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Subchronic Toxicity of *Curcuma longa* (Turmeric) Rhizoma Extract on Rats

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ABSTRACT

Toxicity is a condition that indicates a harmful effect contained in a substance such as turmeric, which is an effective traditional medicinal plant used for antihypertensive treatment. Therefore, this study aims to determine the effect of repeated dosing of turmeric (*Curcuma longa* L.) rhizome extract. This subchronic toxicity study was divided into 4 groups, namely 1 normal treated with 0.5% Na-CMC, and 3 treatments with turmeric extract at doses of 50, 100, and 200 mg/kg BW for 28 days with each group consisting of 5 males and 5 female Wistar rats. The results showed that the turmeric extract at doses of 50, 100, and 200 mg/kg BW did not cause toxicity to liver and kidney biochemistry nor contain any toxic substances that might cause anemia or other abnormalities. Furthermore, histopathological examination showed that the tissues were normal. This indicates that the turmeric rhizome extract at all dose variations indicate non-toxic when used in traditional medicine.

Keywords : Biochemical; *Curcuma longa*; Hematology; Histopathology; Toxicity.

INTRODUCTION

Medicines obtained from plants have long been used in all civilizations and cultures, therefore, plants play an important role in healthcare worldwide. Medicinal plants have been used in traditional medicine to maintain health and treat diseases since ancient times, due to the adverse side effects of synthetic drugs. However, recent surveys showed that medicinal plants also have side effects. Due to the several concerns raised about the potentially toxic effects caused by using these plants, an evaluation of the toxicological impact for clinical use or preclinical studies was carried out (Porwal et al., 2017).

One of the medicinal plants used by Indonesians is the turmeric plant, which has the Latin name *Curcuma longa* Linn and is widely known by the public to possess diverse medicinal uses (Winarsih et al., 2012). The plant has pharmacological activity, one of which is used in antihypertensive treatment (Hasimun et al., 2019). Meanwhile, hypertension is a major risk factor for cardiovascular disease (Mozaffarian et al., 2015) which is one of the most common causes of death in the world (WHO, 2018).

Turmeric has antihypertensive properties, hence, its safety needs to be ensured, also, Indonesians use this plant for treating various diseases, as well as a cooking spice. The toxicity level of turmeric has not been ascertained, especially in the liver, because there are approximately 64 compounds in this plant that are thought to be hepatotoxic (Balaji & Chempakam, 2010), given that the liver is the main target of drugs and xenobiotics (Kim et al., 2014) consequently, there is a need to test the toxicity of turmeric.

Therefore, this study aims to determine the subchronic toxicity of turmeric extract for 28 days to improve public safety in treating various diseases.

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METHODOLOGY

Extract Preparation

Turmeric dry powder was extracted for 72 hours using 70% ethanol (1:10 b/v) by maceration. The extract was obtained from PT. BALITRO (Research Institute for Spices and Medicines) Bogor, West Java, and plant determination was carried out at the Biological Research Center, Indonesian Institute of Sciences (LIPI), Bogor, West Java, with the number B-3896/IPH.3/KS/XI/2019.

Ethical Considerations

Approval for the use of animals in the study was obtained from the animal ethics committee of the Padjadjaran University, Bandung, Indonesia (No. 246/UN6.KEP/EC/2020).

Preparation and Grouping of Experimental Animals

Healthy male and female Wistar rats that were not pregnant at 6 to 8 weeks of age and weighing 100 g-200 g were obtained from a test

animal provider in Majalaya, Bandung, Indonesia. The rats were placed in standard cages and kept under standard conditions at 22°C ± 3°C, 30-70% relative humidity, 12 hours light and dark cycle, with access to standard food and water ad libitum.

Subchronic toxicity study (28 days)

The rats were divided into 4 groups, each consisting of 5 female and 5 male rats divided randomly and previously adapted for ± 7 days to allow acclimatization to the cage conditions. Group, I was a normal control given 1 mL of 0,5% Na-CMC solution, group II was given a test preparation at a dose of 50 mg/kg BW, group III with 100 mg/kg, and group IV with 200 mg/kg BW. The treatments were given once a day for 28 days orally, then, on the 29th day, the rats were euthanized using a chamber filled with CO_2 gas, while blood was drawn from the orbital sinus of the eye. Blood samples were collected for clinical and hematological biochemical examination. Furthermore, the rats were isolated in the neck and operated on for histopathological examination.

Biochemical Analysis

On the 29th day, blood samples were taken from the orbital sinus, placed in an Eppendorf tube, centrifuged, and then the serum was taken to analyze the activity of SGOT (Serum Glutamic Oxaloacetic Transaminase), SGPT (Serum Glutamic Pyruvate Transaminase), BUN (Blood Urea Nitrogen), and Creatinine. SGOT - SGPT levels were determined based on enzymatic reactions, 100 µL of the sample solution was added with 1000 µL of the reagent kit. For the BUN analysis, 10 µL of the sample solution was added with 1000 µL of reagent kit, while for creatinine analysis, 50 µL sample solution was added with 1000 µL reagent kit which was then read using a Microlab 300 tool at a wavelength of 340 nm.

