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Evaluating Acute Toxicity of *Jamu Cekok* in Rats: A Histopathological Approach Based on OECD 425

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Abstract

Jamu cekok, an Indonesian traditional medicine to increase children appetite, has not passed preclinical or clinical trials. The rhizomes that make up *jamu cekok* such as pakai bahasa latinnya contain ethyl p-methoxycinnamate which is toxic, thus potentially harmful to the children. Therefore in this study we aim to determine the Lethal Dose (LD50) value of *jamu cekok* using the OECD 425 method as well as to observed the histopathological change in the liver in female Rattus norvegicus L. This study used an analytical experimental design with the OECD 425 method to test the acute toxicity of *jamu cekok*. The test rats used were in accordance with the OECD 425 protocol with an additional 2 negative control rats. *Jamu cekok* in the form of decocta were given once on the first day of the acute toxicity test and observed for 14 days. On the 15th day, rats were euthanized, dissected, and hepatic organs were taken to make histopathological preparations with Hematoxylin-Eosin staining. Analysis of hepatic damage was assessed using Histopathology Scoring Manja Roenigk and tested by Kruskal Wallis method. The results showed the LD50 value of decocta extract of *jamu cekok* was >5000 mg/kgBB. Observations of body weight, behavioral tests, organ index, and macroscopic hepar of test rats showed no signs of toxicity. However, The statistical analysis for histopathology test showed that there was a significant difference ($p<0.05$) between the negative control group and the 5000 mg/kgBB treatment group. In conclusion, the administration of *jamu cekok* was categorized as practically non-toxic but it can caused reversible hepatic damage at a dose of 5000 mg/kgBB.

Keywords: Acute Toxicity Test; *Jamu Cekok*; Liver; Pathology; OECD 425.

INTRODUCTION

In Indonesia, the use of traditional medicine is still believed by some people to treat various diseases. Traditional medicine or also known as herbal medicine is a concoction consisting of natural ingredients mixed for consumption and is believed to be able to treat diseases for generations (BPOM, 2019). According to BPOM Indonesia, traditional medicine is categorized into several groups, namely herbal medicine, standardized herbal medicines, and phytopharmaceuticals. Jamu is one of the three groups that is commonly known by the public and utilized by the public to overcome health problems (BPOM, 2023). Data from the 2018 Indonesia Basic Health Survey shows that 59.12% of Indonesians still consume herbal medicine (Kemenkes, 2018).

The definition of *jamu cekok* refers to the method or method of administration, namely *dicekokkan* or *dicangar* (forcibly inserted) into the child's mouth (Rini & Endah, 2019). The types of plants used in *jamu cekok* are *temu ireng* (*Curcuma aeruginosa* Roxb.), *kunir* (*Curcuma longa* L.*), temulawak* (*Curcuma xanthorrhiza* Roxb.), *babakan pule* (Al*stonia scholaris* (L.) R. Br.), *meniran* (*Phyllanthus niruri* L.), *asam jawa* (*Tamarindus indica* L.), *bayem lemah* (*Amaranthus tricolor* L.), *jambu krikil* (*Psidium guajava* L.), *puyang* (*Zingiber zerumbet* L.), *kates* (*Carica papaya* L.), and *krokot* (*Portulaca oleracea* L.). Some use additional ingredients in *jamu cekok* such as *tempe mendem* (rotten) and salt (Bhagawan et al., 2023).

The many plants used in *jamu cekok* are believed to have a positive impact on the health of its users. However, research conducted on one of the components in *jamu cekok* showed that there are ingredients that fall into the moderately toxic category. Toxicity tests on ethyl p-methoxycinnamate compounds in *Kaempferia galanga* L. found that the compounds were categorized as moderately toxic (Nurmala, 2017). Phenolic compounds contained in the rhizomes of *jamu cekok* can also have toxic effects on the human body (Kyselova, 2011). Phenolic compounds that enter the body in large concentrations can activate the caspase pathway which results in apoptosis (cytotoxicity) (Kong et al., 2000). In addition, there are still few or no studies on toxicity tests

involving *jamu cekok*. More importantly the main costumer of *jamu cekok* is children below 5 years old, thus make the analysis of its safety became important.

The United States of Food and Drug Administration (FDA) states that testing is conducted to help develop compounds that are potentially medicinal or toxic in animals. Toxicity testing is important to determine the cumulative effects and dose of a compound that can cause toxic effects on the human body. Toxicity tests consist of acute toxicity tests, subacute toxicity tests, and chronic toxicity tests. An acute toxicity test is a preliminary method used to detect toxic effects, including potential mortality, resulting from the administration of a single dose of a compound to experimental animals over a short observation period. To measure acute toxicity, parameters such as Lethal Dose (LD50) measurements and liver histopathology images are used. The LD50 value obtained will be used as a reference in determining the dose level for further toxicity tests (BPOM, 2022). Although histopathological analysis is not mandatory under OECD 425, it was included in this study to explore potential organ-specific tissue changes following acute exposure.

