Available online at www.ujpronline.com
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)

RESEARCH ARTICLE

HISTOPATHOLOGICAL FEATURES OF MICE LIVER AND KIDNEY ON ORAL ACUTE TESTING OF WHITE RADISH TUBER LYOPHILISATE

Nurzadrina Wahyuddin[1]*, Yuri Pratiwi Utami[2], Noer Fauziah Rahman[3], Andi Rainun Puspa Nodjeng[4], Kezya Ratu Marewa[5]

[1]*Laboratory of Pharmacology and Clinical Pharmacy, Universitas Almarisah Madani, Indonesia.*
[2]*Laboratory of Biology Pharmacy, Universitas Almarisah Madani, Indonesia.*
[3]*Laboratory of Pharmaceutical analysis and medicinal chemistry, Universitas Almarisah Madani, Indonesia.*
[4]*Laboratory of Pharmacology and Clinical Pharmacy, Universitas Almarisah Madani, Indonesia.*
[5]*Laboratory of Pharmacology and Clinical Pharmacy, Universitas Almarisah Madani, Indonesia.*

Article Info:

Article History:
Received: 3 December 2023
Reviewed: 29 January 2024
Accepted: 27 February 2024
Published: 15 March 2024

Cite this article:
Wahyuddin N, Utami YP, Rahman NF, Nodjeng ARP, Marewa KR. Histopathological features of mice liver and kidney on oral acute testing of white radish tuber lyophilisate. Universal Journal of Pharmaceutical Research 2024; 9(1): 22-29. ***http://doi.org/10.22270/ujpr.v9i1.1058***

***Address for Correspondence:**
Nurzadrina Wahyuddin, Laboratory of Pharmacology and Clinical Pharmacy, Universitas Almarisah Madani, Indonesia. Tel: +6281947348269.
E-mail: *apt.nona8890@gmail.com*

Abstract

Background and aim: Radish tuber (*Raphanus sativus L.*) is one of the plant species often consumed by society as a vegetable. Currently, its use is beginning to expand as a medicinal plant, including its use for diabetes, pharyngitis, sinusitis, cardiac fibrosis, diuretics, and bacterial infections. This study aims to determine the histopathology of the liver and kidney organs in male albino mice (*Mus musculus*) by testing the oral pungency of white radish (*Raphanus sativus L.*).
Methods: This experimental study used 12 mice weighing 25–30 grams, divided into 4 treatment groups: Group 1 was administered aquadest, Group 2 received a dose of 400 mg, Group 3 received 800 mg, and Group 4 received 1600 mg of the medication. The treatment was carried out for 14 days, and then the mice were euthanized to see the histopathology of the hepatic and kidney tissues.
Results: In the histopathology of the liver and kidney in treatment groups 1, 2, 3, and 4, the results were normal, mild, moderate, and severe damage, respectively. In kidney histopathology, there are signs of damaged tissue, such as bleeding, edema, and inflamation cells.
Conclusion: It can be concluded that the higher the dose of white radish lyophilizate, the greater the potential to cause toxicity to the liver and kidney organs.
Keywords: hepar, histopathology, kidney, white radish lyophilizate.

INTRODUCTION

Radish tuber (*Raphanus sativus* L.) is one type of plant that is often consumed as a vegetable by the community. Currently, its use is starting to expand as a medicinal plant, including being used for diabetes mellitus, pharyngitis, sinusitis, cardiac cirrhosis, diuretics, and bacterial infections. The content in radish tubers includes active substances such as raphanin, flavonoids, polyphenols, and saponins. Recent research by Rahman found that radish tubers have the potential to overcome insomnia, in synergy with previous research reporting that glucosinolates in the *Brassica* genus can provide neuroprotective effects through modulation of inflammatory responses in the central nervous system. Many studies have discussed the sedative mechanism in radish tubers. The main pharmacological mechanism of action shared by most sedatives is to act through the neurotransmitter Gamma Aminobutyric Acid (GABA) or through stimulation of GABA-AA receptors[1].

In addition, radish tubers have antibacterial properties. Phenol compounds have antibacterial properties that cause bacterial cell walls to be damaged, inhibiting cell growth and ultimately leading to cell death. This is achieved by denaturing proteins, rupturing cell membranes, and stimulating enzymes. The way that this compound works is by interfering with the formation of the peptidoglycan in the bacterial cell wall, which prevents the cell from strengthening fully.

The use of herbal remedies made from plant extracts to treat a wide range of clinical conditions is growing[2].