Hematology Analysis

The blood samples were placed in a test tube containing an anticoagulant, namely ethylenediaminetetraacetic acid (EDTA) to determine the hematological parameters of hemoglobin concentration, erythrocyte count, leukocytes, hematocrit, platelets, MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration), Segment Neutrophils, and lymphocytes.

Histopathological Analysis

On the 29th day, the liver, spleen, heart, kidney, lungs, testes from the male and ovaries from females rats were isolated for histopathological observation and calculated the score. The test animals were dissected, and the organs were removed, washed, and then soaked using 10% formalin. Furthermore, the histopathological examination was carried out by making tissue preparations, stained with Hematoxylin and Eosin (HE), and examined microscopically.

Statistical Analysis

Statistical analysis was performed with one-way analysis (ANOVA), if there was a significant difference, multiple comparisons of Post Hoc LSD were carried out with a P-value <0.05 which was considered statistically significant.

RESULT AND DISCUSSION

Phytochemical screening and characterization of *Curcuma longa*

Phytochemical screening of the turmeric ethanol extract showed the presence of saponins, phenolics, flavonoids, triterpenoids, and glycosides (Table I), while the characterization results are shown in (Table II).

Biochemical analysis of rats given turmeric extract for 28 days

The administration of turmeric extract showed no significant difference in SGPT and BUN results with p>0.05 while creatinine and SGOT showed a significant difference as indicated by p<0.05 at a dose of 200 mg /kg BW against the normal group (Table III).

The biochemical examination did not show a significant difference in the levels of SGPT and BUN, but there was an increase in the levels of SGOT and creatinine at a dose of 200 mg/kg BW. An increase in the plasma creatinine levels mostly indicates a decrease in excretion caused by impaired renal function (Anugerah et al., 2018), hence, creatinine is a good indicator of kidney function (Mulyani Yani, Sukmawati Ika K, 2017). However, this increase was categorized as normal because the value did not exceed the predetermined normal range, namely 0,2-0,6 mg/dL (Devi, 2015). High levels of AST are not only limited to the liver but also influenced by the pancreas, lungs, leukocytes, and erythrocytes (Longo et al., 2012), while a small part is also produced by the muscles, heart, brain, and kidney cells, hence, the physical health condition of the rat affects the SGOT activities. However, the increase in SGOT showed no toxicity because the activity value was still in the normal range, namely 69-191 U/L (Anugerah et al., 2018). This shows that turmeric does not cause toxicity to kidney and liver biochemistry because the compounds contained in

Table I. Results of Phytochemical Screening of Turmeric Rhizome Extract

Phytochemical components	Inference
Alkaloid	-
Saponin	+
Tannin	-
Phenolic	+
Flavonoid	+
Triterpenoid	+
Steroid	-
Glycosides	+

(+ detected, - not detected)

Table II. Characterization Results of Turmeric Rhizome Extract

Characterization	Inference	Methods
Water content	11,43%	Aufhasuer
Ash content	1,32%	Gravimeter
Soluble in ethanol	62,29%	Gravimeter
Curcumin	99,51µg/mL extract	Spectrophotometry

Table III. Effects of turmeric extract and normal on biochemical parameters in rats for 28 days

Parameter	Treatment			
	Normal	Dose 50mg/Kg	Dose 100mg/Kg	Dose 200mg/Kg
Creatinine (mg/dL)	0.27 ± 0.05	0.19 ± 0.09	0.35 ± 0.17	0.51 ± 0.08*
BUN (mg/dL)	17.33 ± 5,78	13.18 ± 3.65	14.96 ± 3.45	19.52 ± 5.62
SHOT(U/L)	92.40 ± 8.71	108.60 ± 6.12	98.23 ± 15.48	158.30 ± 15.46*
SGPT(U/L)	52.46 ± 7.66	45.18 ± 7.14	43.46 ± 8.20	53.22 ± 12.26

*P<0.05 compared with a normal group; Values represent mean ± SD (Standard deviation) for n=5

the extract, namely curcuminoids, have an antioxidant effect that reduces kidney toxicity and hepatotoxicity (Sharma et al., 2011). In addition, the presence of flavonoids which also have antioxidant effects inhibits liver damage by binding radicals (Kresnadipayana et al., 2019).