MATERIALS AND METHODS

Research Type

This study design falls under experimental research using a Post-Test Only Control Group Design. The research was conducted at Laboratory of Universitas Tanjungpura and Anatomical Pathology Laboratory of Rumah Sakit Universitas Tanjungpura. The test animals used in this research were female white rats (*Rattus norvegicus L.*) Wistar strain aged 2-3 months. In this study, a total of 10 white female rats were used, divided into 3 (three) groups, control group, 2000 mg/kgBW limit test group, and 5000 mg/kgBW limit test group, with the rats used were in accordance to OECD 425 methods and 2 female rats for control group.

Procedures

Collection and Preparation of Jamu Cekok

Jamu cekok was prepared using *temulawak*, *kunyit*, *jahe*, and *kencur* rhizomes that have been cleaned using clean water. The rhizomes of *jamu cekok* were obtained by direct purchase from the market in Pontianak City, West Kalimantan. Temulawak, turmeric, ginger, and kencur each amounted to 100g.

Preparation of Extract

Decocta extract of *jamu cekok* was made from Javanese ginger, turmeric, ginger, and galangal (*temulawak*, *kunyit*, *jahe*, and *kencur)* that have been blended with 400 ml of distilled water and heated for 30 minutes at 90ºC. *Jamu cekok* was then evaporated using *rotary evaporator* to obtain a thick extract.

Preparation of Experimental Animals

The experimental animals used were healthy female wistar rats aged 2-3 months and no >20% weight loss after the acclimatization period. Before use, the female rats were acclimatized for 14 days under the experimental conditions.

Preparation of Materials

The materials used included distilled water as a negative control and decocta extract of *jamu cekok* as the test material.

Acute Toxicity Test Using OECD 425 Methods

Acute toxicity test were performed in accordance with OECD 425 guidelines. The toxicity test begins with a limit test with a dose of 2000 mg/kgBW. One test animal was given a dose of 2000 mg/kgBW and observed for 48 hours. If after 48 hours of observation the animal does not show any mortality sign, then the same dose is given to one more test animal. The step is repeated until the maximum test animals used are 5. Dosing was stopped if there were test animals that showed mortality. After 5 test animals were dosed and there was no mortality, dosing was stopped and all test animals were observed for 14 days. If 3 or more of the 5 test animals died, the LD50 of the sample was less than 2000 mg/kgBB. If 3 or more test animals are alive, then a limit test with a dose of 5000 mg/kgBB is performed. The parameters observed for acute toxicity test included behavioral and motor activity observations, body weight observations, macroscopic and organ index observations. On the 15th day of the acute toxicity test, termination was carried out and the liver organ of the test rats were extracted.

Behavioral and Motoric Activities Observation

Behavioral observation were conducted before and after the administration of the test material. If the test animals do not show mortality, observation will be continued for 14 days.

Histopathological Examination

The liver of the rats in the study were surgically harvested and fixed in buffered formalin. They were then processed and stained with hematotoxylin and eosin (HE) according to the standard procedures at the Anatomical Pathology Laboratory of Rumah Sakit Universitas Tanjungpura. Observation was performed under a microscope with a magnification of 400x in 5 field of view. Observations were made in five random fields of view on each mount. Hepatic cell in each field of view were assessed and counted using Manja Roenigk histopathology scoring model, by counting the number of normal cells (A), parenchymal degeneration cells (B), hydropic degeneration (C), and necrosis cells (D) from each field of view of the preparation. The measurement results are obtained from (Ax1)+(Bx2)+(Cx3)+(Dx4) then the results are divided by (A+B+C+D) to obtain the

average value in one treatment group, the average value in each field of view is divided by the number of samples from each treatment.

Table 1. Manja Roenigk Histopathology Scoring (Uche et al., 2022).

Rate of Change	Score
Normal	1
Parenchymatous degeneration	2
Hydropic degeneration	3
Necrosis	4

Data analysis

Data obtained from the toxicity test using the OECD method were then analyzed using the AoT425 program. Data from the observation of hepatic histopathology preparations were tested for normality by Shapiro Wilk. Data distribution was found to be abnormal ($p<0.05$), so the Kruskal Wallis non-parametric test was conducted to determine the significant difference ($p>0.05$) between the three test groups and the Dunn Post Hoc test to determine the significant difference between the medians of each test group using the SPSS 26 program.

RESULTS AND DISCUSSION

Acute Toxicity Test

The results of the limit test with dose of 2000 mg/kgBW and 5000 mg/kgBW on female Wistar rats exhibited no mortality. LD_{50} value is obtained through data processing with AOT 425 StatPgm software and showed that LD_{50} of *jamu cekok* decocta extract was higher than 5000 mg/kgBW (Figure 1 and Figure 2). According to globally harmonized classification system, decocta extract of *jamu cekok* is categorized as practically non-toxic.

