

# The Antioxidant Activity and Acute Toxicity of Jamu *Cekok*: Kidney Histopathology Analysis of Female Wistar

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## Abstract

Jamu *cekok* is a traditional Indonesian herbal remedy for toddlers to improve appetite. While previous studies have shown benefits such as weight gain, its antioxidant activity and safety profile remain underexplored. This study evaluated the antioxidant potential and acute toxicity of jamu *cekok*, composed of *Curcuma xanthorrhiza*, *Curcuma longa*, *Zingiber officinale*, and *Kaempferia galanga*. Extracts were extracted with Aquadest and n-hexane, and antioxidant activity was assessed via the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Results showed weak antioxidant activity in both extracts (IC50: 481.59 ppm for decoction, 127.12 ppm for n-hexane), lower than some individual herbs. Moreover, an acute toxicity tests followed OECD 425 guidelines using female Wistar rats. No mortality occurred at doses up to 5,000 mg/kgBW, categorizing it as practically non-toxic. However, elevated creatinine levels and histopathological kidney changes, including degeneration and Bowman's capsule dilation, were observed at higher doses. These findings suggest jamu *cekok* is safe at high doses but may induce temporary renal effects. Further research is needed to optimize formulations and evaluate long-term safety.

**Keywords:** Acute Toxicity; Antioxidant; Cekok; Jamu.

## INTRODUCTION

Indonesia, home to millions of medicinal herbs, has a long history of utilizing them for health purposes, both preventive and curative, spanning thousands of years. Indonesian herbal plants are commonly consumed as herbal medicine or *jamu*, widely believed by the community to impact health positively. These herbal remedies are typically made from a combination of leaves, stems, or roots of a single plant or a mix of multiple herbs (Nurmajesty et al., 2022). Examples of these *jamu* include "Subur Kandungan Madura," "Pahitan," "Beras Kencur," "Kunir Asam," "Gondhongkates," "Temulawak," "Kunci suruh," "Gula Asem," "Cabepuyeng," and "Cekok" (Sumarni et al., 2019). Among these, "jamu cekok" is a herbal remedy specifically administered to toddlers.

The term "cekok" originates from Javanese and refers to the method of giving herbal medicine to toddlers. According to the Indonesian Dictionary (KBBI), "cekok" is defined as a traditional medicine made from a blend of ground spices, wrapped in cloth, and administered orally to toddlers by squeezing the liquid into their mouths. Jamu *cekok* is typically given to toddlers to improve their appetite. A study by Handajani and Widhiastuti (2019) conducted in Surakarta involving 72 toddlers aged 12–36 months reported weight gain after two months of

receiving jamu *cekok* (administered four times per month) (Rini Handajani & Widhiastuti, 2019). Similar findings were observed in 22 toddlers in Sragen in 2017 (Nurfieni, 2017).

Although studies suggest that jamu *cekok* can enhance weight gain in toddlers, research on its mechanism of action and safety is limited. Jamu *cekok* is generally composed of a mixture of herbs, such as temulawak (*Curcuma xanthorrhiza* Roxb.), kencur (*Kaempferia galanga* L.), ginger (*Zingiber officinale* Rosc.), turmeric (*Curcuma longa* Linn), temu ireng (*Curcuma aeruginosa* Roxb.), lempuyang emprit (*Zingiber americanus* L.), sambiloto (*Andrographis paniculata* Nees.), brotowali (*Tinospora tuberculata* Beumee.), cardamom (*Amomum cardamomum* Willd.), fennel (*Foeniculum vulgare* Mill.), and papaya leaves (*Carica papaya* L.) (Limananti & Triratnawati, 2010). These herbs contain bioactive components with antioxidant properties that benefit health (Widyowati & Agil, 2018).

Research indicates that herbal mixtures exhibit higher antioxidant activity compared to individual herbs (Septiawan et al., 2020; Sufiana, 2014). For instance, studies on Kampo medicine (a Japanese herbal therapy) showed that herbal combinations were more effective in elderly patients than single herbs (Naviglio et al., 2023).

Similarly, a study on herbs like rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), laurel (*Laurus nobilis*), gentian (*Gentiana lutea*), dandelion (*Taraxacum officinale*), and rhubarb (*Rheum palmatum*) demonstrated enhanced antioxidant activity in mixtures compared to individual plants (Caesar & Cech, 2019). This enhanced effect is likely due to the synergistic interactions among the combined herbs (Caesar & Cech, 2019), which can also neutralize the potential negative effects of certain bioactive substances (Wang SuNan et al., 2015).