In actuality, using medicinal herbs can have unfavorable effects. This situation can be caused by products that may be toxic or contaminated. The World Health Organization (WHO) states that the toxic effect of a compound depends on the dose and duration of drug use. Therefore, it is important to know the safety

level of nutritious plants so that they can be used as raw materials for herbal medicines[3] For this purpose, a toxicity test examination is needed. The toxicity test conducted in this study was an acute toxicity test. According to Sargent *et al.*[4], the oral acute toxicity test is a test to detect toxic effects that appear after administration of test preparations with repeated doses given orally to test animals during part of the animal's life, but not more than 10% of the entire animal's life. The purpose of the oral acute toxicity test is to obtain information on the toxic effects of substances that are not detected in the acute toxicity test, information on the possibility of toxic effects after repeated exposure to the test preparation over a period of time, information on the dose that does not cause toxic effects (No Observed Adverse Effect Level/NOAEL), and study the cumulative effects and reversibility of the substance[4].

Acute toxicity of a compound can be found in the blood and organ tissues through which the compound passes. In this observation, what is considered in the acute toxicity test is the function of the hepatic organ after the administration of white radish lyophilisat for 14 days. Hepar is an organ that has the potential to be damaged by various chemicals and the environment because of its function in metabolizing and detoxifying chemicals that enter the body. Administration of toxic compounds can cause changes such as hemorrhage, congestion, degeneration to necrosis[5].

The liver is an organ that has the function of detoxifying compounds, so it has a significant risk of experiencing damage or hepatotoxicity from compounds that are exposed to the body[6]. The parameters that can be used in the examination are SGOT (Serum Glutamic Oxaloacetic Transaminase) and SGPT (Serum Glutamic Pyruvic Transaminase). In addition, the kidney is also a target organ for testing toxic effects because the kidney produces urine, which is the main route of excretion of toxins, and has a high blood flow volume. One indicator of kidney damage is an increase or decrease in creatinine levels and kidney histopathology in the body, so that clinical interpretation will be more likely to disrupt kidney function[7]. In this study, we investigated the acute toxic effects of white radish tuber lyophilisate *in vivo* on the liver and kidney of mice using varying doses.

MATERIALS AND METHODS

The sample used in this study was white radish (*Raphanus sativus* L.) in a fresh state taken in Malino, Tinggimoncong District, Gowa Regency, South Sulawesi Province.

Methods

This is an experimental study using 12 mice weighing 25–30 grams, divided into 4 treatment groups: group 1 was given aquadest, group 2 was given a dose of 400 mg, group 3 was given a dose of 800 mg, and group 4 was given a dose of 1600 mg. The treatment was carried out for 14 days, and then the mice were euthanized to see the histopathology of the hepatic and kidney tissues. The health research Ethics Committee Sekolah Tinggi Ilmu Farmasi Makassar with ethical approval number: 082/EC.1.1.B/I/KEPK/2022.

Preparation of sample

White radish tubers weighing as much as 1 kg (1,000 g) were wet-sorted beforehand and then cut into small pieces. Next, the sample was blended so as to produce a filtrate (juice), then filtered and separated the filtrate (juice) and residue.

Freeze-drying method

The obtained white radish filtrate was put into the freezer, then dried using freeze drying at -55ºC. The chamber connected to the freeze-drying rubber was opened, and then the pressure variation (P) of 0.1 MB and time (L) were set for 24 hours. Freeze drying is done until brownish-white flakes are formed. The lyophilizate obtained was then weighed with an analytical balance, and 50 g was obtained.

Animal test treatment

The male white mice used in this study were 12 male white mice. The test animals were acclimatized for 7 days before the study. After acclimatization, mice were grouped according to treatment and placed in clean cages that had been cleaned every 3 days. Furthermore, male mice were orally given Aquadest to group 1 as a healthy control, group 2 at a dose of 400 mg, group 3 at a dose of 800 mg, and group 4 at a dose of 1600 mg as a positive control. Dose selection is based on previously conducted studies using the same dose on anti-insomnia activity testing. It is therefore continued to test the safety of the dose used. The mice were given white radish lyophilizate according to a predetermined dose using a cannula, and administration was carried out 1 x 24 hours for 14 days. Sampling of hepatic organs was carried out on the 15th day of treatment. Mice were taken to the Maros Veterinary Center in Maros District of South Sulawesi Province for histopathology testing.