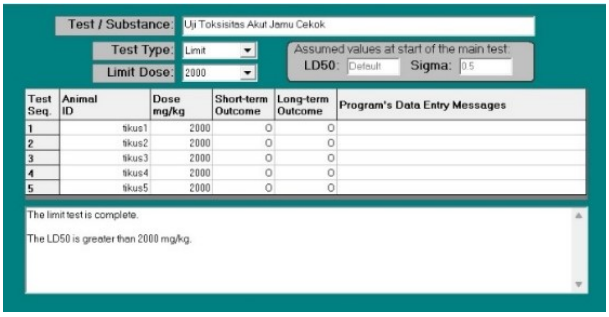


Figure 1. Testing Results Dose 2000 mg/kgBB.

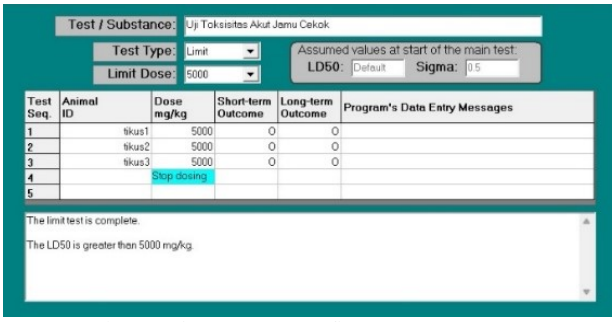


Figure 2. Testing Results Dose 2000 mg/kgBB.

Body Weight Observation

The body weights of all the animals both in dose of 2000 mg/kgBW and 5000 mg/kgBW are shown in figure 3. Statistical analysis results showed insignificant differences between the body weight of test rats in each test group.

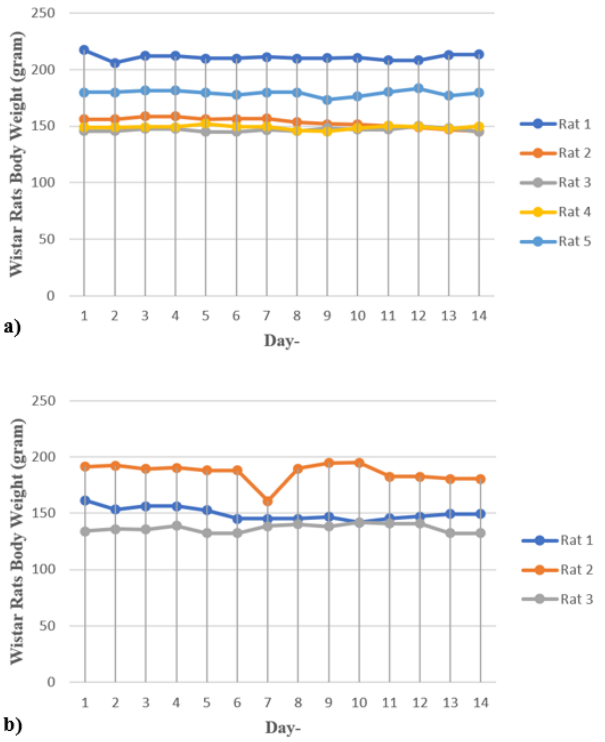


Figure 3. Effect of *Jamu Cekok* Decocta Extract on Body Weight of Wistar Female Rats in Acute Toxicity Studies: a) 2000 mg/kgBW dose group; b) 5000 mg/kgBW dose group.

Behavioral and Motoric Activities Observation

The test animals were observed for 30 min, 60 min, 120 min, and then for 24 h and continued for 14 days. Behavioral observation for the dose of 2000 mg/kgBW showed that all parameters were normal except the platform and motoric activity. All rats in the 2000 mg/kgBW group had a decreased platform value but did not differ significantly when compared to before being given the preparation. The motor activity of the five rats decreased at 30 minutes, 1 hour, and 2 hours after being given the test preparation, but returned to normal after 24 hours until termination (Table 2).

The results of behavioral observation in the 5000 mg/kgBB dose group also showed decreased platform values and motor activity but were not significantly different from before being given the preparation. There was a decrease in motor activity of the three rats at 30 minutes, 1 hour, and 2 hours after administration of the test preparation and returned to normal after 24 hours until termination. Other behavioral parameters showed normal results (Table 3).

Table 2. Effect of 2000 mg/kgBW *jamu cekok* on the behavior of white Wistar female rats in acute toxicity studies.