Given its diverse composition, jamu *cekok* may contain superior bioactives compared to individual herbs. However, studies investigating the antioxidant properties of jamu *cekok*, particularly in comparison with its constituent herbs, remain scarce. This study aims to evaluate the antioxidant potential of jamu *cekok* relative to its components and conduct an acute toxicity assessment.

## MATERIALS AND METHODS

### Study Design and Timeline

This observational analytical laboratory study compared the antioxidant activity of jamu *cekok* with its constituent herbs. Antioxidant activity was assessed using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) method. To replicate the traditional formulation of jamu *cekok*, four key rhizomes—*temulawak* (*Curcuma xanthorrhiza* Roxb.), *kencur* (*Kaempferia galanga* L.), ginger (*Zingiber officinale* Rosc.), and turmeric (*Curcuma longa* Linn), were selected. In this study, jamu *cekok* refers to the combination of these four herbs. Acute toxicity testing was conducted following the OECD (Organisation for Economic Co-operation and Development) 425 guidelines. The study was carried out from April to November 2024 at the Faculty of Medicine, Universitas Tanjungpura.

### Material

The jamu *cekok* ingredients, sourced from the Flamboyan Market in Pontianak City, were prepared as mixtures dissolved in distilled water (aquadest) and n-hexane. For the acute toxicity test, nulliparous female Wistar rats (*Rattus norvegicus* L.), aged 2–3 months and weighing 100–150 grams, were used. The sample size was determined according to OECD 425 guidelines, with 10 rats included in the study. The herbal decoction extract was used for toxicity testing. This research has received ethical approval from the Faculty of Medicine, Universitas Tanjungpura, with Ethical-Clearance No: 5328/UN22.9/PG/2024.

### Procedures

#### Sample Preparation

##### Preparation of the Decoction Extract

The decoction extract of jamu *cekok* was prepared by blending 250 grams each of rhizomes after washing them

thoroughly. The blended mixture was placed in a decoction pan with 1000 mL of distilled water and heated at 90°C for 30 minutes. The decoction was filtered, concentrated using a rotary evaporator at 50°C, and stored as a thick extract. The dosage variation of the decoction solution was calculated based on the dosage to be given to the test animals according to the OECD 425 guideline and the conversion of units from decoction extract (mL) to dosage units in test rats (mg).

##### Preparation of the n-Hexane Extract

The n-hexane extract was prepared by macerating 250 grams of each rhizome in 2500 mL of n-hexane solvent at room temperature over three days, with the solvent replaced daily. The extract was then concentrated using a rotary evaporator and oven-dried at 40–50°C to yield a thick extract. The final jamu *cekok* extract was prepared by combining single herb extracts in a 1:1:1:1 ratio.

##### Antioxidant Testing Using the DPPH Method

Antioxidant activity was tested using the DPPH method. A 0.05 mM DPPH solution was prepared by dissolving 0.98 mg of DPPH in ethanol to a final volume of 50 mL (Kartikasari et al., 2018). The maximum wavelength was determined by mixing 2 mL of the DPPH solution with 2 mL of distilled water and leaving it in the dark for 30 minutes. The mixture's absorbance was measured using a UV-Vis spectrophotometer, and the wavelength with the highest absorbance was used for subsequent tests. Jamu *cekok* and its constituent extracts were prepared as stock solutions of 1000 ppm. Each sample was diluted to 20, 40, 60, 80, and 100 ppm, mixed with an equal volume of DPPH solution, and incubated in the dark for 30 minutes before measuring absorbance with a UV-Vis spectrophotometer (Kartikasari et al., 2018). Antioxidant activity was present in the inhibition concentration (IC) 50, then categorized (Blois, 1958).

##### Toxicity Testing Using the OECD 425 Method

Acute toxicity testing followed OECD 425 guidelines and adhered to the principles of replacement, reduction, and refinement (3R) (Cheluvappa et al., 2017). Before the experiment began, all rats underwent a five-day adaptation period to ensure environmental acclimatization, during which their body weight, physical condition, and behavioral patterns were closely monitored to confirm that only healthy animals were used. A limit test at 2000 mg/kg BW was performed by administering the dose to a single rat. If no mortality occurred within 48 hours, the same dose was administered to up to five rats. Observations continued for 14 days. If three or more rats survived, a higher dose of 5000 mg/kg BW was administered to assess toxicity. The LD50 value was determined based on mortality rates.

### Euthanasia and Sample Collection

On day 14<sup>th</sup>, blood samples were collected. Furthermore, all animals were euthanized, and liver tissue was collected for further analysis (Carbone et al., 2012). Blood creatinine levels were measured using a spectrophotometer, while histopathological analysis of the kidney tissue was conducted after hematoxylin-eosin staining to assess organ damage (Kuswara, 2015).