RESULTS AND DISCUSSION

Histopathology results of hepar with healthy control administered aquadest. In the observations obtained from healthy controls, the results showed that the hepatocytes and sinusoids were still good and did not experience narrowing or widening, which did not have a significant effect on the hepatic mice. Hepatocytes are the main cells that form hepatic tissue, which are parenchymatous cells that make up the hepatic mass. It plays a role in metabolic processes, detoxification, bile production and for storing nutrients. Hepatocytes also have the ability to regenerate themselves when damaged[8]. If hepatic cells are damaged by several factors, there will be a series of morphological changes in hepatic cells that are sublethal, namely degenerative or lethal in the form of necrotic.

Sinusoids in mice hepatic histology refer to the structure of blood vessels that are between hepatic cells (Hepatosid), that is an important part of the hepatic structure. Sinusoids play a role in the exchange of substances between blood and hepatic cells.

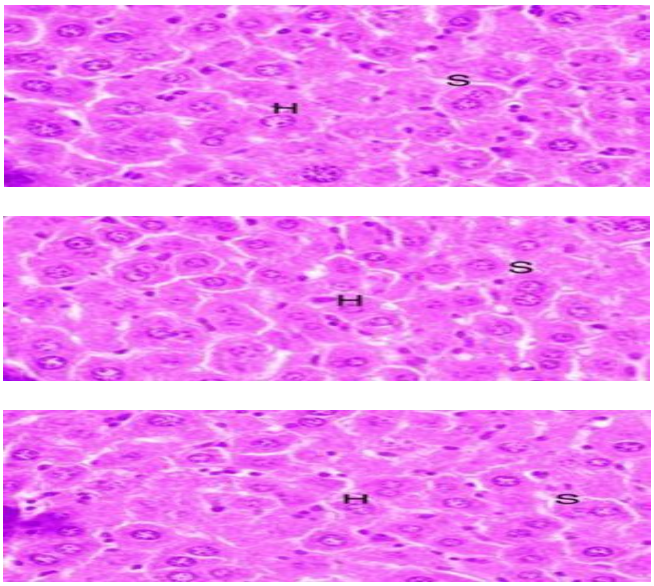

Figure 1: Group 1 histopathological of the hepatic was given aquadest.

In the histology picture of mice hepar, sinusoids are seen as spaces surrounding hepatocytes that play a role in metabolic and detoxification processes. Several histopathological studies of mice showed changes in sinusoids, such as widening of sinusoids and an increase in the number of cells that degenerate and necrosis due to various treatments[9]. The results of group 2 treatment with a 400 mg dose caused mild damage characterized by the appearance of edema between hepatocid cells. In this group, the damage is more visible and is characterized by sinusoids experiencing cell dilation or swelling. According to the results of hepatic histopathology, mice experience edema or swelling, which is due to the accumulation of fluid in the tissue.

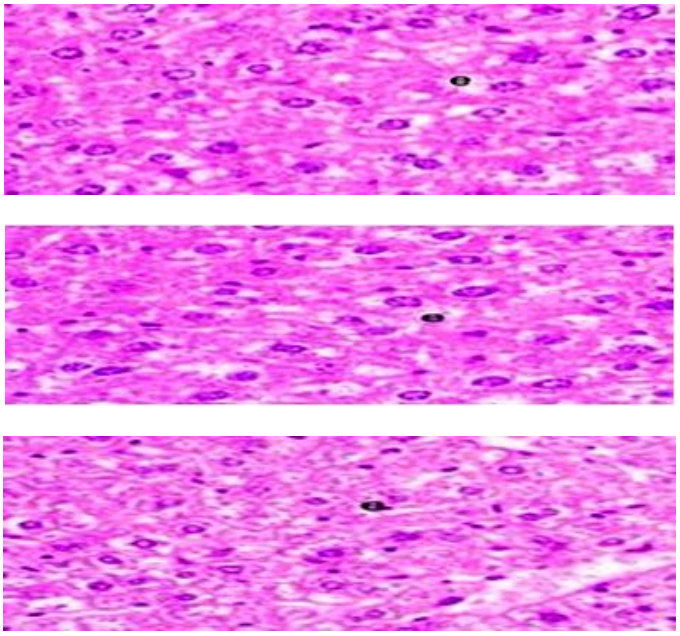

Figure 2: Group 2 histopathological of the hepatic was given dose of 400 mg.
e: intercellular edema of hepatocytes

Edema is caused by bacterial infiltration into the liver, which causes the cells to be irritative so that they swell. According to Irfai, edema is a disorder in the liver characterized by an increase in the size and weight of the liver where there is swelling and thickening of one of the liver lobules due to exposure to doses or extracts[10]. The results of group 3 treatment with an 800 mg dose caused moderate damage characterized by edema, inflammation, cell degeneration, hemorrhage, and fibrin. Also seen in liver damage to hepatocyte cells, sinusoids appear dilated and appear to increase cells that degenerate or hemorrhage. The occurrence of hemorrhage is due to the rupture of blood vessels in the sinusoids, which causes blood to flow out of the blood vessels. Blood flow in the sinusoids comes from the terminal branches of the portal vein and hepatic artery.