Parameters of Behavioral Observation		The Average of *Jamu Cekok* Behavioral Observation Dose of 2000 mg/kgBW					
		0	**30 minutes**	**60 minutes**	**120 minutes**	**24 hours**	**Day-15**
Platform		7,4	6,4	5,8	6	6,8	6,6
Motoric Activity	Up (%)	0	0	0	0	0	0
	Normal (%)	100	0	0	0	100	100
	Down (%)	0	80	80	100	0	0
	No Activity (%)	0	20	20	0	0	0
Straub (%)		0	0	0	0	0	0
Piloerection (%)		0	0	0	0	0	0
Ptosis (%)		0	0	0	0	0	0
Pineal Reflex (%)		100	100	100	100	100	100
Corneal Reflex (%)		100	100	100	100	100	100
Lacrimation (%)		0	0	0	0	0	0
Catelepsy (%)		0	0	0	0	0	0
Gestures	Normal (%)	100	100	100	100	100	100
	Not Normal (%)	0	0	0	0	0	0
Hanging (%)		100	80	80	100	100	100
Retablishment (%)		100	80	80	100	100	100
Flexion (%)		0	0	0	0	0	0
Hafner (%)		100	100	100	100	100	100
Mortality (%)		0	0	0	0	0	0
Grooming (%)		100	100	100	60	80	100
Defecation (%)		100	80	20	0	100	0
Urination (%)		100	20	20	40	100	40
Respiration	Fast (%)	0	0	0	0	0	0
	Normal (%)	100	100	100	100	100	100
	Breathless (%)	0	0	0	0	0	0
Salivation (%)		0	0	0	0	0	0
Vocalization (%)		0	0	0	0	0	0
Tremor (%)		0	0	0	0	0	0
Convulsions (%)		0	0	0	0	0	0
Writhing (%)		0	0	0	0	0	0

Table 3. Effect of 5000 mg/kgBW *jamu cekok* on the behavior of white Wistar female rats in acute toxicity studies.

Parameters of Behavioral Observation		The Average of *Jamu Cekok* Behavioral Observation Dose of 5000 mg/kgBW					
		0	**30 minutes**	**60 minutes**	**120 minutes**	**24 hours**	**Day-15**
Platform		7,3	5,6	6,6	6	6,3	6,6
Motoric Activity	Up (%)	0	0	0	0	0	0
	Normal (%)	100	0	0	0	100	100
	Down (%)	0	66,6	100	100	0	0
	No Activity (%)	0	33,3		0	0	0
Straub (%)		0	0	0	0	0	0
Piloerection (%)		0	0	0	0	0	0
Ptosis (%)		0	0	0	0	0	0
Pineal Reflex (%)		100	100	100	100	100	100
Corneal Reflex (%)		100	100	100	100	100	100
Lacrimation (%)		0	0	0	0	0	0
Catelepsy (%)		0	0	0	0	0	0
Gestures	Normal (%)	100	100	100	100	100	100
	Not Normal (%)	0	0	0	0	0	0
Hanging (%)		100	80	80	100	100	100
Retablishment (%)		100	80	80	100	100	100
Flexion (%)		0	0	0	0	0	0
Hafner (%)		100	100	100	100	100	100
Mortality (%)		0	0	0	0	0	0

Parameters of Behavioral Observation		The Average of *Jamu Cekok* Behavioral Observation Dose of 5000 mg/kgBW					
		0	**30 minutes**	**60 minutes**	**120 minutes**	**24 hours**	**Day-15**
Grooming (%)		100	66,6	33,3	100	100	33,3
Defecation (%)		33,3	66,6	33,3	0	0	100
Urination (%)		66,6	100	33,3	33,3	0	100
Respiration	Fast (%)	0	0	0	0	0	0
	Normal (%)	100	100	100	100	100	100
	Breathless (%)	0	0	0	0	0	0
Salivation (%)		0	0	0	0	0	0
Vocalization (%)		0	0	0	0	0	0
Tremor (%)		0	0	0	0	0	0
Convulsions (%)		0	0	0	0	0	0
Writhing (%)		0	0	0	0	0	0

Liver Organ Index

The value of liver organ index in the dose of 2000 mg/kgBB is greater than the dose of 5000 mg/kgBB. Statistical test results obtained p value = 0.724 (p>0.05). This indicates that there is no significant difference between the organ index of the 2000 mg/kgBB treatment group and the 5000 mg/kgBB treatment group. This shows that there is no significant enlargement or shrinkage in the liver organs of rats (Figure 4).

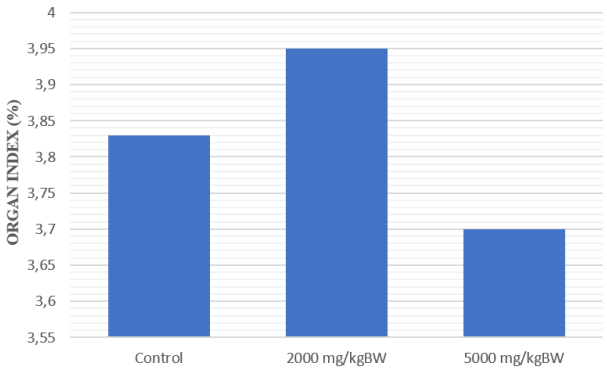


Figure 4. The Percentage of Organ Index After Being Given *Jamu Cekok* Decocta Extract.

The macroscopic examination of liver organ in all treatment groups showed brownish-red in color. The decocta extract of *jamu cekok* did not cause any lesions or nodules in the liver macroscopically (Figure 5).