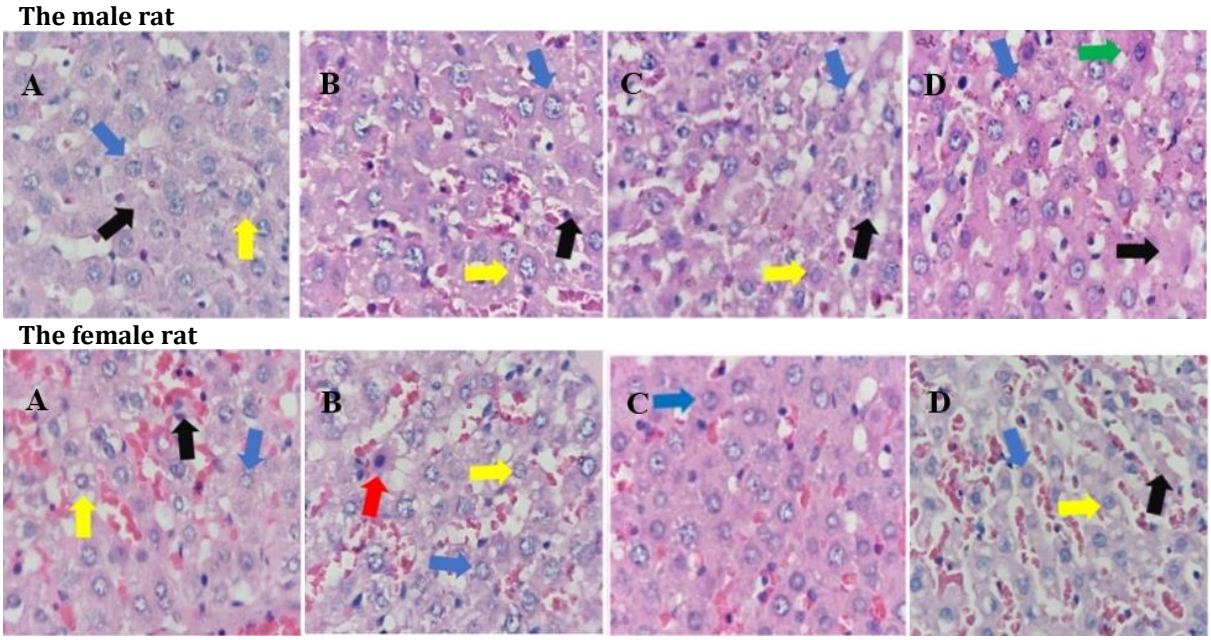
Hematological changes of rats given turmeric extract for 28 days

Administration of turmeric extract for 28 days showed no significant difference with p>0.05 in leucocytes, platelets, MCV, MCH, MCHC, segment neutrophils, and lymphocytes, while hemoglobin, hematocrit, and erythrocytes showed a significant difference indicated by p<0.05 (Table IV).

Hematological parameter assessment is used to determine the toxicity level of foreign compounds present in plant material on blood components. The examination showed that there were no significant differences in leucocytes, platelets, MCV, MCH, MCHC, segment neutrophils, and lymphocytes. Meanwhile, hemoglobin, hematocrit, and erythrocytes showed an increase in levels, hence, it was concluded that turmeric affects red blood cells. This is consistent with a previous study that stated that the number of erythrocytes, hematocrit, and hemoglobin levels increase altogether when there is a change (Meyer & Harvey, 2004). These variables increase due to the influence of curcumin which elevates stamina, immunostimulant, antioxidant effects and induces specific and non-specific immune responses (Saragih et al., 2015). This suggests that turmeric rhizome extract does not have toxic substances that might cause anemia or other disorders.

Histopathological changes of rats given turmeric extract for 28 days

The administration of turmeric ethanol extract showed damage to the liver in both male (Figure 1) and female rats (Figure 2). There were cell changes in the form of parenchymal and hydropic degeneration, as well as necrosis in both the normal and treatment group but the cells were generally normal. Furthermore, the kidneys showed necrosis in male (Figure 3) and female rats (Figure 4) but generally had normal cells. In the spleen, generally normal cells were found in



(A) normal group; **(B)** 50 mg/kg; **(C)** 100 mg/kg: **(D)** 200 mg/kg (treated group of turmeric extract). Blue Arrow: normal; Yellow Arrow: hydropic degeneration; Green Arrow: parenchymal degeneration; Red Arrow: Parenchymal Degeneration; Black Arrow: necrosis. (H&E x400)

Figure 1. Liver histology

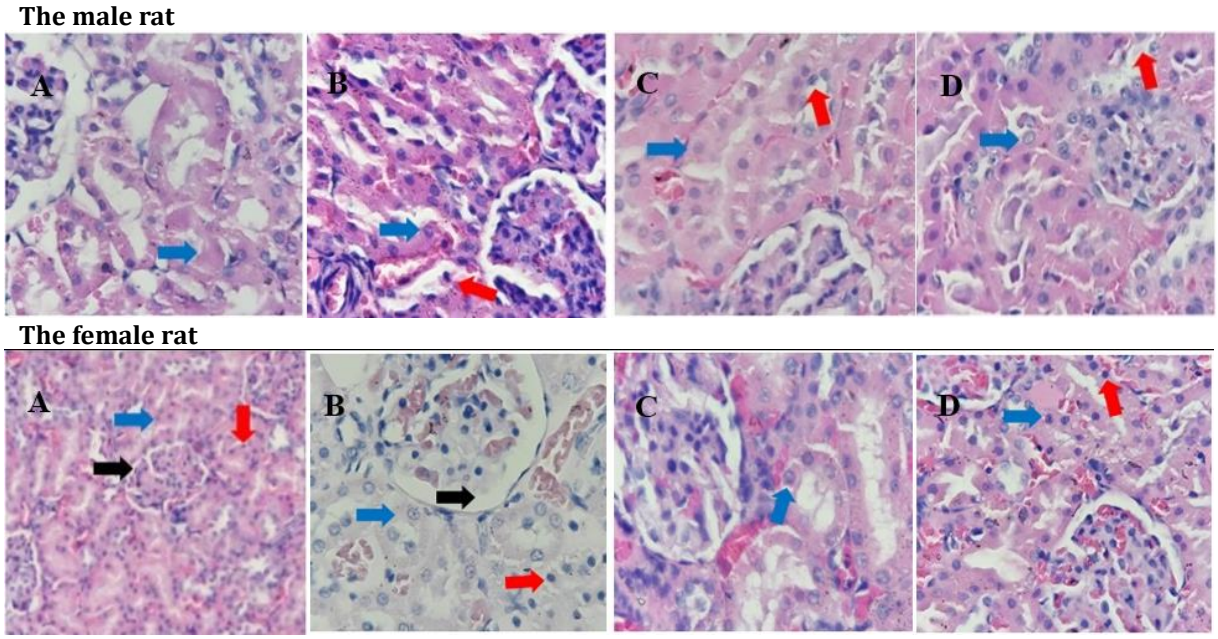
Table IV. Effects of turmeric extract and normal group on hematological parameters in rats for 28 days