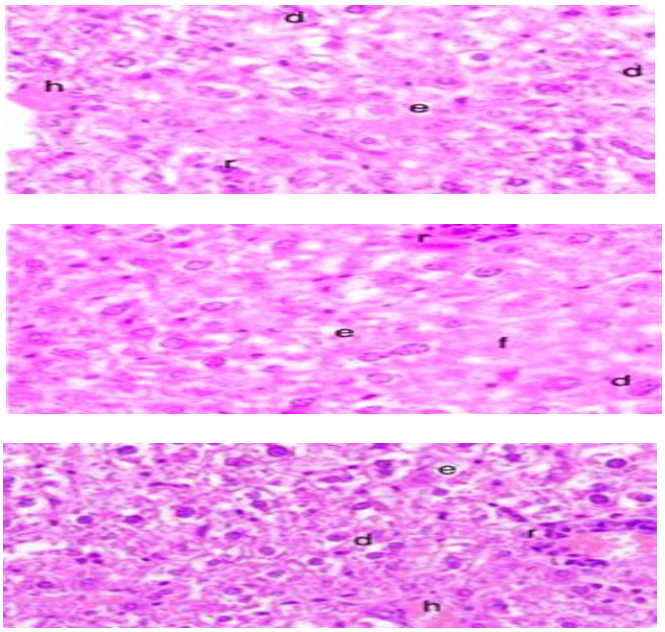

Figure 3: Group 3 histopathological of the hepatic was given dose of 800 mg.
e: edema between hepatocyte cells; r: inflammatory cells; f: fibrin; h: hemorrhage; d: cell degeneration

Hemorrhage is also the release of blood from blood vessels, namely red cells, especially in hematoxylin-eosin staining[11]. The presence of inflammatory cells found in the liver indicates that an inflammatory process occurs[12]. Inflammatory cells are the response of the body's defense mechanism to damage that affects tissues, both locally and throughout the body. If damming continues, there is a disturbance in blood circulation that can cause degeneration and liver necrosis. One of the causes of degeneration in the liver occurs due to the accumulation of toxic materials and other metabolites in the liver. Hydrophilic degeneration is a reversible cellular lesion with more severe intracellular accumulation when compared to parenchymal degeneration.

The etiology is considered the same as parenchymal degeneration, except that the intensity of pathologic stimuli is more severe and the period of exposure is longer. Parenchymal degeneration is the mildest degeneration where there is swelling and turbidity of the cytoplasm[13]. The results of group 4 treatment with a 1600 mg dose caused severe damage. The presence of edema between hepatocyte cells, inflammatory cells, hemorrhage, fibrin, fibrous cells, and necrotic cells.

Necrosis is the death of cells or tissues in living organisms. Necrosis can occur due to toxic materials, microorganism activity, feed deficiency, and metabolic disorders. Cells or tissues that undergo necrosis show changes in the nucleus and cytoplasm, characterized by cytoplasm containing eosinophilic deposits in the form of proteins.