### Data analysis

SPSS (Statistical Products and Services Solutions) Version 20.0 was utilized to analyse the data.

## RESULTS AND DISCUSSION

### RESULTS

#### Antioxidant Measurement Using the DPPH Method

In this study, we used two solvents, Aquadest and n-hexane. Aquadest is a polar solvent, making it suitable

for extracting polar compounds, whereas n-hexane, a non-polar solvent, is better for extracting non-polar compounds and has lower polarity than aquadest (Altemimi et al., 2017). The decoction method involves continuous hot extraction using a specific volume of water for a fixed time (Ingle et al., 2017; Sakti et al., 2024). All rhizomes were extracted using maceration, yielding an average of 19.2% and 10.3% for decoction and n-hexane extracts, respectively.

The antioxidant activity of the jamu cekok extracts and individual rhizomes was analyzed using aquadest and n-hexane as solvents by using DPPH method. Quercetin, a potent antioxidant, was used as a positive control. The results are presented in Table 1.

**Table 1.** Antioxidant activity of Jamu cekok and its constituents by using DPPH Method.

Solvent	Sample	IC50	Category of Antioxidant
-	Quercetin as positive control	9,00 ppm	Very Strong
Decoctiona	Temulawak ( <i>Curcuma xanthorrhiza</i> Roxb.)	798,64 ppm	Very weak
	Turmeric ( <i>Curcuma longa</i> Linn)	39,29 ppm	Very Strong
	Ginger ( <i>Zingiber officinale</i> Rosc.)	154,76 ppm	Weak
	Kencur ( <i>Kaempferia galanga</i> L.)	3390,00 ppm	Very Weak
	Jamu cekok	481,59 ppm	Very Weak
	n-Hexane	Temulawak ( <i>Curcuma xanthorrhiza</i> Roxb.),	191,76 ppm
Turmeric ( <i>Curcuma longa</i> Linn)		177,09 ppm	Weak
Ginger ( <i>Zingiber officinale</i> Rosc.)		16,52 ppm	Very Strong
Kencur ( <i>Kaempferia galanga</i> L.)		1339,72 ppm	Very Weak
Jamu cekok		127,12 ppm	Mild

Based on Table 1, the type of solvent significantly influenced the antioxidant activity of each sample. Turmeric showed very strong antioxidant activity when extracted as a decoction, but exhibited weak activity when extracted with n-hexane. Ginger (*Zingiber officinale* Rosc.) showed the opposite pattern. When extracted with aquadest (decoction), ginger demonstrated weak antioxidant activity, whereas extraction with n-hexane resulted in very strong antioxidant activity. The antioxidant activity of jamu cekok—both in decoction and n-hexane extracts—was lower than that of its individual herbal components. The decoction form exhibited very weak antioxidant activity, similar to temulawak and kencur. In n-hexane, jamu cekok demonstrated mild antioxidant activity. Although turmeric (in decoction) and ginger (in n-hexane) displayed very strong antioxidant activity individually, their combination within jamu cekok resulted in reduced antioxidant activity.

#### Acute Toxicity of Jamu Cekok

We next examined the acute toxicity and renal effects of jamu cekok. This study used the decoction form to reflect its traditional application in society. Acute toxicity was assessed using the OECD 425 method, represented by the LD50 (lethal dose) value. No physical or behavioral abnormalities were observed in the sample rats during the acclimatization and experimental periods. Rats were administered doses of 2,000 mg/kgBW and 5,000 mg/kgBW, as per OECD 425 guidelines, and observed for 14 days. No mortality was recorded at either dose.

#### Kidney Analysis Result

In this study, creatinine concentration measurements were carried out, which are an indicator of kidney health. The following are the results of the creatinine concentration test.

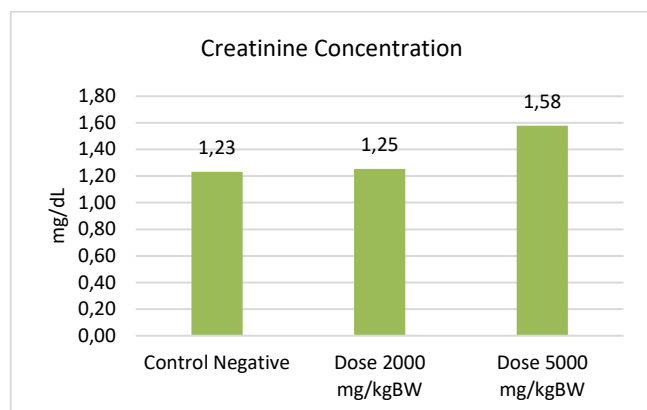


Figure 1. Creatinine concentration after acute toxicity experiment.

