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Acute Toxicity Testing of White Turmeric Extract (*Curcuma zedoaria*) on Histopathological Imaging of the Lungs

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ABSTRACT

White turmeric (*Curcuma zedoaria*) is a type of plant whose extract contains compounds that can inhibit carcinogenesis. Acute toxicity test was conducted to determine the safe dose and lethal dose (LD) 50 from the use of a drug substance. This research aimed to determine the effect of the acute toxicity test of white turmeric extract on the histopathological imaging of the lungs. This study is an experimental study with a post test only control group design. A total of 30 Wistar rats was divided into six groups. Data analysis was using one-way ANOVA statistical test, while for lung histopathology using ordinal data which were analyzed descriptively. In conclusion, the acute toxicity test of white turmeric extract on Wistar rats was not toxic and there was no death and no toxic symptoms and no necrosis, congestion and inflammation were found on the histopathological picture of the lungs.

1. Introduction

Healthy living is everyone's dream. However, this hope has been hampered by the increasing price of modern medicines and the possible side effects. Nowadays, people tend to explore experiences from ancestral culture, as a back to nature trends. Furthermore, they start to use herbal medicine. Traditional medicines and medicinal plants are widely used in the lower middle class, especially in preventive, promotive, and rehabilitative efforts. Meanwhile, many people think that the ingredients for using traditional medicines are relatively safer than synthetic drugs. Generally, the efficacy of traditional medicines is only based on practical experience and has not been scientifically tested ².

White turmeric (*Curcuma zedoaria*) is one of the genus *Curcuma* has the characteristics of light green round leaves; flowers grow in clusters on pseudostems as high as 30-70 cm.³ The shape of the rhizome is long like a finger, the outside parts are brownish-white, and the inside is yellowish-white. White turmeric rhizome contains the primary substance, namely curcumin, which contains diferuloylmethane as a natural antioxidant. Curcumin also has anti-inflammatory, anticancer, and antifungal functions. It is also known that white turmeric extracted has natural antioxidant substances such as total phenols and total flavonoids and has antioxidant activity, namely free radical scavenging capacity and reactive oxygen species.⁴ White turmeric extract contains compounds that can



inhibit carcinogenesis. These compounds include essential oils, polysaccharides, and curcuminoids identified and isolated, including curcumin, dimethoxy curcumin, and bisdemethoxycurcumin.^{5,6} Research by Murwanti et al. showed that curcuminoid compounds isolated from white turmeric rhizome extract at dose of 500 mg/kg BW had the best carcinogenesis inhibitory activity, which acts as antiproliferation and stimulates apoptosis⁷.

The lungs are internal organs that function in the exchange of gases (oxygen and carbon dioxide). The airflow will carry environmental oxygen to the alveoli, diffuses into the alveolar-capillary membrane, then oxygen enters the capillary network to the blood. Meanwhile, carbon dioxide, a metabolic waste, will be removed from the blood into the tissues and then released into the environment. If there is impaired lung function, the metabolism in the body will be disrupted⁵.

One of the stages of developing traditional medicine is preclinical testing, namely toxicity testing. A toxicity test is a test that detects the toxic effect of a substance on a biological system, and typical dose-response data from the test preparation, the data obtained can be used to provide information about the degree of danger of the test preparation in the event of exposure to humans, so that it can be used for human safety⁸. An acute toxicity test was conducted to determine the safe and lethal doses (LD) 50 from using a drug substance. Acute toxicity testing is carried out to determine the toxic effect of a drug or drug substance. Toxicity tests are also carried out to determine the safety of a substance or drug⁹. The interpretation of the safety of drugs/materials for humans is carried out through a series of toxicity experiments on animals. Therefore, this study aimed to determine the results of the toxicity test of white turmeric extract (*Curcuma zedoaria*) on histopathological features of the lungs with an acute toxicity test in Wistar rats (*Rattus norvegicus*).

2. Methods

The design of this research is experimental research

with a post-test-only approach with a control group design. A total of 30 Wistar rats were included in this study. The animals were acclimatized for seven days before being given treatment. They were reared in standardized room conditions with a temperature of 24-25°C, a light-dark cycle for 12 hours, and were given access to food and drink ad libitum. The maintenance and treatment of experimental animals were carried out at the Pharmacy Laboratory of the University of North Sumatra (USU). The Ethics Committee has approved this research of the Faculty of Medicine, University of Prima Indonesia (2809/SK/UNPRI.F1.1/SPPKI.1/III/2021).

A total of 500 grams of white turmeric simplicia were extracted using the maceration method for 3 x 24 hours with 70% ethanol as solvent. Then, the macerate was evaporated using a rotary evaporator so that a thick extract was obtained for other weighing and preparation of the extract to be given to the rats. After acclimatization, rats were grouped into six groups (each group consisted of five rats): group 1 (rats were given aqua dest), group 2 (rats were given NaCMC), group 3 (rats were given white turmeric extract 250 mg/kg BW (WTE250)), group 4 (rats were given white turmeric extract 500 mg/kg BW (WTE500)), group 5 (rats were given white turmeric extract 750 mg/kg BW (WTE750)), group 6 (rats were given white turmeric extract 2000 mg/kg BW (WTE2000)). The treatment was carried out for 14 days, where the treatment was given using an intragastric sonde.

