

IAI SPECIAL EDITION

RESEARCH ARTICLE

Acute toxicity test of tablets containing α -mangosteen, piperine, curcumin, methyl cinnamate and vitamin C in female wistar rats

Nia Kurnia Sari^{1,2}, Sriwidodo³, Novi Irwan Fauzi² , Ajeng Diantini⁴, Diki Prayugo W²

¹ Doctoral Programme in Pharmaceutical Science, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, Indonesia

² Sekolah Tinggi Farmasi Indonesia, Bandung, Indonesia

³ Department of Pharmaceutical, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, Indonesia

⁴ Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Sumedang, Indonesia

Keywords

Curcumin
Isolate
Mangosteen
Methyl cinnamate
Piperin

Correspondence

Novi Irwan Fauzi
Sekolah Tinggi Farmasi Indonesia
Bandung
Indonesia
noviirwan@stfi.ac.id

Abstract

Background: Traditional medicine has long been used and contributed to health services. Supplement tablets containing isolated α -mangosteen, piperine, curcumin, methyl cinnamate, and vitamin C are potential antivirals. **Objective:** This study determined the LD₅₀ of these supplement tablets and their influence on the liver, stomach, kidneys, spleen, and lungs after the acute oral administration of 5000 mg/kg to female Wistar rats, according to the OECD 425 *Up and Down Procedure*. **Method:** The test animals were observed for signs of toxicity for 14 days, the first 48 hours, with observation especially in the first 4 hours every 30 minutes. **Results:** They showed no signs of toxicity and non-irritating death and had an LD₅₀ toxicity value >5000 mg/kg body weight. Therefore, the tablets were deemed practically non-toxic. Histopathology revealed no changes in the liver, spleen, and lungs. However, desquamation and ulceration of the stomach glomerular degeneration and atrophy in the kidney were light and reversible. **Conclusion:** The tablets containing isolated α -mangosteen, piperine, curcumin, methyl cinnamate and vitamin C were practically non-toxic.

Introduction

Traditional medicine can consist of one or more compounds or several isolates of the active ingredient. Supplements in tablets containing isolated mangosteen, piperine, curcumin, methyl cinnamate, and vitamin C can be used as supplement enhancers to protect against various diseases. Mangosteen from fruit skin (*Garcinia mangostana* L.) contains xanthenes such as α -mangosteen, γ -mangosteen, and garsinon-E, which have antioxidant, anti-inflammatory, antibacterial, and antifungal activities (Rohman *et al.*, 2019). Piperine is a major compound in the fruit pepper (*Piper nigrum* L.). The alkaloid content ranges from 5.3% to 9.2% and has antioxidant, anti-inflammatory, and carminative properties (Rosa *et al.*, 2013). Curcumin is a nutritious compound of turmeric (*Curcuma domestica* Val.). Curcumin and its derivatives

are potential antioxidants, anti-inflammatories, antibacterials, antivirals, and antifungals (Alsamydai & Nisrein, 2018). Other components include methyl cinnamate or ester of sour cinnamate, the major component of the essential oil rhizomes galanga (*Alpinia galanga* L.). Vitamin C is a water-soluble vitamin required for growth and development (FI IV, 1995). It protects white blood cells from enzymes released during digestion of ingested bacteria, synthesises steroid hormones and cholesterol, helps form collagen, heals canker sores, and promotes healing and protects against infection and oxidative stress (Chambial, 2013).

The utilisation of tablet preparations as drug formulations is an approach that is often used in drug administration. Tablets have advantages such as ease of storage, distribution, and consumption. The use of

tablet formulations can provide effective dose control and increase stability. With the right formulation, tablets will provide comfortable, efficient and practical usage (Nagar *et al.*, 2011). Therefore, toxicity testing is required to evaluate the security of a drug or a substance used as a supplement. The acute toxicity test aims to determine the short-term effects and appropriate duration and dosage to protect the public from possible detrimental effects.

Methods

Acute toxicity test

The test material was a tablet supplement containing isolated mangosteen, piperine, curcumin, methyl cinnamate, and vitamin C. The acute toxicity test was based on OECD guideline 425: *Acute Oral Toxicity Up and Down Procedure* (reference).