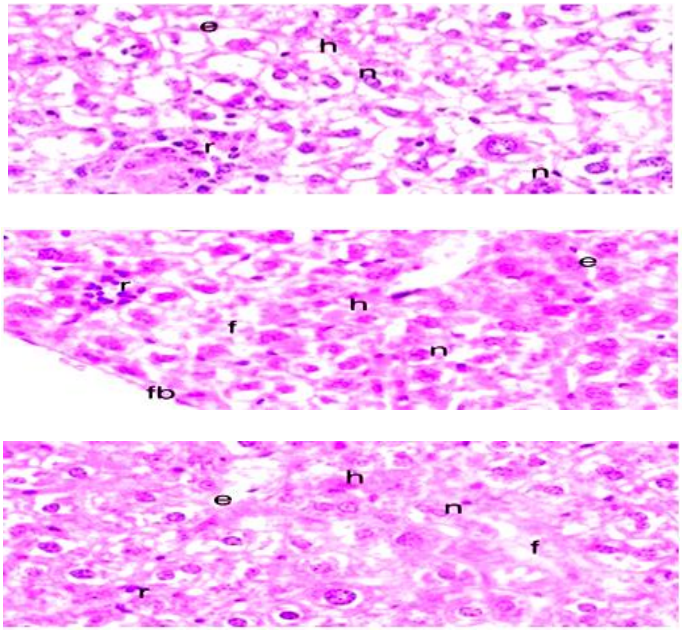

Figure 4: Group 4 histopathological of the hepatic was given dose of 1600 mg.
e: edema between hepatocyte cells; r: inflammatory cells; f: fibrin; h: hemorrhage; d: cell degeneration

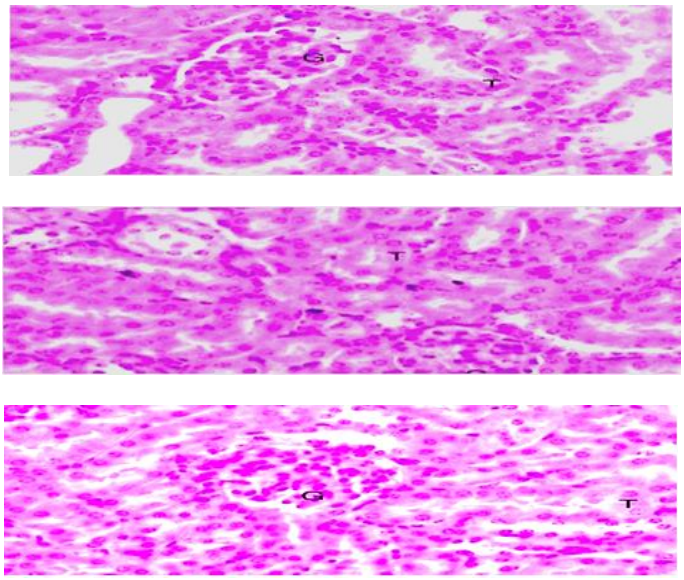

Figure 5: Group 1 histopathological of the kidney was given aquadest.
G : glomelurus; T : Tubules

Microscopically, the nucleus turns black (pycnosis), the nucleus is granulated or divided into fragments (cariorexis), and the nucleus no longer takes on much color or is pale and disappears (cariolysis). This cell damage occurs because more and more chemicals enter the body, where the liver will work harder to detoxify the compounds that enter the body[14].

Histopathology results of kidneys with healthy controls administered aquadest. In the observations obtained from healthy controls, the results indicated the presence of glomeruli and tubules, which are the first steps in urine formation. Water, ions, food substances, and solutes are removed from the blood and sent to the proximal tubules[15].

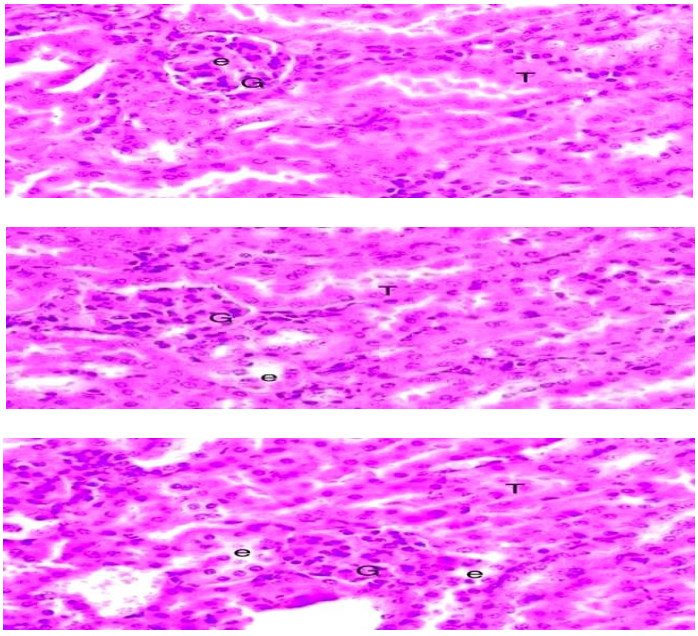

Figure 6: Group 2 histopathological of the kidney was given dose of 400 mg
G : glomelurus; **T** : tubules **e:**edema