Furthermore, clinical observations were carried out to assess the toxic symptoms of the treatment by assessing the rat skin, lethargy, diarrhea, tremor, and death. After 14 days, euthanasia was performed using ketamine 100 mg/kg BW intraperitoneally, and then the lungs were evacuated. Next, the lungs were put into a 10% Neutral Buffer Formalin solution (1:10). Then the dehydration process was carried out by gradually entering the lungs from 70% to 99%, followed by making paraffin blocks. Furthermore, the staining process was carried out with hematoxylin-eosin, and histopathological examination was carried out to



assess changes and damage to lung tissue.

Data analysis was carried out using SPSS 25. The data were presented descriptively and narratively to explain the observations. Univariate and bivariate analyses were performed to assess differences in clinical symptoms and lung tissue damage, with $p < 0.05$.

3. Results

Observations of toxic symptoms were carried out every day after being induced by white turmeric extract (*Curcuma zedoria*) for 14 days. Symptoms of toxicity that occurred in rats during acute toxicity tests are

presented in table 1. Observation of toxic symptoms showed that the condition was in good condition, there was no indication of skin damage, did not experience lethargy, diarrhea or tremors. The results of the observation of toxic effects until day 14 of the rats did not experience death.

The results of microscopic observation of the lungs of rats showed no histopathological changes in the lungs (table 2). The examination of histopathological changes of the lungs showed no necrosis, no inflammation and no congestion in all treatment group (figure 1).

Table 1. Observation of toxicity symptoms

Treatment	Toxic Symptoms				
	Skin	Lethargy	Diarrhea	Tremor	Death
aquadest	Normal	No indication	No indication	No indication	No
NaCMC	Normal	No indication	No indication	No indication	No
WTE 250	Normal	No indication	No indication	No indication	No
WTE 500	Normal	No indication	No indication	No indication	No
WTE 750	Normal	No indication	No indication	No indication	No
WTE 2000	Normal	No indication	No indication	No indication	No

Table 2. Histopathological changes

Necrosis	Treatment						Total
	aquadest	NaCMC	WTE 250	WTE 500	WTE 750	WTE 2000	
No necrosis	5	5	5	5	5	5	30
focal necrosis (mild)	0	0	0	0	0	0	0
multifocal necrosis (moderate)	0	0	0	0	0	0	0
diffuse necrosis (severe)	0	0	0	0	0	0	0
Inflammation							
No inflammation	5	5	5	5	5	5	30
focal inflammation (mild)	0	0	0	0	0	0	0
multifocal (moderate) inflammation	0	0	0	0	0	0	0
diffuse inflammation (severe)	0	0	0	0	0	0	0
Congestion							
No congestion	5	5	5	5	5	5	30
focal (mild) congestion	0	0	0	0	0	0	0
multifocal (moderate) congestion	0	0	0	0	0	0	0
diffuse congestion (severe)	0	0	0	0	0	0	0



