe for oral consumption.

## CONCLUSIONS

Based on the results of the research conducted, it can be concluded that no mortality was observed in any of the test animals at doses of 2000 mg/kg BW or 5000 mg/kg BW during the 14-day observation. Therefore, based on the OECD 425 method, the estimated LD<sub>50</sub> value of *jamu cekok* is greater than 5000 mg/kg BW. The administration of a *jamu cekok* decoction extract at doses of 2000 mg/kg BW and 5000 mg/kg BW in female Wistar strain white rats (*Rattus norvegicus* L.) did not cause significant differences in SGOT and SGPT levels compared to the control group. The SGOT levels at these doses were 140.93 U/L and 100.89 U/L, respectively, while the SGPT levels were 51.87 U/L and 60.89 U/L none of which differed significantly from the control. Furthermore, no mortality was observed in the test rats, indicating that the LD<sub>50</sub> is estimated to be greater than 5000 mg/kg BW and is categorized as practically non-toxic.

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