Parameter	Treatment			
	Normal	Dose 50mg/Kg	Dose100mg/Kg	Dose 200mg/Kg
Hemoglobin (g/dL)	11.17 ± 0.75	13.00 ± 0.20*	12.96 ± 0.30*	11.57 ± 1.20
Hematocrit (%)	36.67 ± 2.08	42.67 ± 1.53*	42.3 ± 1.53*	39.30 ± 2.89
Leukocytes (10^3/µL)	21.98 ± 4.56	26.13 ± 11.96	27.22 ± 9.73	27.50 ± 3.27
Platelets (10^3/µL)	364.00 ± 57.69	350.00 ± 35.16	328.00 ± 79.77	334.00 ± 108.88
Erythrocytes (fl)	3.87 ± 0.25	4.26 ± 0.15	4.50 ± 0.30*	3.90 ± 0.26
MCV(pg)	94.66 ± 2.52	99.33 ± 1.15	94.67 ± 9.073	100.67 ± 1.15
MCH(gr/dL)	29.00 ± 0.00	30.33 ± 0.58	29.00 ± 1.00	29.67 ± 1.15
MCHC (%)	30.67 ± 0.57	29.67 ± 0.58	30.67 ± 1.15	30.17 ± 1.03
Segment neutrophils (%)	17.00 ± 8.18	11.67 ± 4.51	8.33 ± 2.08	20.33 ± 8.74
Lymphocytes (%)	83.00 ± 8.18	88.33 ± 4.51	90.67 ± 2.52	78.33 ± 10.26

*P<0.05 compared with a normal group; Values represent mean ± SD (Standard deviation) for n=5

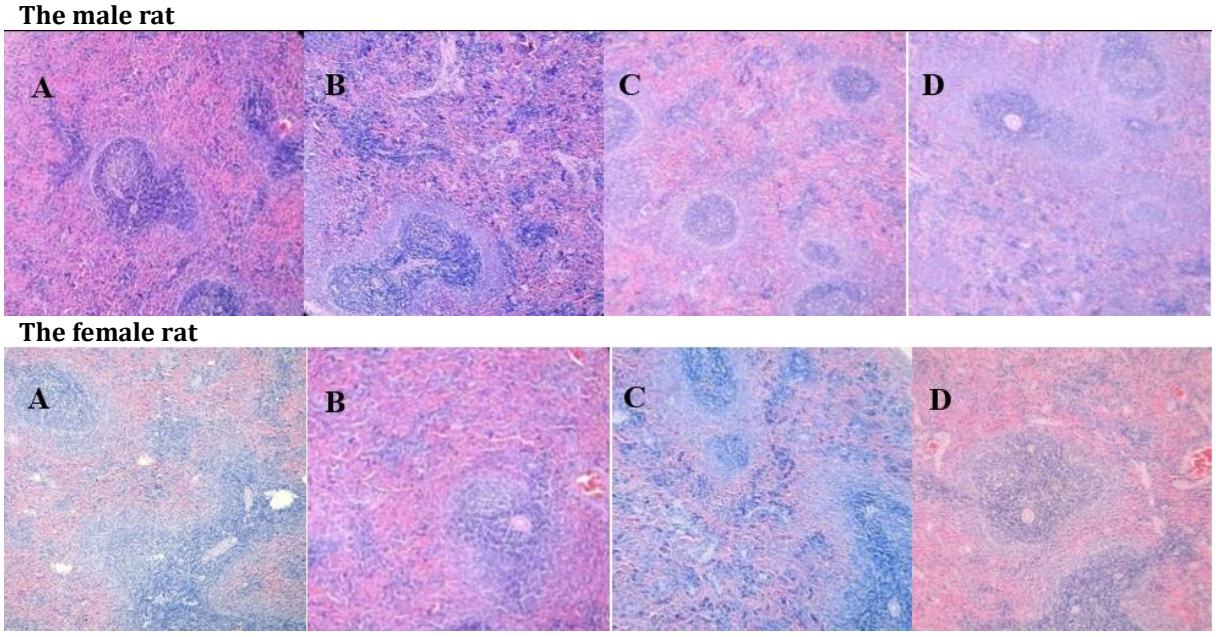
both male (Figure 5) and female rats (Figre 6). Meanwhile, there was an infiltration of inflammatory cells in the heart both in male (Figure 7) and female rats (Figure 8) at a dose of 200 mg/kg BW. The lungs of both male (Figure 9) and female rats (Figure 10) showed the presence of inflammatory cells in all dose groups but generally normal cells, while the male testis (Figure 11) and female ovary (Figure 11) show normal cells.