Figure 1 illustrates the creatinine concentrations measured in the negative control group and in rats administered *jamu cekok* decoction at doses of 2,000 mg/kgBW and 5,000 mg/kgBW. The negative control

group showed an average creatinine level of 1.23 mg/dL, while the 2,000 mg/kgBW group exhibited a slightly higher level of 1.25 mg/dL. A more pronounced increase was observed in the 5,000 mg/kgBW group, with a creatinine concentration of 1.58 mg/dL. This upward trend suggests a dose-dependent elevation in creatinine levels following administration of *jamu cekok*.

The Shapiro–Wilk normality test indicated that the creatinine data were not normally distributed; therefore a non-parametric test was applied. The Kruskal–Wallis test yielded a p-value of 0.000 ( $p < \alpha$ ), demonstrating a statistically significant difference in creatinine concentrations among the three groups. These findings indicate that higher doses of *jamu cekok* decoction, particularly at 5,000 mg/kgBW, may contribute to impaired renal function as reflected by elevated creatinine levels.

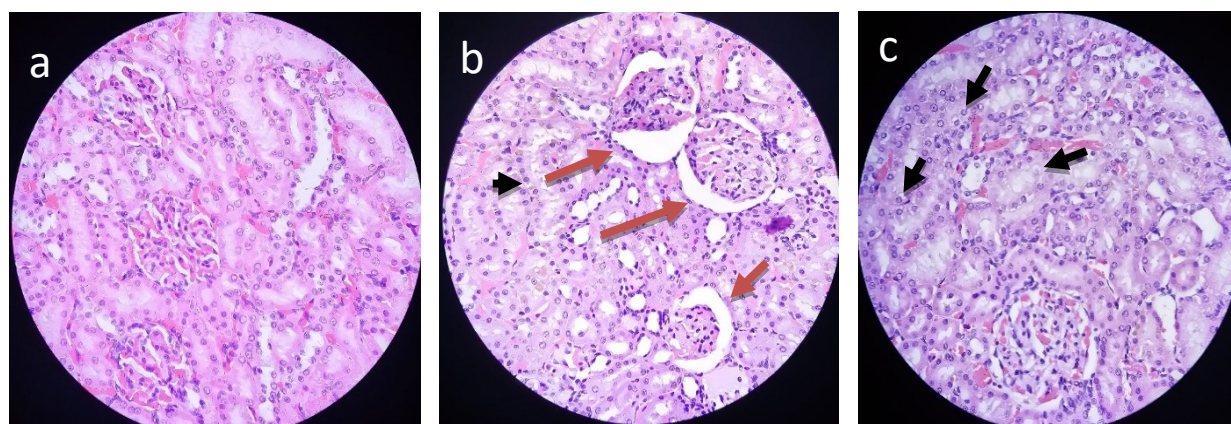


Figure 2. Histopathological result of kidneys. a) Negative control; b) treatment group, Dose 2.000 mg/kgBW; c) treatment group, dose 5.000 mg/kgBW. The red arrow indicates a Bowman's capsule dilated and black arrow indicates a degeneration cell.

Kidney analysis revealed no abnormalities in the negative control group. However, both treatment groups exhibited abnormalities, including degeneration (marked by black arrows) and dilation of Bowman's capsule (marked by orange arrows). These findings align with the creatinine test results, where a significant increase in creatinine levels was observed in the 5,000 mg/kgBW treatment group.

## DISCUSSION

The contrasting antioxidant activities between decoction and n-hexane extracts highlight the critical role of solvent polarity in extracting bioactive compounds. Turmeric contains over 200 phytoconstituents, primarily terpenoids and polyphenols, with curcumin as the dominant compound (Ashraf, 2017). Because curcumin is hydrophilic, it is more efficiently extracted using high-polarity solvents such as ethanol or water, resulting in strong antioxidant activity (AINA RAHMAWATI, 2021a; Brglez Mojzer et al., 2016; Rakhmayanti & Rusita, 2022; Setyowati & Suryani, 2013; Zamzam et al., 2023). Meanwhile, low-polarity solvents like n-hexane

predominantly extract essential oils such as tumerone and curlone, which explains the lower antioxidant activity observed in the n-hexane extract (Fahmy et al., 2023). Ginger displayed the opposite behavior. Its main antioxidant compounds—gingerols, shogaols, and diarylheptanoids—are phenolic but not efficiently extracted in water-based solvents (Liu et al., 2012). Instead, these compounds are more concentrated in non-polar or low-polarity solvents such as n-hexane, explaining the very strong antioxidant activity in the n-hexane extract (Anggraini et al., 2023; Liu et al., 2019).