Limit tests

The experimental animals were divided into two groups, namely the test group (combination α -mangosteen isolate 20 mg, curcumin 6 mg, piperine 2 mg, methyl cinnamate 2 mg, and vitamin C 50 mg contained in supplement tablets) and the normal control (suspension NaCMC 0.5%). Initially, the dose was 5000 mg/kg body weight administered orally. The rats were fasted first for 12 hours but were allowed water ad libum. The animals were observed for any signs of toxicity every 30 minutes for the first four hours with a time interval of 48 hours. If the mouse dies during the first test, proceed directly to the main test. If the rats survived 48 hours, they were given the same dose and monitored to return for the same procedure. If three out of five rats lived, the testing was stopped, but if three out of five rats died, it was necessary to continue the main test.

Main test

Rats were fasted first for 12 hours but were given enough to drink. The test animals were divided into two groups: the normal control group (given suspension NaCMC 0.5%) and the test group with the dose starting at 175 mg/kg BW. The test animals were given doses at 48-hour intervals and observed for 14 days. If the rat lived, the dose was increased, but if the rat died or was near death, the dose was reduced in the following order determined by OECD 425, namely 1.75; 5.5; 17.5; 55; 175; 550; 1750; 5000 mg/kg BW. The test was terminated when one of the following criteria was met:

1. Three animals lived beyond the limit of the test dose

2. Five repetitions occurred in every six animals tested in a row
3. If three deaths occurred in the same four concentrations

Toxicity parameters evaluation

The animals were observed for signs of poisoning, including tremors (shaking), convulsions (seizures), salivation (drooling), diarrhoea, lethargy (sluggishness), sedatives, coma, and death.

The appearance of the liver, kidneys, stomach, spleen, and lungs was also assessed by observing the colour, structure surface, and consistency. The weight of the organs from the test animals was also compared to the control animals (Santana *et al.*, 2019).

Results

During the seven-day acclimatisation, the rats demonstrated healthy physical behaviour and gained weight within 200-250 g (Figure 1). The observations for signs of toxicity are presented in Table I for the test substance dose of 5000 mg/kg BW, showing no evidence of toxicity in all test animals. There was no significant difference in the weight of the test animals' liver, stomach, kidneys, spleen, and lungs. Furthermore, the appearance of these organs was scored (Piao *et al.*, 2013), showing no significant differences between the test and control animals (Table II). The histopathology of the liver (Figure 2), kidney (Figure 3), stomach (Figure 4), spleen (Figure 5), and lung (Figure 6) showed some minor damage to the liver and kidneys that tends to be reversible, which means it can be recovered if the exposure to the test substance is stopped.

The tablets did not affect the gastric organs of the rats but caused focal (mild) congestion in the lung tissue. Overall, the tablets did not affect the histopathology of the major rat organs.

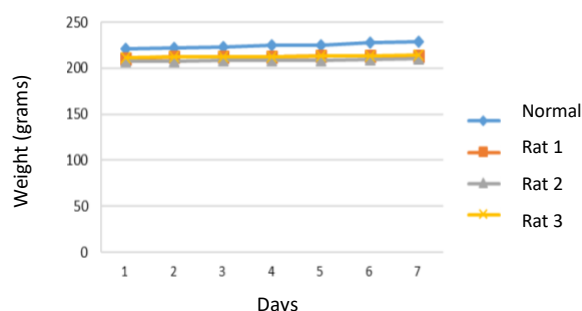


Figure 1: Graph of rat body weight during the acclimatisation period

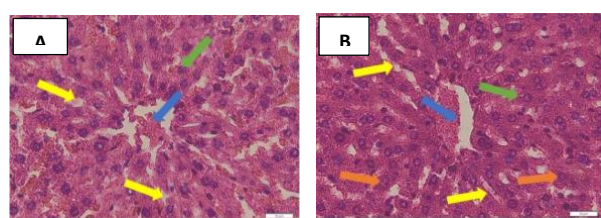
Table I: Observations of signs of toxicity

Toxicity signs	Normal rat				Rat 1				Rat 2				Rat 3			
	Days 1-2			Days 3-14	Days 1-2			Days 3-14	Days 1-2			Days 3-14	Days 1-2			Days 3-14
	4 hrs	24 hrs	48 hrs		4 hrs	24 hrs	48 hrs		4 hrs	24 hrs	48 hrs		4 hrs	24 hrs	48 hrs	
Pyrolection	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lethargy	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Red eyes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salivation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhoea	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Hyperactivity	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dead	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: (+) = There is a reaction; (-) = Normal/no reaction