The observations made on the liver of both male and female rats found that there were cell changes in the form of parenchymal and hydropic degeneration, as well as necrosis in the normal and treatment group. The damage to the normal group was the only minor, while the necrosis observed was not a pathological event, but rather physiological (Cheville, 1999). Turmeric rhizome extract is known to provide numerous benefits against liver damage because it contains curcumin which is used as an anti-inflammatory and antioxidant (Sardjiman, 2000). Based on the results, all groups experienced cell degeneration in the parenchyma and hydropic cells probably due to



(A) normal group; **(B)** 50 mg/kg; **(C)** 100 mg/kg: **(D)** 200 mg/kg (treated group of turmeric extract). Blue Arrow: normal; Black Arrow: normal distance; Red Arrow: necrosis. (H&E x400)

Figure 2. Kidney histology

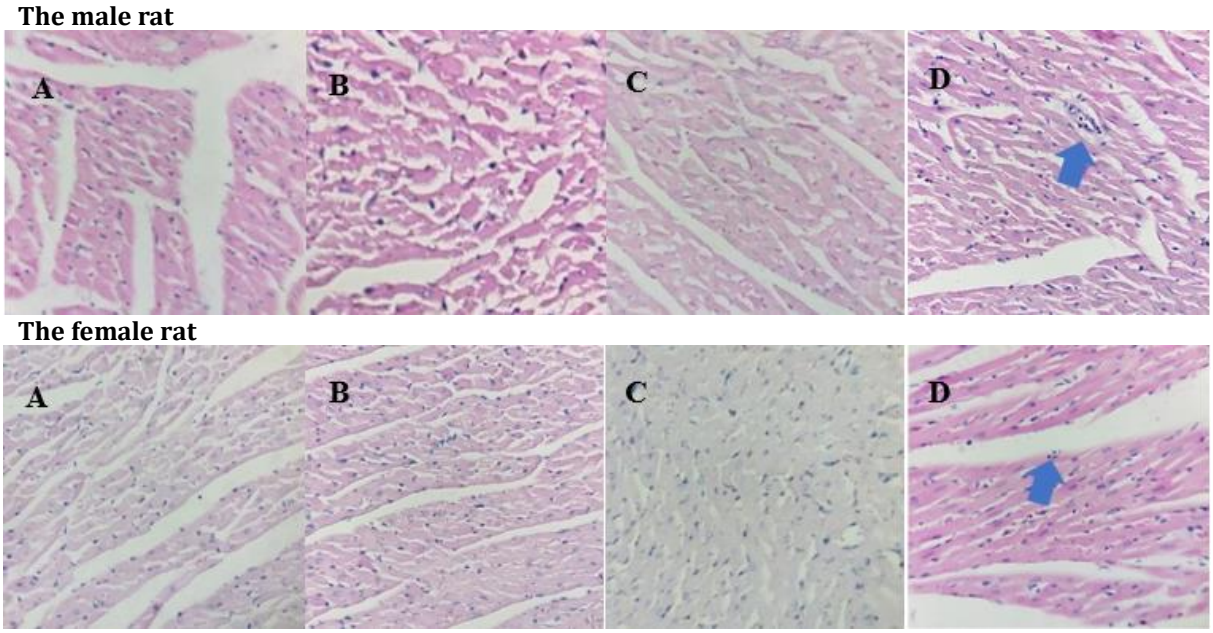


(A) normal group; **(B)** 50 mg/kg; **(C)** 100 mg/kg: **(D)** 200 mg/kg (treated group of turmeric extract). (H&E x400)