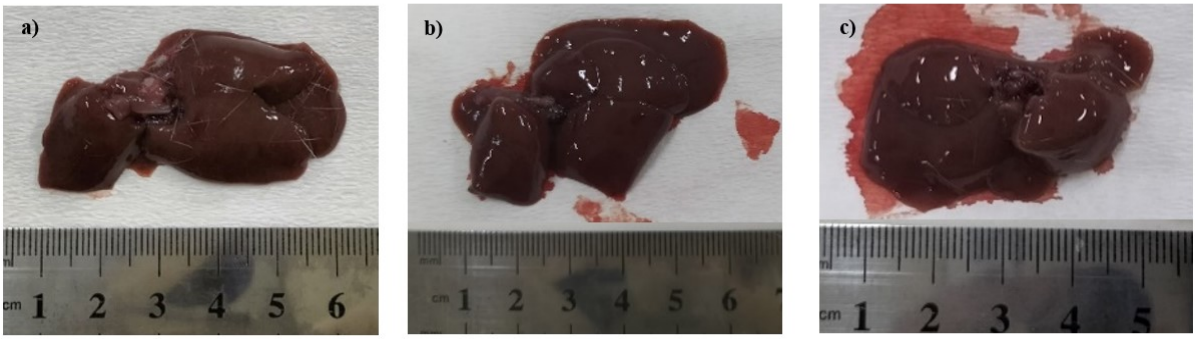


Figure 5. Macroscopic View of Liver Organ: a) control group; b) 2000 mg/kgBW dose group; b) 5000 mg/kgBW dose group.

Microscopic Observation of Liver

Microscopic observation of hepatic organs showed different results from each treatment group (Figure 6). Figure 6(a) is a histopathological picture of the negative control group which shows a picture of normal hepatic cells with few cells experiencing parenchymal degeneration. Hepatic cells seen in this group generally have characteristics such as round nuclei, clear boundaries, full cytoplasm, and regular sinusoids. Figure 6(b) is a histopathological picture of the 2000 mg/kgBB treatment group showing damage to hepatic cells.

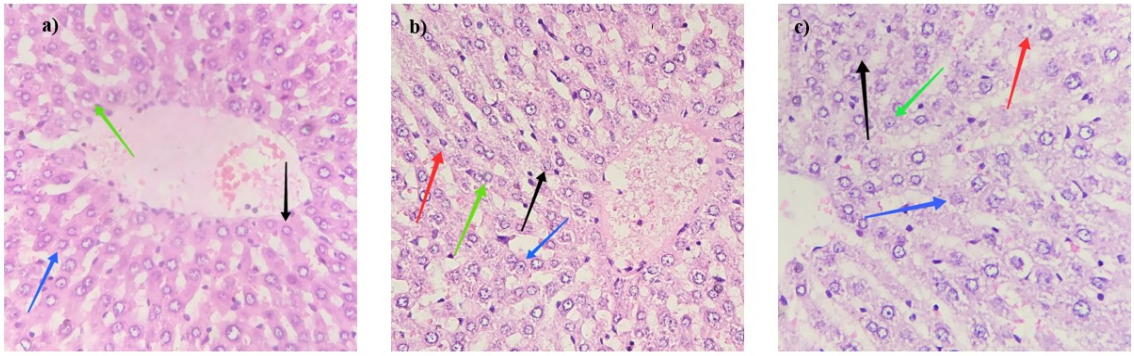


Figure 6. Macroscopic View of Liver Organ: a) control group; b) 2000 mg/kgBW dose group; b) 5000 mg/kgBW dose group.

Hepatic cell damage seen is parenchymatous degeneration (cytoplasm begins to cloudy), hydropic degeneration (cells swell with a typical picture of small to large vacuoles), and necrosis cells such as piknotik (purple to black nucleus with a reduced size due to cell wall compaction), or karyolysis which has a picture of empty cells without cell nuclei. The microscopic picture of the 5000 mg/kgBB treatment group (Figure 6(c)) shows cells that have more damage than the other two groups. In Figure 6(c), it can be seen that almost every cell has degeneration both parenchymatous and hydropic, also seen necrotizing hepatic cells with karyolysis cell nuclei.

Further observations were made in quantitative measurements by counting the number of normal hepatic cells (A), parenchymal degeneration cells (B), hydropic degeneration cells (C), and necrosis cells (D) in 5 field of view in each preparation using the Manja Roenigk Histopatholgy Scoring model. The determination of the observed field of view was determined randomly to represent each rat hepatic lobe. The calculation results shows that the negative control group has the lowest value of 1.14, followed by the 2000 mg/kgBB treatment group and the 5000 mg/kgBB treatment group (Table 4).

Table 4. Hepatic Cell Calculation Result Using Manja Roenigk Histopathology Scoring Model.

Group	Number of Samples (n)	Average	SD
Negative Control	10	1,14	0,04
2000 mg/kgBW Treatment	25	1,33	0,14
5000 mg/kgBW Treatment	15	2,85	0,63

The closer the value to 1, the more the number of normal hepatic cells. The closer the value is to 2, the more the number of hepatic cells that have parenchymal degeneration. The closer the value is to 3, the more the number of hepatic cells that have hydropic degeneration. The closer the value is to 4, the more the number of hepatic cells that experience necrosis. The data shows that an increase in the dose of *jamu cekok* extract is directly proportional to the increase in hepatic damage value (Table 4).