Blood cells and some large proteins or negatively charged proteins, such as albumin, are effectively retained due to the size and charge of the glomerular filtration membrane. The main goal of glomerular filtration is the formation of primary filtrate in the proximal tubule. The pressures that play a role in the glomerular filtration rate process are glomerular capillary blood pressure, plasma colloid oncotic pressure, and Bowman's capsule hydrostatic pressure. This increased glomerular blood pressure pushes fluid out of the glomerulus to enter the Bowman's capsule along the glomerular capillaries and is the main force that produces glomerular filtration. It can be seen that the results of renal histopathology with the administration of a dose of 400 mg caused damage to the kidneys, characterized by the discovery of edema cells, glomeruli, and tubules. All metabolic processes in the body will end with the excretion process in the kidneys.

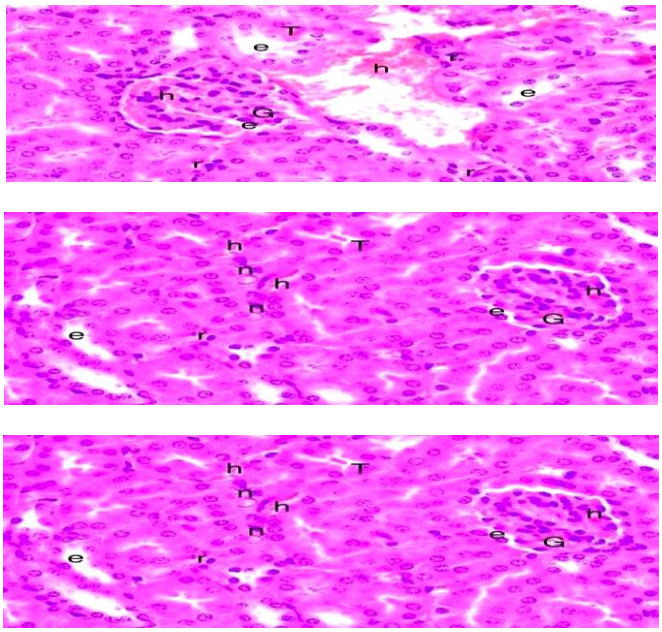

Figure 7: Group 3 histopathological of the kidney was given dose of 800 mg.
G: glomerulus; T: Tubule; n: necrotic; r: inflammatory cells; h: hemorrhage

Metabolic substances will undergo filtration in the glomerulus and reabsorption in the proximal tubulus, ansa henle, and distal tubules, which will then continue to the tubule collectivus to be excreted as urine. All of these processes occur in the kidney and can cause histopathological changes caused by dosing radish[16]. In addition to the glomerular appearance, there was also edema in the bowman space edema in the bowman space occurred due to impaired renal blood circulation, which was thought to be the effect of the administration of lyophilizate 400 mg. Edema that occurs is characterized by the expansion of the Bowman space because it is filled with fluid. Disruption of the normal balance of blood, interstitial, and lymphatic fluid complements is also a cause of edema. Fat accumulation occurs due to decreased cellular enzyme activity, resulting in the inability of non-adipose tissues to metabolize some lipids. In addition, the presence of toxic substances cause's lipoproteins not to form because protein production is disrupted[17]. It can be seen that the results of histopathological observations in the control group given a dose of 800 mg showed moderate damage to the kidneys and were characterized by edema cells, hemorrhage, inflammatory cells, and necrosis[18-21]. Changes in the form of hemorrhage were seen in the histopathological picture of all treatments. Hemorrhage or bleeding is a condition characterized by blood coming out of blood vessels due to damage to their walls, which is pathologically characterized by the presence of red blood cells outside the blood vessels or in the tissue[22,23].