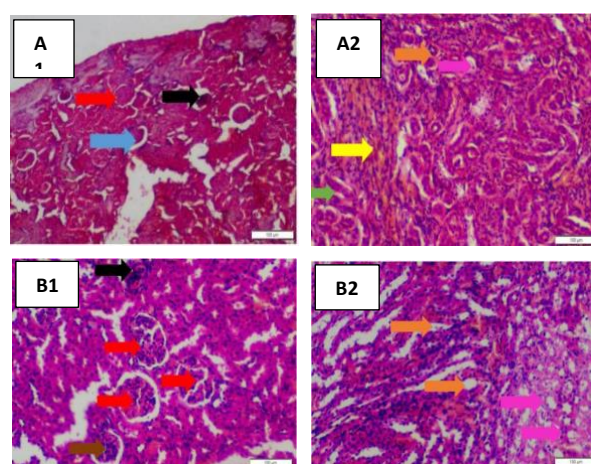
Table II: Macroscopic observations of the organs

Organ	Test animals	Parameter		
		Colour	Structure surface	Consistency
Heart	Normal control rats	Brownish red	Slippery	Springy
	All test rats	Brownish red	Slippery	Springy
Kidney	Normal control rats	Brownish red	Like peanuts and small	Springy
	All test rats	Brownish red	Like peanuts and small	Springy
Stomach	Normal control rats	Pink	No ulcers	Springy
	All test rats	Pink	No ulcers	Springy
Spleen	Normal control rats	Dark red	Shaped edges taper	Springy
	All test rats	Dark red	Shaped edges taper	Springy
Lungs	Normal control rats	Pink	Conical and solid shape	Springy
	All test rats	Pink	Conical and solid shape	Springy



A. control rat, B. test rat
 (←) hepatocytes (←) sinusoids (←) central veins
 (←) degeneration parenchymatous

Figure 2: Liver histopathology



A1. rats glomerulus normal control, A2. rats tubules normal control,
 B1. test rats glomerulus, B2. test rats tubules
 (←) glomerulus (←) tubules proximal (←) distal tubule
 (←) edema (←) atrophy (←) degeneration parenchyma
 (←) degeneration hydropic (←) inflammatory cells