The calculation data were then processed statistically to see meaningful differences. Normality test was conducted first. The normality test results of the negative control group (p=0.146) and the 2000 mg/kgBB treatment group (p=0.106) were normally distributed (p>0.05). Meanwhile, the significance value of the 5000 mg/kgBB treatment group is p = 0.020 which indicates that the data is not normally distributed (p <0.05). Therefore, data processing was continued in the Kruskal Wallis non-parametric test.

Table 5. Effect of Dekokta *Jamu Cekok* Extract on Hepatic Cell Damage.

Group	Number of Samples (n)	p-Value
Negative Control	10	
2000 mg/kgBW Treatment	25	0,000
5000 mg/kgBW Treatment	15	

Note: Kruskal Wallis Test Result

The statistical analysis result shows that the p-value obtained from the Kruskal Wallis test is p=0.000 which indicates a significant difference (p<0.05). These results indicate that there are significant differences in hepatic

cell damage from each group. Because there is a significant difference, it is continued with the Post Hoc Dunn Test. The results of the Post Hoc Dunn Test can be seen in Table 6.

Table 6. Effect of Dekokta *Jamu Cekok* Extract on Hepatic Cell Damage.

Group	Negative Control	2000 mg/kgBW Treatment	5000 mg/kgBW Treatment
Negative Control			
2000 mg/kgBW Treatment			
5000 mg/kgBW Treatment			

Note: Post Hoc Dunn Test. The shaded part shows the significant difference between the two groups.

The negative control group compared to the 2000 mg/kgBB treatment group has a p value of 0.077 which indicates no significant difference ($p>0.05$) between the two groups. The negative control group compared to the 5000 mg/kgBB treatment group and the 2000 mg/kgBB treatment group compared to the 5000 mg/kgBB treatment group had a $p=0.000$ value indicating a significant difference ($p<0.05$) of hepatic cell damage between the two groups.

Discussion

Jamu cekok as a preparation tested for acute toxicity in this study used 4 types of rhizomes, namely temulawak (*Curcuma xanthorriza Roxb*.), turmeric (*Curcuma domestica*), ginger (*Zingiber offcinale Rosc*.) and kencur (*Kaempferia galanga L*.). Among one of the four rhizomes, kencur, has the main content of ethyl p-methoxycinnamate compounds which are moderately toxic (Nurmala, 2017). Ethyl p-methoxycinnamate has pharmacological activities including as an analgesic and anti-inflammatory and has potential as a sunscreen (Sinarsih et al., 2023). In addition, phenolic compounds contained in the four rhizomes of *jamu cekok* also have a toxic effect if present in excess in the human body. Phenolic compounds can work as pro-oxidants in the body system that contains redox active metals. In the presence of oxygen (O2), transition metals such as copper and iron will catalyze the phenolate redox cycle, leading to the formation of ROS and phenoxyl radicals that can damage DNA, lipids, and other biological molecules. Oxidation of flavonoid compounds with phenol ring-B by peroxidase/H_2O_2 can form phenoxyl radicals and generate ROS. In addition, dietary flavonoids containing phenol rings and other phenolics also cause oxidation of glutathione in hepatocytes (Kyselova, 2011 & Kong, 2000).

This study used an acute toxicity test with the OECD 425 method: Up and Down Procedure (UDP). This method is an alternative method in acute toxicity testing. When compared to the conventional LD50 value determination method, the Up and Down Procedure uses fewer test animals (OECD, 2022). Acute toxicity testing using the UDP method was conducted in the form of a limit test. This is because *jamu cekok* has been consumed by the public, so it can be assumed that the decoction extract of *jamu cekok* has a low dose level that fits the limit test criteria (Rini & Endah, 2019 & Bhagawan et al., 2023). In the limit test, doses of 2000 mg/kgBB and 5000 mg/kgBB were used because the single dose acute toxicity test should be done with the top dose of the limit dose.

This acute toxicity test uses female wistar rats as test animals. The selection of female rats in general, female sex animals are more sensitive compared to males, thus allowing for chemicals that act directly in their toxic mechanisms and female rats have a low detoxification capacity than males (Lipnick et al., 1995). In addition, the use of female rats is also recommended in the OECD acute toxicity testing guideline (OECD, 2022). Wistar rats were used in this study because they have been widely used in various research fields such as toxicology and pharmacology studies, have good growth, are easy to obtain in large quantities, are easy to maintain, are relatively cheap and have fast metabolic abilities (Ridwan, 2013 & Sutrisno et al., 2014). In this study, the administration of test preparations was carried out orally using a sonde. Oral administration of the test preparation was chosen to adjust the method of administration that is usually used in humans in the administration of *jamu cekok*.

