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TOXICITY TEST OF *Eusideroxylon zwageri* BARK EXTRACT BASED ON KIDNEY HISTOPATHOLOGY OF BLEEDING AND NECROSIS

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ABSTRACT

Background*: Ironwood plant (Eusideroxylon zwageri) can potentially be an herbal medicine that accelerates wound healing because of the antioxidant compounds contained in it. The dominant compounds contained in ironwood bark are flavonoids 30.48 mgCE/g, phenolics 31.28 mgCE/g, and proanthocyanidins 183.3 mgCE/g. Before used as an herbal medicine, it is necessary to do an acute toxicity test on ironwood bark at doses of 1.250 mg/kgBW, 2.750 mg/kgBW, and 4.750 mg/kgBW to consider the right dose. Acute toxicity tests can be seen using histopathological appearance in the kidney based on bleeding and necrosis.* ***Purpose:*** *Determine the toxic effects of giving Eusideroxylon zwageri doses of 1.250 mg/kgBW, 2.750 mg/kgBW, and 4.750 mg/kgBW on the kidneys of Wistar rats (Rattus norvegicus) based on bleeding and necrosis in histopathological appearance.* ***Methods****: Research is true experimental and posttest-only with control design. The study was divided into a control group given aquades and a treatment group given ironbark extract dose of 1.250 mg/kgBW, 2.750 mg/kgBW, and 4.750 mg/kgBW as much as 1 ml given 2 times a day orally for 14 days.* ***Results:*** *The percentage of histopathological appearances of bleeding and necrosis score is 1 or mild. Analysis of bleeding and necrosis did not show significant differences of ironwood bark extract doses of 1.250 mg/kgBW, 2.750 mg/kgBW, and 4.750 mg/kgBW.* ***Conclusion:*** *There were no toxic effects of ironwood bark extract doses of 1.250 mg/kgBW, 2.750 mg/kgBW, and 4.750 mg/kgBW on the kidney organs of wistar rats based on bleeding and necrosis in histopathological appearances.*

Keywords : *Bleeding, Ironwood bark extract, Kidney, Necrosis, Toxicity test.*

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INTRODUCTION

Based on data from the World Health Organization in 2018, there are as many as 91% of the population of countries in the Southeast Asian region have used herbal medicines because they are believed to have minimal side effects compared to synthetic drugs.[1,2] One plant that can be used as an herbal medicine is Ulin *(Eusideroxylon zwageri)*.[3]

Ulin is a tropical forest plant native to the island of Borneo which is a wetland resource.[3] Ironwood plants contain high metabolic compounds, especially in the bark of the tree trunk.[4] It is proven that the yield of ironwood bark ethanol extract is 21.63%, where this yield is higher than the yield of other parts such as leaves which only ranges from 4.33-8.99%.[4] Ironwood bark extract contains flavonoids, phenolics, proanthocyanidins, tannins, alkaloids, saponins, and terpenoids.[4]

The dominant compounds contained in ironwood bark are flavonoids 30.48 mgCE / g, phenolics 31.28 mgCE / g, and proanthocyanidins 183.3 mgCE / g.[5] Flavonoids, phenolics, and proanthocyanidins are natural antioxidant compounds derived from plants, function to reduce reactive oxygen species (ROS) so that the amount is controlled and prevents oxidative stress.[6] Nevertheless, there is a high concentration of activity of phenolic and flavonoids is oftenlost and converted into prooxidants.[7] Therefore, it is necessary to test the toxicity of ironwood bark extract before being used as an herbal medicine.[8]

Toxicity tests aim to detect the toxic effects of a substance after acute treatment of the extract for 14 days so that the results can provide information about the dangers of the substance if exposed to humans.[8] One of the parameters that can be observed from the results of this acute toxicity test is the histopathology of the kidney organs, the destruction of the kidneys due to toxic substances can be with changes in histological structure in the form of reversible changes such as bleeding and also irreversible changes such as necrosis.[9]

Bleeding is defined by the discovery of blood cells outside the blood vessels.[9] Bleeding is a pathological reaction that presents as a manifestation of inflammation.[10] Inflamation occurs due to oxidative stress, triggered by high ROS such as hydroxyl radicals that can form as a result of high levels of flavonoid compounds.[7,10,11] Oxidative stress activates NOD-like receptor protein 3 (NLRP3) triggering inflammation in the kidneys.[11] The inflammation then causes vasodilation, and increases interstitial bleeding.[10]