Figure 3: Kidney histopathology

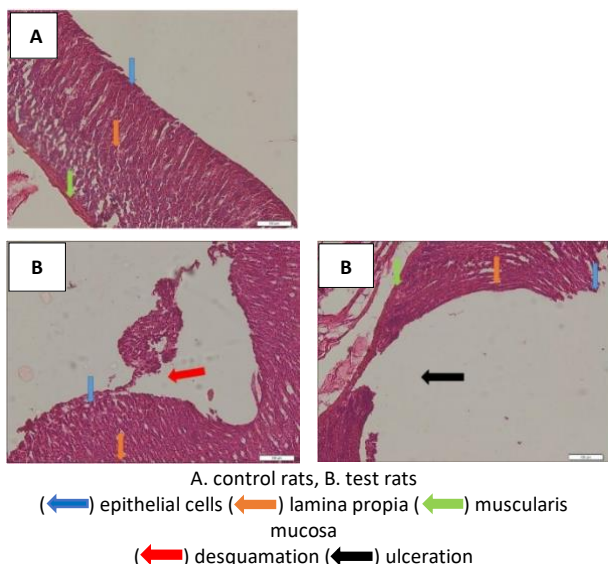


Figure 4: Stomach histopathology

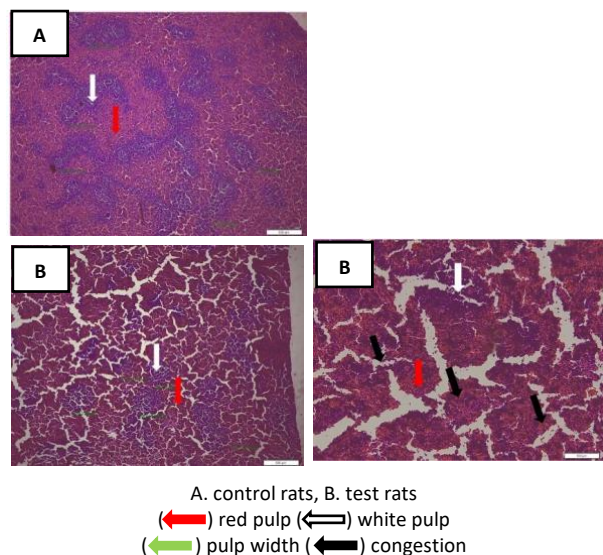


Figure 5: Spleen histopathology

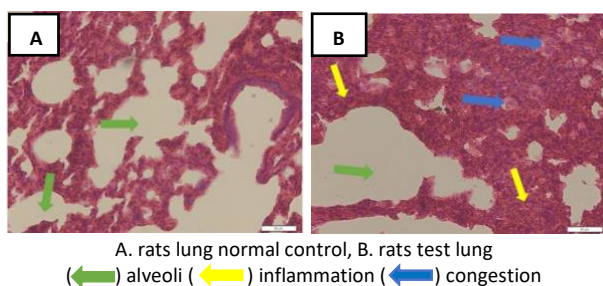


Figure 6: Lung histopathology

Discussion

During acclimatisation, the rats are given 15 grams of food per animal and enough water to drink. Husks are placed every three days to keep the rats' cages clean, keeping the rats comfortable and protected from disease. During acclimatisation, body weight and physical condition must also be monitored daily. Changes in rats' body weight during seven days of acclimatisation can be seen in Figure 1.

In Table I, the toxicity test observations show that one rat had diarrhoea on day three, which resolved from day four to day 14 and possibly was due to the spicy taste from isolated piperine. Piperine is the main alkaloid in fruit pepper and an aromatic nitrogen compound with a sharp and spicy taste. Changes in rat body weight can be described as a toxic effect. After treatment on day one to day 14, the body weight did not change beyond 10%, and they gained 200-250 g due to increased age. This indicates that the combination of mangosteen, piperine, curcumin, methyl cinnamic and vitamin C does not affect the body weight of the test animals. The AOT425 statPgm software (version 1.0) revealed that no test animals died from day one to day 14. Based on toxicity classification level according to BPOM, the tablets are classified as practically non-toxic.

The next toxicity test parameter is microscopically observing signs of toxicity in the test animals' organs. The organs observed are the liver, stomach, kidneys, spleen, and lungs. Organ observations are carried out to check for signs of organ toxicity. Organs that show signs of toxicity will experience cell damage. Histopathological changes in organs are observed under a microscope with sufficient magnification so that the morphological structure of the tissues in the organ is visible. A magnification of up to 400x is used for liver histopathology, 100x for kidney histopathology, 100x for stomach histopathology, 20x and 100x for spleen histopathology, and up to 200x for lung histopathology.

The results of histopathological observations of the liver on normal control rat preparations did not reveal any pathological or normal changes. The results of histopathological observations of the kidneys in the preparations of normal control and test rats found pathological changes. Apart from that, atrophy, which is a decrease in tissue size caused by a decrease in the number of cells or a decrease in cell size, was also found in the glomerulus. Atrophy is characterised by shrinking the glomerulus in the Bowman's capsule so that the space between the glomerulus and the Bowman's capsule becomes wider. Apart from the influence of toxicants or exposure to test substances, kidney damage is also influenced by factors outside the

kidneys, which can affect blood volume and pressure, making it difficult to confirm that the damage that occurs is caused by toxicants or exposure to test substances (Colvin & Chang, 2019). This was proven in normal control rats not exposed to the test substance, but there were pathological changes. Based on Figure 4, microscopic observation of the stomach organ by comparing the results from normal rats with test rats, namely the test rats, showed damage to the stomach tissue in the form of desquamation and ulceration in several fields of view of the gastric tissue observed. Desquamation is the detachment of epithelial cells from the tissue surface. Observation of spleen tissue found congestion of 50% (moderate) in the test rats. Tissue congestion in test rats was characterised by stretched white pulp (Smital *et al.*, 2020). The results of histopathological observations of the lung organs from normal control rats and test rats found focal (mild) congestion. Congestion is a lesion that reflects circulation disorders and can also indicate tissue repair (Tomashefski, 2000). The histopathology of the liver, kidney, stomach, spleen, and lung showed some minor damage to the liver and kidneys that tends to be reversible, such as acute renal or liver failure, a related reversible condition (Akindele *et al.*, 2014). This means it can be recovered if the exposure to the test substance is stopped.