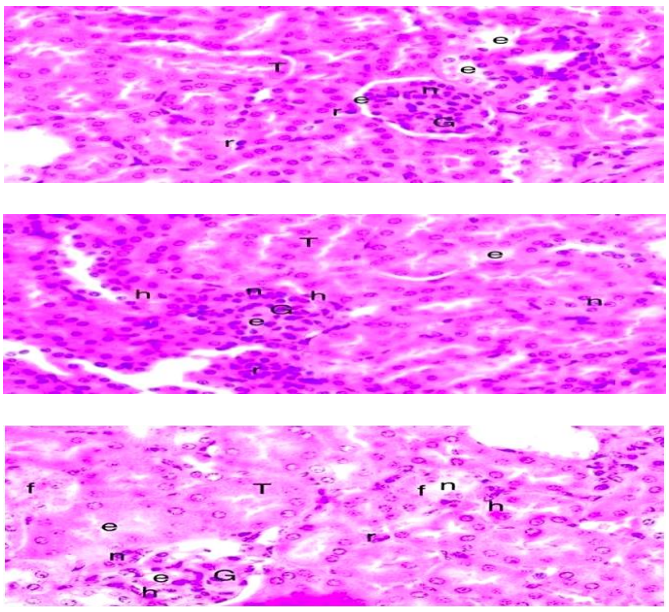

Figure 8: Group 4 histopathological of the kidney was given dose of 1600 mg.
e: edema; G: glomerulus; T: Tubule; n: necrotic; r: inflammatory cells; h: hemorrhage;
f.: fibrin

High blood flow to the kidneys and increased excreted products, followed by the reabsorption of water from the tubular fluid, are the main factors involved in sensitizing the kidneys to toxic substances[18]. In test animals 1, 2, and 3, there are inflammatory cells. According to Taek *et al*.[13], inflammation can occur due to the influence of parasites, bacteria, acids, and strong bases. Inflammatory cells are the body's defense mechanism in response to damage that affects the tissue[13].

Other factors that cause kidney damage are environmental factors and feed and drinking factors that are not hygienically provided so that they can cause health problems. Inappropriate oral treatment is also a factor that causes damage to the kidneys. Giving oral treatment can cause test animals to experience stress that triggers blood sugar, which causes an increase in kidney metabolism and results in damage to the kidneys[19].

Limitations of the study

The limitation in this study is that in the process of making slides of liver and kidney organs, researchers are not involved because of the rules in the laboratory, therefore researchers only know the description of the process of making slides and parts of organs that will be subjected to histopathological testing. It is important of course to be directly involved in the manufacturing process, so that researchers can describe in detail and clearly the flow in this study, up to the results obtained during this study.

CONCLUSION

Histopathological examination of rat liver and kidneys during the acute toxicity test showed that the highest dose of white radish tuber lyophilisate caused significant damage to liver cells and kidney cells.

ACKNOWLEDGEMENTS

The author would like to extend our thanks to Department Pharmacology and Clinical Pharmacy of University Almarisah Madani (UNIVERAL) for supporting this study.

AUTHORS CONTRIBUTIONS

Wahyuddin N: conceptualization, data curation, methodology, writing original draft. **Utami YP:** Data curation, writing, review, and editing. **Rahman NF:** Writing-review and editing. **Nodjeng ARP:** Writing-review and editing. **Marewa KR:** Writing, review, and editing. All authors revised the article and approved the final version.

DATA AVAILABILITY

Data will be made available on request.

CONFLICT OF INTEREST

None to declare.