Figure 3. Spleen histology

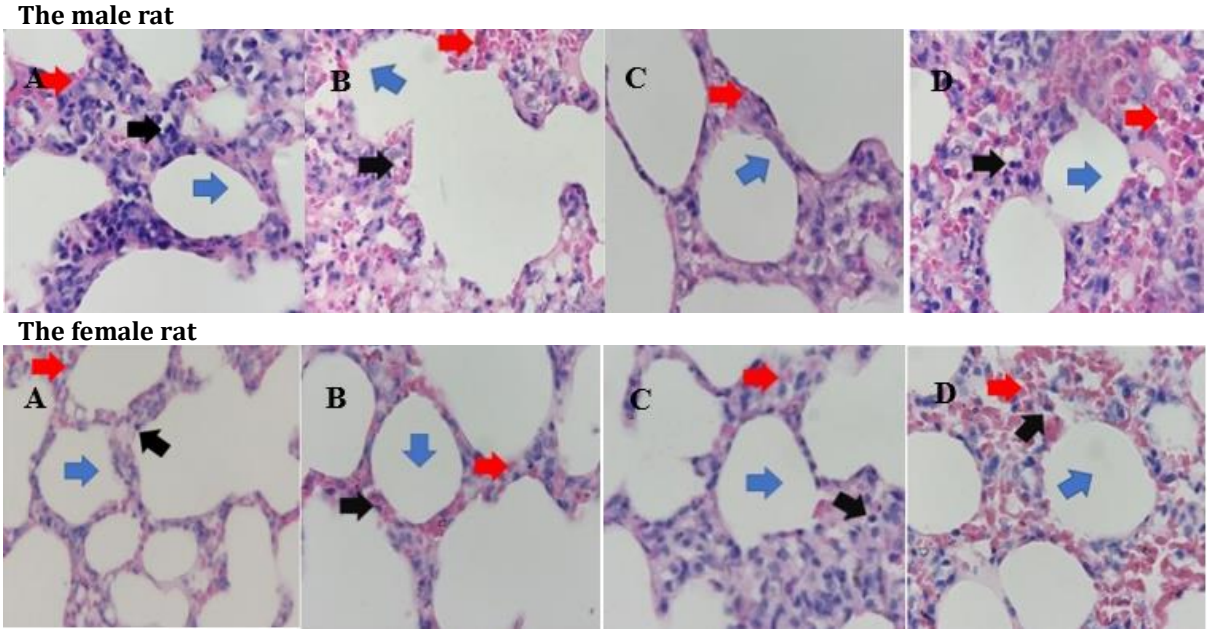
malnutrition, aging tissue, lack of oxygen, and the presence of intoxication (Corwin & Patofisiologi, 2001).

The toxic impact of a compound on the kidneys is usually in the form of damage to the tubules and glomerulus. The shrinkage of the



(A) normal group; **(B)** 50 mg/kg; **(C)** 100 mg/kg: **(D)** 200 mg/kg (treated group of turmeric extract). Blue arrow: inflammatory cell infiltration. (H&E x400)

Figure 4. Heart histology

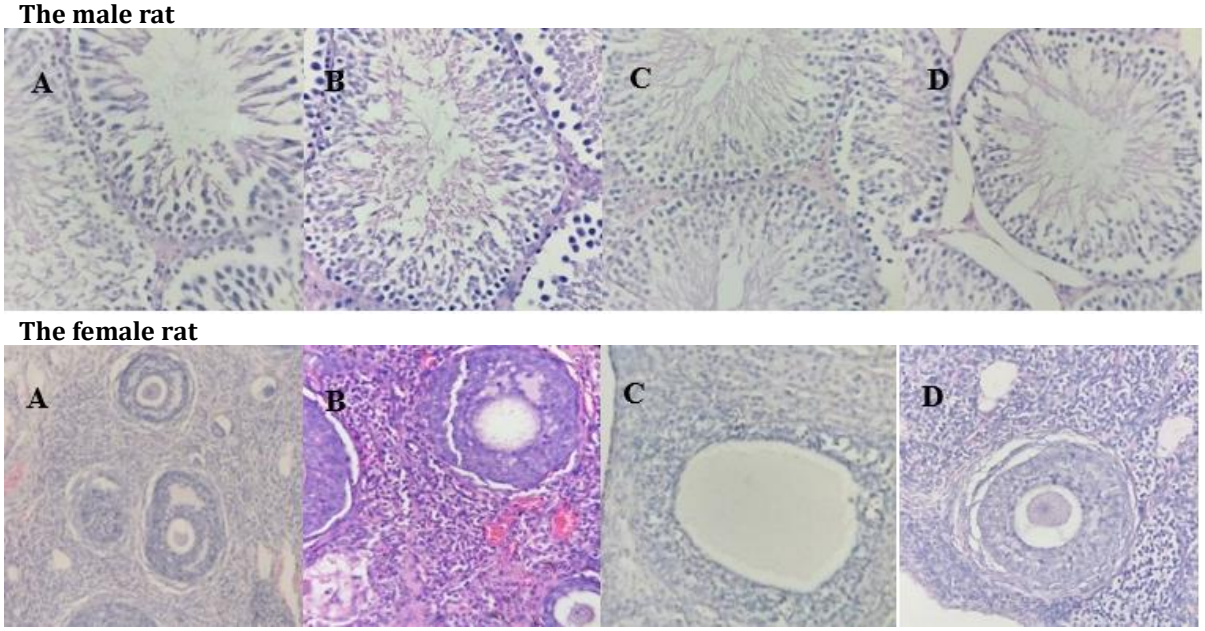


(A) normal group; **(B)** 50 mg/kg; **(C)** 100 mg/kg: **(D)** 200 mg/kg (treated group of turmeric extract). Blue Arrow: alveolar; Black arrow: inflammatory cells; Red arrow: erythrocytes. (H&E x400)

Figure 5. Lungs histology

glomerular size caused the Bowman space to widen slightly (Olagunju et al., 2009), but in this study, the glomerular distance had normal spacing. Furthermore, there were a few necroses observed in the normal and treatment groups, but the tissue was generally normal because the curcumin contained in turmeric has antioxidants that reduce damage to the kidneys (Sharma et al., 2011).