In addition, histopathological changes in the kidneys due to toxic substances can be necrosis.[9] Necrosis is a passive cell death event characterized bycell swelling, lysis and exit of intracellular components into surrounding tissues.[9] Necrosis can be induced by high hydroxyl radicals that cause one of the biomarkers of oxidative stress, namely lipid peroxidation in kidney cell membranes.[12,13] The lipid bilayer on the renal cell membrane is very sensitive to hydroxyl radicals.[13] In the event of prolonged oxidative stress in the kidneys, necrosis-inducing factors occur, namely mitochondrial swelling, dysfunction, failed ATP production, lipid peroxidation, and loss of membrane integrity, as well as the induction of poly(ADP-ribosyl) polyperase (PARP1) as a necrosis promoter.[9.12]

Previously, research was conducted by Salwa (2021) on the toxicity test of ironwood bark extract (*Eusideroxylon zwageri*) concentrations of 5%, 15%, 25%, 35%, 45%, 55%, 65%, 75%, 85%, and 95% in *vitro* against fibroblast cells baby hamster kidney-21 (BHK-21) and there were no toxic effects.[3] Based on the description above, it is necessary to do in *vivo* research on ironbark extract (*Eusideroxylon zwageri*) with concentrations of 25%, 55%, and 95% which are converted to doses of 1,250 mg/kgBW, 2,750 mg/kgBW, and 4,750 mg/kgBW to determine the effect of toxicity ironbark extract on the kidneys of wistar rats *(Rattus norvegicus)* based on the histopathological appearance of hemorrhages and necrosis.

METHODS

This research began with obtaining an ethical permit from the Ethics Commission of the Faculty of Dentistry, Lambung Mangkurat University No. 024 / KEPKG-FKGULM / EC / II / 2023. The research is true experimental design with posttest-only with control design. Toxicity test using male wistar rats (*Rattus norvegicus*) aged 8-12 weeks, body weight 200-250 grams. The number of samples used is 16 calculated based on an unpaired comparative analytical formula, the samples are divided into 4 groups. Then it is randomly selected by simple random sampling.

Table 1. Table of Test Animal Groups

Group	Treatment	Samples
C	Aquades	4
P1	Ironwood bark extract 1,250 mg/kgBW	4
P2	Ironwood bark extract 2,750 mg/kgBW	4
P3	Ironwood bark extract 4,750 mg/kgBW	4
Total		16

Ironwood bark *(Eusideroxylon zwageri)* was taken in Paau Village, Aranio District, Banjar Regency, South Kalimantan. Brownish-red bark is taken without injuring the cambium at a height of >50 cm with a diameter of >60 cm as much as 14 kilograms, cleaned and cut into small pieces using wood planer machine and dried in an oven for about ±4 hours with a 40ºC temperature. After drying, the ironwood stem is made into powder and produced 2.1 kilograms of simplisia. Furthermore, extraction was carried out using the maceration method by means of ironwood bark powder soaked in 96% ethanol solvent. Maceration is carried out for 3x24 hours with solvents that are changed every 24 hours. The extract results were filtered using WH40 filter paper. Then, the maserat is evaporated using a 60ºC rotary evaporator vacuum for 4-6 hours, then heated in a waterbath so that a thick brownish extract of 102 grams is obtained. The extract tested for ethanol-free using potassium dichromate ($K_2Cr_2O_7$). Making concentrations of 25%, 55%, and 95% ironwood bark extract is done by adding aquades as a solvent to obtain a homogeneous mixture. Then, the concentration convert to dose of 1,250 mg/kgBW, 2,750 mg/kgBW, and 4,750 mg/kgBW.

Before being given treatment, rats were satisfied for ±6 hours. Rats in each group were given 1 ml which was 2 times a day orally for 14 days. During treatment, rats are given BR2 feed. On day 15, rats were euthanized with ketamine-xylazine doses of 0.6 ml/200 g for kidney collection. Before euthanasia, rats were

not fed, but still given water for 8-12 hours. Rats that have been sacrificed are buried in the ground to a depth of ±75 cm. The kidney organs that have been taken are fixed with 10% Buffer Neutral Formalin (BNF) followed by making histological slides and then stained by Hematoxylin-Eosin (HE).

Kidney preparations with cross sectional cut will be observed using a microscope in 5 different fields of view with a magnification of 400x. Scoring of bleeding and necrosis appearance is seen in the presence of focal, multifocal, and diffuse lesions. The percentage of bleeding appearance is obtained by calculating the area of bleeding divided by all areas in one field of view multiplied by 100% then calculated the average percentage of 5 fields of view. Then, the number of necrosis cells in one field of view is divided by the total number of cells and multiplied by 100% to then calculate the average percentage of each field of view.