The test mice were acclimatized for 14 days with the aim that the test mice could adjust to the laboratory environment. During the acclimatization period, the test rats were given food and water. Before conducting acute toxicity testing on test rats, behavioral testing and motor activity of test rats were carried out. Behavioral observations were made on each rat after 30 minutes, 1 hour, 2 hours, and 24 hours of treatment. This behavioral observation aims to see whether decocta extract of *jamu cekok* can affect changes in behavior and motor activity in test animals. This test was carried out for 2 minutes for each test animal except hanging and retablismen which were carried out for 5 seconds (Mustarichie et al., 2017 & Sutjiatmo et al., 2015). Overall platform observations showed a decrease in the 30th, 60th, and 120th minutes after administration of the test preparation and increased again after 24 hours. In addition, there was also a decrease in the motor activity of rats at minutes 30, 60, and 120 after administration of the test preparation and returned to normal after 24 hours of administration of the test preparation. Behavioral changes can occur due to stress experienced by rats during the administration of

the test preparation resulting in temporary inhibition of motor activity (Fitria, 2019). In addition, the decrease in motor activity is thought to occur due to the flavonoids in the rhizome of *jamu cekok* which have a skeletal muscle relaxant effect and alkaloids that can inhibit the work of the nervous system (Assad & Khan, 2017 & Sulastra, 2020).

Observations of sensory activity, neuromuscular system changes, eyes, respiratory changes, skin, gastrointestinal and urogenital changes, as well as body posture appeared normal in all test animal groups. Other observations such as ptosis, lacrimation, catalepsy, mortality, salivation, vocalization, tremors, seizures, and writhing were not found in any of the test animal groups. Based on the results of behavioural tests on Wistar strain white rats (Rattus norvegicus L.), no signs of toxicity were found (Nur et al., 2022).

This indicates that the decoction extract of cekok herbal medicine does not have a significant effect on the behaviour of the test animals. The first test was conducted on female rats with a dose group of 2000 mg/kgBW who had been fasted for 3 hours while still being given water. The rats were fasted with the aim that when the rats were given the test preparation, the test preparation could directly interact with the digestive system and not be disturbed by the presence of food in the rats' digestive system. This is done to maximize the absorption of the extract in the rat's digestive system (Fu et al., 2024). After being given the test preparation, observations of motor activity and behaviour were conducted every 30 minutes, 1 hour, 2 hours, and 24 hours. Observation of the behaviour and motor activity of the test rats after 24 hours did not show any signs of death or toxicity.

The same treatment was also given to female rats with a dose of 5000 mg/kgBW. The results of the limit test at 2000 mg/kgBW and 5000 mg/kgBW did not show any mortality up to 14 days of observation. Observations of the test rats' body weight were also conducted. Observations were conducted by weighing daily from before, during, to after the treatment. The observation results can be seen in Figure 4.2 and Appendix 6. The observation results show that the test mice experienced weight changes that varied over time. The variation in body weight changes of the test mice was not significantly different, as indicated by the statistical test results which yielded a significance value of $p = 0.781$ ($p>0.05$). This indicates normal metabolism of proteins, carbohydrates, and lipids in the rats' bodies, as well as normal vital organ function in the test rats (Nurbaeti et al., 2021 & Saleem et al., 2017).

Observation was conducted for 14 days and subsequently, surgery was performed on the test rats to examine the organ index of the rats that had been given the preparation orally. Surgery was performed to observe the changes occurring in vital organs, particularly the liver, macroscopically first due to the administration of the decoction extract of cekok herbal medicine. The liver is observed because it is the largest organ and has the most complex metabolism in the body. The liver is involved in the metabolism of nutrients and most drugs and toxic compounds that enter the body (Chiang, 2014). In addition, the liver is also one of the main organs in toxicity testing (BPOM, 2022).

According to BPOM standards, the organ index is one of the parameters of acute toxicity. The organ index is a parameter that can provide a general overview of the compound's effects, whether there is enlargement or shrinkage of the organ (Katrin et al., 2014). Although, the results of the macroscopic observation showed that no lesions or damage were found in the organs. However, another parameter, the organ index, shows that the liver organ is larger in the 2000 mg/kgBW dose group compared to the negative control group and the 5000 mg/kgBW dose group. The decrease in liver organ index occurred in the group with a dosage of 5000 mg/kgBW. The liver organ index in the 2000 mg/kgBB dose group increased when compared to normal. The increase in the organ index is associated with the enlargement of the liver organ. Changes in the weight, physiological, and morphological aspects of the liver are related to the feed consumed, health, and intake of toxic substances in the animal's body (Wahyuningtyas et al., 2018). Hepatomegaly occurs when the liver experiences inflammation, storage of certain substances, or increased workload. One of the unique abilities of the liver is regeneration, which is the ability to repair or replace damaged cells. When a mild injury occurs to the liver, enlargement can be a sign of the liver cell regeneration process (Safithri, 2018). Additionally, differences in liver organ index values may occur due to the use of different numbers of rats in each group and the varying body weights of the test rats. However, the differences in organ index values were not statistically significant. These results also indicate that *jamu cekok* is not toxic because one of the functions of the liver is to neutralize toxins that enter the body.