Conclusion

The tablets containing isolated α -mangosteen, piperine, curcumin, methyl cinnamate and vitamin C were practically non-toxic to female Wistar rats. They did not cause histopathological changes to the liver, spleen, or lungs. Still, they caused desquamation and ulceration of the stomach and mild and reversible glomerular degeneration and atrophy in the kidney.

Acknowledgement

The authors thank Sekolah Tinggi Farmasi Indonesia Bandung for providing Laboratory facilities and technical assistance.

References

Akindele, A. J., Adeneye, A. A., Salau, O. S., Sofidiya, M. O., & Benebo, A. S. (2014). Dose and time-dependent sub-chronic

toxicity study of hydroethanolic leaf extract of *Flabellaria paniculata* Cav. (Malpighiaceae) in rodents. *Frontiers in pharmacology*, *5*, 78. <https://doi.org/10.3389/fphar>

Alsamydai, A., & Jaber, N. (2018). Pharmacological aspects of curcumin. *Int J Pharm*, *5*(6), 313–326.

BPOM RI. (2014). *Pedoman uji toksisitas nonklinik secara in vivo*. Badan Pengawas Obat dan Makanan RI.

Chambial, S., Dwivedi, S., Shukla, K. K., John, P. J., & Sharma, P. (2013). Vitamin C in disease prevention and cure: an overview. *Indian Journal of Clinical Biochemistry*, *28*, 314–328. <https://doi.org/10.1007/s12291-013-0375-3>

Colvin, R. B., & Chang, A. (2019). *Diagnostic pathology: kidney disease* (3rd ed.). Elsevier Health Sciences.

Tomashefski Jr, J. F. (2000). Pulmonary pathology of acute respiratory distress syndrome. *Clinics in Chest Medicine*, *21*(3), 435–466. [https://doi.org/10.1016/S0272-5231\(05\)70158-1](https://doi.org/10.1016/S0272-5231(05)70158-1)

Nagar, P., Singh, K., Chauhan, I., Verma, M., Yasir, M., Khan, A., Sharma, R., & Gupta, N. (2011). Orally disintegrating tablets: Formulation, preparation techniques and evaluation. *Journal of Applied Pharmaceutical Science*, *1*(4), 35–45.

OECD. (2022). Test No. 425: Acute oral toxicity: up-and-down procedure. OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing.

Piao Y., Liu, Y., & Xie, X. (2013). Change trends of organ weight background data in sprague dawley rats at different ages. *Journal of Toxicologic Pathology*, *1*, 29–34. <https://doi.org/10.1293/tox.26.29>

Rohman, A., Rafi, M., Alam, G., Muchtaridi, M., & Windarsih, A. (2019). Chemical composition and antioxidant studies of underutilized part of mangosteen (*Garcinia mangostana* L.) fruit. *Journal of Applied Pharmaceutical Science*, *9*(8), 47–52. <http://dx.doi.org/10.7324/JAPS.2019.90807>

Martha Perez Gutierrez, R., Maria Neira Gonzalez, A., & Hoyo-Vadillo, C. (2013). Alkaloids from piper: A review of its phytochemistry and pharmacology. *Mini Reviews in Medicinal Chemistry*, *13*(2), 163–193. <https://doi.org/10.2174/138955713804805148>

Silva-Santana, G., Aguiar-Alves, F., Silva, L. E., Barreto, M. L., Silva, J. F. R., Gonçalves, A., Mattos-Guaraldi, A. L., & Lenzi-Almeida, K. C. (2019). Compared anatomy and histology between mus musculus mice (swiss) and rattus norvegicus rats (wistar). Preprints, 2019070306. <https://doi.org/10.20944/preprints201907.0306.v1>

Smital, K., Niharika, S., & Mansee, T. (2020). Sub-acute toxicity assessment of green synthesized hematite nanoparticles (α -Fe₂O₃ NPs) using Wistar rat. *Research Journal of Biotechnology*, *15*(4).