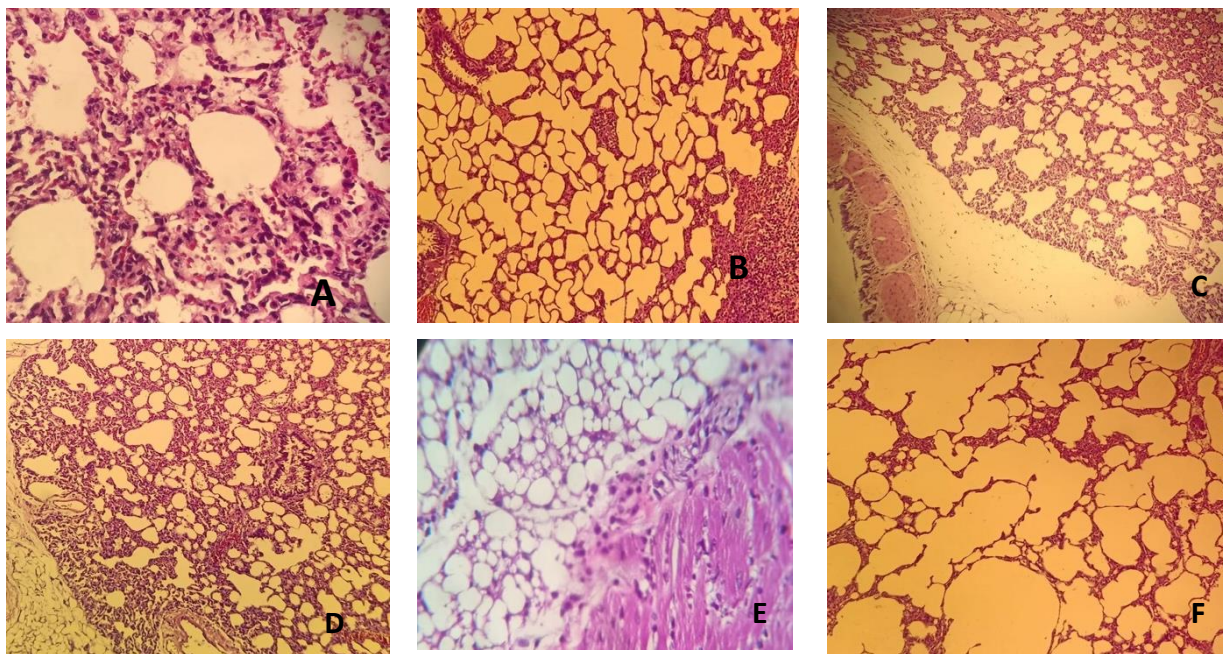


Figure 1. Microscopic imaging of lung tissue (400x magnification). Notes: A. aquadest, B. NaCMC, C. WTE 250, D. WTE 750, E. WTE 2000.

4. Discussion

Symptoms of toxicity after treatment

The toxicity test in this study used an extract of white turmeric (*Curcuma zedoria*) on Wistar rats (*Rattus norvegicus*) for 14 days for observation on each treatment of test animals. Wistar rats are experimental animals often used in research because they are easy to breed. Besides that, their anatomy and physiology are similar to humans¹⁰⁻¹¹. The acute toxicity test results with the administration of white turmeric extract for 14 days were found not to cause death in rats. This indicates that the test preparation in white turmeric extract (*Curcuma zedoria*) is not toxic.

Toxicity symptoms are parameters for the presence of toxic effects that are easily observed. Stressed rats may exhibit coarse hair, sunken eyes, slow or unresponsive movements, slouching, large abdominal masses, and aggressive behavior. While rats that are not stressed and indicate no pain will be seen in well-groomed hair, in a state of calm or sleep, normal appetite, spinal vertebrae, and dorsal pelvis are not prominent¹². The results showed that the rats showed no stress and did not experience pain in all treatment

groups. This indicated that the administration of white turmeric extract did not affect rats, indicated by the absence of indications of toxicity symptoms that appeared either in the form of changes in the skin, experiencing lethargy, diarrhea, or tremors.

Toxic substances that enter the body can cause disturbances to the body's organs, but this also depends on the number of toxic substances that enter and how long the organs are exposed to these toxic substances. Chemical substances in the liver that are too much and are in for a long time can cause various changes, including necrosis, pyknosis, degeneration, and congestion¹³, and in the lungs, the histopathological changes that often occur in the form of necrosis, degeneration, thickening and the possibility of inflammatory cell infiltration¹⁴.

Histopathological features of the lungs

The lungs are a pair of cone-shaped organs in the thoracic cavity, separated by the heart and other structures in the mediastinum, which divides the thoracic cavity into two distinct anatomical spaces. Each lung is covered and protected by a double-layered



serous membrane called the pleural membrane. This pleural fluid reduces friction between the membranes, allowing them to rub against each other easily during the respiratory process. The characteristics of normal lungs are pink, smooth, and shiny¹⁵.

Lungs obtained from the surgical process were observed by looking at the parameters of histopathological changes in the lungs, including necrosis, inflammation, and congestion. Based on the study results, it was found that the group of test animals treated with distilled water and NaCMC did not experience histopathological changes or damage in the form of necrosis, inflammation, or congestion. Likewise, at various doses, the rats did not experience multifocal necrosis (moderate) or diffuse necrosis (severe). This proves that the administration of white turmeric extract does not affect the lung histopathology of rats.

Necrosis is cell death caused by acute and irreversible cell damage, and cells cannot carry out metabolism, which is caused by the presence of toxic substances that enter along with the blood flow to the organs. Necrosis of the lungs can be caused by the direct influence of toxic agents such as chemicals or bacterial toxins or a lack of factors needed by cells such as oxygen and nutrients¹⁶.

Changes that occur in the histopathological picture of the lungs of rats in the treatment can be triggered by several things; it can be due to an excessive immune response that causes a toxin effect, due to an immune response resulting in histopathological changes in the form of necrosis and inflammation, as well as changes in metabolism due to infection. Increased hematological levels in the form of increased levels of glucose, uric acid, and cholesterol can trigger changes in the viscosity of blood fluids which can cause congestion so that it interferes with oncotic and hydrostatic pressure levels, which are one of the causes of lung cell damage. Cell damage can be caused by chemicals, immunological reactions, infectious agents, imbalanced nutrition, and genetics. Various substances can cause damage to cells, even substances

that are not toxic, such as glucose or salt. If given in excess, these substances will damage the osmotic balance so that they can injure or cause cell death¹⁷.

5. Conclusion

Acute toxicity test of white turmeric extract (*Curcuma zedoria*) is not toxic and no death arise in rats. The effect of the acute toxicity test of white turmeric extract on the histopathological picture of the lungs, no necrosis, congestion, and inflammation was found in the administration of white turmeric extract.

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