The LD50 value was obtained through data processing using the AOT425 StatPgm application (Figures 4 and Figure 5) and it was found that the decoction extract of cekok herbal has an LD50 value greater than 5000mg/kgBW. Based on the toxicity levels of a compound listed by the Food and Drug Administration, it can be said that the decoction extract of cekok herbal medicine falls into the practically non-toxic classification (BPOM, 2022).

The liver is the largest and most important metabolic organ in the body; it can be referred to as the main biochemical factory in the body. The liver is a bridge connecting the digestive tract with other organs. Therefore, the liver is an organ that maintains metabolic homeostasis. One of the functions of the liver is as a detoxification organ, which makes the liver susceptible to cellular damage in the form of inflammation,

degeneration, and necrosis (Sherwood, 2014 & Abbas, 2015). One of the contents of the cekok herbal medicine, namely ethyl p-methoxycinnamate, is also metabolized in the liver (Liu et al., 2010). Before damage occurs to the liver cells, the microscopic appearance of the liver shows polygonal-shaped liver cells, with homogeneously red cytoplasm and clearly defined cell walls. Changes in the microscopic appearance of the liver can include parenchymatous degeneration, where there is swelling of the cells accompanied by cloudy and granular cytoplasm. There is also hydropic degeneration, where the cells appear swollen and vacuolated, containing fluid. And the permanent damage to liver cells is that liver cells will undergo necrosis (Indahsari, 2017).

The lowest percentage of damage occurred in the control group given aquades (Figure 6(a)). This is possible because there is no influence of other substances entering the body, so the percentage of damage found is not significant. However, damage was still found in the control group. This could be caused by external factors such as environmental conditions and unhygienic food that leads to health issues in the rats (Fitmawati et al., 2018).

Based on the scoring results of Manja Roenigk, this study found that the average values of the negative control group and the 2000 mg/kgBW treatment group were 1.14 and 1.33, respectively, indicating that the average value of the liver cells is normal. Whereas for the 5000 mg/kgBW treatment group, the value was higher compared to the other two groups, at 2.85, which still indicates reversible liver cell damage. This indicates an increase in damage along with the increase in acute doses of the decoction extract of cekok herbal given to test rats, which is microscopically proven by the presence of various levels of liver cell damage in the preparations. Liver cell damage can occur due to the antioxidant paradox effect. The antioxidant paradox effect occurs due to the total antioxidant capacity of the body becoming unresponsive to antioxidants consumed in large doses (Halliwell, 2013). In this case, the damage is caused by compounds that can act as pro-oxidants. Pro-oxidants can increase the production of free radicals, leading to the accumulation of oxidative stress in the test rats' bodies, resulting in damage to hepatocytes (Solter et al., 2019).

The results of this study are in line with the research by Fitmawati et al. (2018), which examined the traditional concoction of the Malay community in Lingga, Riau Islands, conducted using a bitter herbal decoction administered orally to rats using a syringe. The results of the study showed that the administration of the bitter herbal concoction with varying doses caused increasingly greater damage in line with the increase in dosage. However, the liver cell damage that occurred is still within the normal category (Fitmawati et al., 2018). Whereas, the results are in line with this study which shows that the administration of decoction herbal extract can affect liver cell damage when observed microscopically, and the damage increases with the increase in dosage. However, these changes did not result in mortality in the test rats. This can happen because the more chemical compounds that enter the body, the harder the liver will work, as the liver will detoxify these compounds, especially those that enter through the digestive tract (Klaassen & Amdur, 2013). Moreover, there is a difference between that study, which only assessed the histological structure of the white rat's liver, and this study, which tests acute toxicity.

The administration of the decoction extract of cekok herbal in acute doses may affect the liver cell damage that occurs in the test rats. However, the damage to the liver cells does not affect the macroscopic structure of the liver or the behavior and motor activity of the test rats. The damage found microscopically in liver cells due to the administration of the decoction extract of cekok herbal did not cause any signs of toxicity or death in the test rats and is still considered reversible damage. Overall, this study shows the influence of administering the decoction extract of cekok herbal in acute doses on the histopathological appearance of the livers of female Wistar strain white rats, so the use of cekok herbal by the public needs to consider the dosage. This is in line with WHO research that herbal medicines still have side effects, making dosage-related studies important for herbal medicines (WHO, 2004). The results of this study indicate that although empirically *jamu cekok* is safe to consume, excessive doses may potentially cause microscopic liver organ damage.

CONCLUSIONS

The LD_{50} of the decoction extract of *jamu cekok* administered orally is greater than 5000 mg/kgBW, which is categorized as practically non-toxic. However, administration at high doses can cause microscopic changes in the rat liver cells, such as reversible degeneration and necrosis, with the highest percentage of damage occurring in the 5000 mg/kgBW treatment group.

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