The spleen is one of the body's defense organs and functions to filter blood and coordinate



(A) normal group; **(B)** 50 mg/kg; **(C)** 100 mg/kg; **(D)** 200 mg/kg (treated group of turmeric extract). (H&E x400)

Figure 6. Testes histology

immune responses. Histopathologically, it consists of 2 parts, namely the stroma and parenchyma. The stromal consists of the capsule and trabeculae, while the splenic parenchyma consists of white pulp which is the immune system to fight infection, and the red pulp is responsible for removing unnecessary materials from the blood such as damaged red blood cells (Matheos, 2014). The results showed that all groups had a well-aligned white pulp indicating normal tissue.

At a dose of 200 mg/kg BW, there was an infiltration of inflammatory cells, while no damage was observed in the normal group and at doses of 50 and 100 mg/kg BW (Chang, 2015). Curcumin contained in turmeric is an antioxidant that suppresses the formation of atherosclerosis and reduces cholesterol levels in the blood. It also reduces lipid peroxidation and oxLDL formation, thereby suppressing the inflammatory response and progression of atherosclerosis (Elidiya et al., 2019).

Inflammatory cells were found in both the normal and the dose group of 50, 100, and 200 mg/kg BW, but the results were included in the mild category and the cells were generally normal. Curcumin has antioxidant and anti-inflammatory activity (Higdon et al., 2005), hence, it inhibits the synthesis of the enzymes cyclooxygenase-2, 5-lipoxygenase, nitric oxidase, and also affects arachidonic acid metabolism along with prostaglandin production (Venkatesan et al., 2007).

In the male rats, all groups were normal and doses of 50, 100, and 200 mg/kg BW showed active spermatogenesis, absence of fibrosis, necrosis, and inflammation (Júnior et al., 2014). Curcumin is used to protect against the effects of cadmium in inducing damage to spermatogenic cells, decreasing the number of spermatozoa, and reducing testosterone levels in rats (Salama & EL BAHR, 2007). In the female rats, all groups both normal and doses of 50, 100, and 200 mg/kg BW have a score of 10 indicating normal cells, with no bleeding and inflammation (Asfour et al., 2015). In the testes and ovaries, there was no inflammation because the curcumin contained in turmeric is anti-inflammatory, and inhibits the cyclooxygenase (COX-2) enzyme (Sobolewski et al., 2010), hence, the administration of turmeric extract does not affect the ovaries and testes.

CONCLUSION

The administration of turmeric rhizome extract for 28 days at doses of 50, 100, and 200mg/kg BW does not cause toxicity to liver and kidney biochemistry. In addition, it has no toxic substances that might potentially cause anemia or other abnormalities. The histopathological examination of the heart, lungs, liver, kidney, as well as the ovaries, and testes of the female and

male rats respectively showed normal results, indicating that the turmeric extract used has no toxic effect.

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REFERENCES

Anugerah, A., Pratiwi, W. R., & Wahyuningsih, M. S. H. (2018). Pengaruh Kombinasi Ekstrak Tithonia diversifolia (Hemsley) A.Gray dan Curcuma domestica Val. pada Fungsi Hati dan Ginjal Tikus Model Kanker. *Majalah Farmaseutik*, *14*(1), 1.

Asfour, V., Polson, A., Varma, R., & Menon, P. (2015). *Clinical Obstetrics, Gynecology, and Reproductive Medicine Evaluating ovarian preservation after ovarian torsion using the ovarian preservation score and tissue necrosis score*. *January*.

Balaji, S., & Chempakam, B. (2010). Toxicity prediction of compounds from turmeric (Curcuma longa L). *Food and Chemical Toxicology*, *48*(10), 2951–2959.

Chang, D. (2015). *Cardiac allograft immune activation : current perspectives*. 13–22.

Cheville, N. F. (1999). *Introduction to Veterinary Pathology, Iowa State*. University Press. PP.

Corwin, E. J., & Patofisiologi, B. S. (2001). Edisi 1. *Jakarta: EGC*.

Devi, F. L. (2015). Kadar Kreatinin, Asam Urat, dan Struktur Histologis Ren Tikus Putih *(Rattus norvegicus Berkenhout, 1769)* Dengan Induksi Jelantah dan Perlakuan Jus *Sargassum spp.* Universitas Gadjah Mada.

Elidiya, A., Ayu, P. R., Graharti, R., Kedokteran, F., & Lampung, U. (2019). *Efek Curcumin Sebagai Pengobatan Penyakit Jantung Koroner Effect Of Curcumin As Coronary Heart Disease*. *9*, 244–248.

Hasimun, P., Mulyani, Y., Sulaeman, A., & Embas Sara, D. A. (2019). Prevention of Hypertension and Arterial Stiffness by Combination of Centella asiatica and Curcuma longa in Rats. *Asian Journal of Biological Sciences*, *12*(2), 173–179. https://doi.org/10.3923/ajbs.2019.173.179

Higdon, J., Drake, V. J., Delage, B., & Howells, L. (2005). Curcumin. *Linus Pauling Institute, Updated*, *11*(07).