The reduced antioxidant activity of *jamu cekok* compared with its individual constituents suggests possible antagonistic interactions among the combined herbs (Caesar & Cech, 2019). Similar findings have been reported in other herbal combinations such as honey–*temulawak*–ginger mixtures and rice–*kencur*–turmeric combinations, both of which produced very weak antioxidant activity (Fitriansyah et al., 2024; Septiana et al., 2019). These results indicate that combining multiple herbs does not always enhance bioactivity and may instead diminish antioxidant potential.

The OECD guideline is a widely used international standard for assessing acute toxicity, and our findings align with this framework. Throughout the study, none of the test animals experienced mortality after consuming jamu cekok, even at the highest administered dose. This absence of lethality strongly indicates that jamu *cekok* has very low acute toxicity. Therefore, based on the Food and Drug Supervisory Agency's toxicity classification, jamu *cekok* decoction can be categorized as practically non-toxic. This conclusion is further supported by previous studies reporting no toxic effects of *Curcuma xanthorrhiza* Roxb., *Zingiber officinale* Rosc., and *Curcuma longa* Linn. across different toxicity levels (Hasan et al., 2022; Mulyani et al., 2022; Oktapia, A. D., n.d.; Saputro, 2021).

The kidney is one of the most vulnerable organs to acute toxic insult due to its role in filtering, reabsorbing, and secreting exogenous compounds, which exposes renal tissue to high local concentrations of potentially toxic substances (Bassan et al., 2021; Petejova et al., 2019). Indeed, acute toxic kidney injury is a well-documented consequence of exposure to many toxins, reflecting both structural and functional renal damage (Petejova et al., 2019). Therefore, in our further analysis, we focused on the effect of jamu *cekok* decoction on kidney function. Our data reveal that as the dose of jamu *cekok* decoction increases, kidney function becomes more affected. This was most evident at 5,000 mg/kgBW, where creatinine levels were notably higher than in the control group. Creatinine is a key marker of kidney damage, particularly following toxin exposure. Impaired kidney function reduces filtration capacity, resulting in elevated creatinine levels. In this study, administration of the herbal decoction increased kidney creatinine concentrations, especially in the 5,000 mg/kgBW group. The normal creatinine range is 0.578–1.128 mg/dL (Mulyani et al., 2022).

Kidney histopathological analysis showed normal renal architecture in the negative control group, with no visible structural abnormalities. In contrast, both treatment groups displayed notable alterations, including tubular degeneration (black arrows) and dilation of Bowman's capsule (orange arrows). These structural changes are consistent with the biochemical findings, particularly the significant elevation of creatinine observed in the 5,000 mg/kgBW treatment group. Dilation of Bowman's space is often associated with glomerular atrophy and reduced filtration capacity, which can lead to the accumulation of proteins and red blood cells within the tubules—an indicator of inflammatory processes (Saputro, 2021). Tubular degeneration in the treated groups likely reflects the kidneys' response to bioactive compounds within the decoction, suggesting mild toxic stress that temporarily impairs renal function. Although such damage is generally reversible due to the kidney's inherent capacity for repair, the abnormalities remained detectable 14 days after exposure, indicating that recovery may require a longer duration. While herbal

preparations typically provide antioxidant benefits, excessive intake—particularly of polyphenol-rich components—may exert unintended adverse effects. High concentrations of polyphenols can disrupt iron metabolism, interfere with digestive enzyme activity, alter gut microbiota, and influence hormonal regulation (Duda-Chodak & Tarko, 2023). These mechanisms may contribute to the renal changes observed at higher doses of jamu *cekok*.

## CONCLUSIONS

This study found that the antioxidant activity of jamu *cekok* was lower than its individual components, likely due to antagonistic interactions. Acute toxicity testing using the OECD method with jamu *cekok* decoction on female Wistar rats revealed no toxic effects. Kidney analysis, using creatinine concentration as a parameter, showed elevated creatinine levels (1.59 mg/dL) at the 5,000 mg/kgBW dose compared to the negative control. This finding was consistent with histopathological kidney analysis, which demonstrated abnormalities such as Bowman's capsule dilation and tubular degeneration, both of which were reversible. While jamu *cekok* is generally safe for traditional use, excessive doses may pose renal risks due to potential toxic effects of certain compounds.

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## Authors' Contributions

PA is the first author as well as the main conceptualist, responsible for designing the methodology and conducting the final analysis of this study. VN is the second author who performed the data analysis in this research.

## Competing Interests

The authors declare that there are no competing interests.

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