REFERENCES

1. Rahman NF, Wahyuddin N, Marwati M. *In-silico* study of white radish tuber compounds (*Raphanus sativus* l.) as a candidate for anti-insomnia. Maj Farm Pharmacol 2023; 27: 10–14. *https://doi.org/10.20956/mff.v27i4.30048*
2. Mehana EE, Meki ARMA, Fazili KM. 2012. Ameliorated effects of green tea extract on lead induced liver toxicity in rats. Exp Toxicol Pathol 2012; 64: 291–295. *https://doi.org/10.1016/j.etp.2010.09.001*
3. Hernández-Sánchez LY, González-Trujano ME, Moreno, DA, *et al*. Pharmacological evaluation of the anxiolytic-like effects of an aqueous extract of the *Raphanus sativus* L. sprouts in mice. Biomed. Pharmacother 2023; 162: 114579. *https://doi.org/10.1016/j.biopha.2023.114579*
4. Sargent EV, Naumann BD, Schwartz CS. Occupational toxicology testing, in: McQueen, C.A. (Ed.), Comprehensive Toxicology (Second Edition). Elsevier, Oxford 2010;65–85. *https://doi.org/10.1016/B978-0-08-046884-6.00310-9*
5. Andjelkovic M, Buha Djordjevic A, Antonijevic E, *et al*. Toxic effect of acute cadmium and lead exposure in rat blood, liver, and kidney. Int J Environ Res Public Health 2019; 16: 274. *https://doi.org/10.3390/ijerph16020274*
6. Abou Seif HS. Physiological changes due to hepatotoxicity and the protective role of some medicinal plants. Beni-Suef Univ J Basic Appl Sci 2016; 5: 134–146. *https://doi.org/10.1016/j.bjbas.2016.03.004*
7. Faria J, Ahmed S, Gerritsen KGF, Mihaila SM, Masereeuw R. Kidney-based *in vitro* models for drug-induced toxicity testing. Arch Toxicol 2019; 93: 3397–3418. *https://doi.org/10.1007/s00204-019-02598-0*
8. Michalopoulos GK, Bhushan B. Liver regeneration: Biological and pathological mechanisms and implications. Nat Rev Gastroenterol Hepatol 2021; 18: 40–55. *https://doi.org/10.1038/s41575-020- 0342-4*
9. Bhat N, Kalthur SG, Padmashali S, Monappa V. Toxic effects of different doses of cyclophosphamide on liver and kidney tissue in Swiss albino mice: A Histo-pathological study. Ethiop J Health Sci 2018; 28. *https://doi.org/10.4314/ejhs.v28i6.5*
10. Irfai I. 2013. Effectiveness of giving Kenikir (*Cosmos caudatus* kunth) on carcass weight, digestive organs, liver and cholesterol in meat of village Chickens (*Gallus gallus* domesticus).
11. Furqon, Abdullah Al, Riau, Morina, Luskityowati, Iesje, 2023. Histopathology of kidney and intestine of Tilapia (*Oreochromis niloticus*) fed with herb fermented feed after challenge of *Streptococcus agalactiae*. Asian J Aquat Sci 2023; 6: 293–301.
12. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 2017; 9: 7204–7218. *https://doi.org/10.18632/oncotarget.23208*
13. Taek AY, Ndaong NA, Gaina CD. Histopathological description of the heart of male white rats (*Rattus norvegicus*) post administration of local NTT better (*Momordica charantia* L.) fruit infusa extract. J Vet Nusant 2020; 3: 89–96. *https://doi.org/10.35508/jvn.v3i2.3412*
14. Maftuch M, Sanoesi E, Farichin I, *et al*. Histopathology of gill, muscle, intestine, kidney, and liver on Myxobolus sp.-infected Koi carp (*Cyprinus carpio*). J Parasit Dis Off Organ Indian Soc. Parasitol 2018; 42: 137–143. *https://doi.org/10.1007/s12639-017-0955-x*
15. Benton WW, Lindsay DM, Rubino CM, Villanueva RCQ. Chapter 112-Adverse drug reactions and drug–drug interactions, in: Fuhrman BP, Zimmerman JJ (Eds.), Pediatric Crit Care 2006; (Third Edition). Mosby, Philadelphia 1678–1703. *https://doi.org/10.1016/B978-032301808-1.50115-2*

16. Tojo A, Kinugasa S. Mechanisms of glomerular albumin filtration and tubular reabsorption. Int J Nephrol 2012, 481520. *https://doi.org/10.1155/2012/481520*
17. Engin AB. 2017. What is lipotoxicity? in: Engin, A.B., Engin, A. (Eds.), Obesity and Lipotoxicity, advances in experimental medicine and biology. Springer International Publishing, Cham 2017; 197–220. *https://doi.org/10.1007/978-3-319-48382-5_8*
18. Basile DP, Anderson MD, Sutton TA. Pathophysiology of acute kidney injury. Compr Physiol 2012; 2, 1303–1353. *https://doi.org/10.1002/cphy.c110041*
19. Kellum JA, Romagnani P, Ashuntantang G, Ronco C, Zarbock A, Anders HJ. 2021. Acute kidney injury. Nat Rev Dis Primer 2021; 7: 1–17. *https://doi.org/10.1038/s41572-021-00284-z*
20. Gamba M, Asllanaj E, Raguindin PF, *et al*. Nutritional and phytochemical characterization of radish (*Raphanus sativus*): A systematic review. Trends Food Sci. Technol 2021; 113, 205–218. *https://doi.org/10.1016/j.tifs.2021.04.045*
21. Levenbrown Y, Costarino AT. Chapter 17 - Edema, in: Oh W, Baum M. (Eds.), Nephrology and Fluid/Electrolyte Physiology (Third Edition). Elsevier, Philadelphia 2019; 271–290. *https://doi.org/10.1016/B978-0-323-53367-6.00017-0*
22. Munford RS, Pugin J. Normal responses to injury prevent systemic inflammation and can be immunosuppressive. Am J Respir Crit Care Med 2001; 163: 316–321. *https://doi.org/10.1164/ajrccm.163.2.2007102*
23. Sharma P. Clinical biochemistry of hepatotoxicity. J Clin Toxicol 2014; 04. *https://doi.org/10.4172/2161-0495.S4-001*