Júnior, S. R., Diogo, A., Ii, F., Machado, T., Iii, D. A., Bortoletto, T., Iv, R., & Borges, L. B. (2014). *Testicular histology after intestinal pedicle flap (cecum) apposition in rats Testicular histology after intestinal pedicle flap (cecum) apposition in rats 1 Histologia testicular depois da aposição de um retalho intestinal (ceco) em ratos*. *July*.

Kim, Y., You, Y., Yoon, H. G., Lee, Y. H., Kim, K., Lee, J., Kim, M. S., Kim, J. C., & Jun, W. (2014). Hepatoprotective effects of fermented Curcuma longa L. on carbon tetrachloride-induced oxidative stress in rats. *Food Chemistry*, *151*, 148–153.

Kresnadipayana, D., Subianto, R. H., Faradilla, R., Studi, P., Kesehatan, D. A., Ilmu, F., Budi, U. S., Studi, P., Kesehatan, D. A., Ilmu, F., & Budi, U. S. (2019). *Efek Subkronik Pemberian Ekstrak Etanol Kunyit (Curcuma domestica Val) Terhadap Hati Tikus Galur Wistar dengan Pemeriksaan SGOT dan SGPT Pendahuluan digunakan berupa rimpangnya untuk uji toksisitas adalah hati . Hati merupakan Glutamic Oxalacetic Transa*. 77–85.

Longo, D. L., Fauci, A. S., Kasper, D. L., Hauser, S. L., Jameson, J. L., & Loscalzo, J. (2012). *Harrison's principles of internal medicine* (Vol. 2012). Mcgraw-hill New York.

Matheos, C. (2014). Gambaran Histologik Jaringan Limpa Tikus Putih (Rattus norvegicus) yang Diinfeksi Eschericia Coli Dan Diberi Madu. *Jurnal E-Biomedik*, *1*(2).

Meyer, D. J., & Harvey, J. W. (2004). Veterinary Laboratory Medicine: Interpretation and Diagnosis, Saunders. *St. Louis*.

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., de Ferranti, S., Després, J.-P., Fullerton, H. J., & Howard, V. J. (2015). Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation*, *131*(4), e29–e322.

Mulyani Yani, Sukmawati Ika K, P. T. S. (2017). International Journal for Pharmaceutical Research Scholars (IJPRS) Effect from Repeated Dose of Ethanol Extract of Singawalang Leaves (Petiveria. *International Journal for Pharmaceutical Research Scholars*, *6*(1–4), 20–24.

Olagunju, J. A., Adeneye, A. A., Fagbohunka, B. S., Bisuga, N. A., Ketiku, A. O., Benebo, A. S., Olufowobi, O. M., Adeoye, A. G., Alimi, M. A., & Adeleke, A. G. (2009). Nephroprotective activities of the aqueous seed extract of Carica papaya Linn. In carbon tetrachloride induced renal injured wistar rats: A dose- and time-dependent study. *Biology and*

Medicine, *1*(1), 11–19.

Porwal, M., Khan, N. A., & Maheshwari, K. K. (2017). *Evaluation of Acute and Subacute Oral Toxicity Induced by Ethanolic Extract of Marsdenia tenacissima Leaves in Experimental Rats*.

Salama, A., & EL BAHR, S. (2007). *Effect of curcumin on cadmium-induced oxidative testicular damage in rats*.

Saragih, S. P., Syawal, H., & Rauwaty, M. (2015). Total of Erythrocytes, Haematocrit, and Haemoglobin Changes of *Pangasius hypophthalmus* That Were Immersed In Curcumin Extract and That Were In Infected *BY Aeromonas hydrophila*. 1–14.

Sardjiman, S. (2000). Synthesis of some new series of curcumin analogues, antioxidative, antiinflammatory, antibacterial activities and qualitative-structure activity relationships [Thesis]. Yogyakarta: Universitas Gadjah Mada.

Sharma, V., Sharma, C., Paliwal, R., Pracheta, S. S., & Sharma, S. (2011). Ameliorative effects of Curcuma longa and curcumin on aflatoxin B1 induced serological and biochemical changes in kidney of male mice. *Asian Journal of Biochemical and Pharmaceutical Research*, *1*(2), 338–351.

Sobolewski, C., Cerella, C., Dicato, M., Ghibelli, L., & Diederich, M. (2010). The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. *International Journal of Cell Biology*, *2010*.

Venkatesan, N., Punithavathi, D., & Babu, M. (2007). Protection from acute and chronic lung diseases by curcumin. In *The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease* (pp. 379–405). Springer.

WHO. (2018). monitoring health for the SDGs, sustainable development goals. In *World Health Organization 2018*.

Winarsih, W., Wientarsih, I., Sulistyawati, N. P., & Wahyudina, I. (2012). Uji Toksisitas Akut Ekstrak Rimpang Kunyit pada Mencit : Kajian Histopatologis Lambung, Hati dan Ginjal. *Jurnal Veteriner*, *Vol. 13*(4), 402–409.