Table 2. Scoring of Bleeding and Nekrosis

Bleeding	**Score**	**Information**
No bleeding	0	Normal
Focal lesions, hemorrhages <25%	1	Mild
Multifocal lesions, hemorrhages 25-50%	2	Moderate
Diffuse lesions, bleeding >50%	3	Severe
Necrosis	**Score**	**Information**
No necrosis	0	Normal
Focal lesions, necrosis <25%	1	Mild
Multifocal lesions, necrosis <50%	2	Moderate
Diffuse lesions, necrosis >50%,	3	Severe

RESULTS

Histopathological changes in bleeding were found in the control group, P1, P2, and P3 in minimal amounts with focal lesions categorized in a score of 1 or mild due to the average percentage of bleeding appearance found <25%.

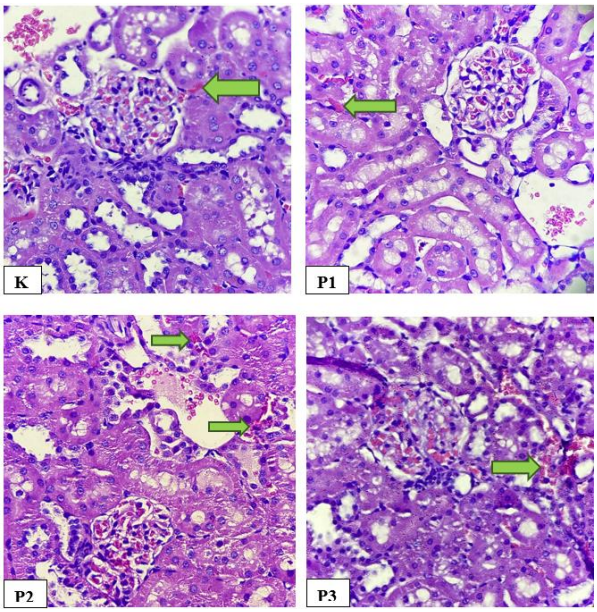

Figure 1. Histopathological appearance of renal tissue hemorrhage. (K) control; (P1) ironwood bark extract dose 1,250 mg/kgBW; (P2) ironwood bark extract dose 2.750 mg/kgBW; (P3) ironwood bark extract dose 4,750 mg/kgBW. Using an *Olympus CX43* microscope (400x magnification, HE; green arrow: hemorrhage)

Table 3. The results of data analysis mean percentage of bleeding appearance and standard deviation

Group	**Bleeding ± SD**	**Score**
C	0.7 ± 0.94^{a}	1
P1	0.5 ± 0.41^{a}	1
P2	1.4 ± 0.74^{a}	1
P3	1.7 ± 0.62^{a}	1

Description: Superscript letters in the same column indicate no significant difference (p>0.05).

The average percentage of histopathological appearance of bleeding were tested for normality using Saphiro-Wilk and obtained p values of group K p = 0.220, group P1 p = 0.995, group P2 p = 0.783, and group P3 p = 0.240 concluded that the data was normally distributed because the value of p > 0.05. The average percentage of bleeding appearance was declared homogeneous after being tested with Levene's Test because the p value obtained was p = 0.578 (p > 0.05). The histopathological data of bleeding was tested using the One Way ANOVA test which resulted in a value of p = 0.104 (p > 0.05) which means that there were no significant differences between groups so it was not continued to the Post Hoc Bonferroni test.

Histopathological changes in bleeding were found in the control group, P1, P2, and P3 in minimal amounts with focal lesions categorized in a score of 1 or mild (<25%).

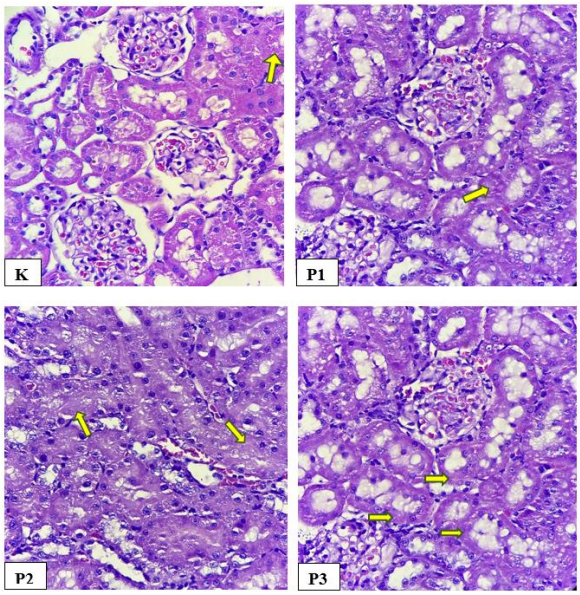

Figure 2. Histopathological appearance of renal tissue necrosis. (K) control; (P1) ironwood bark extract 1,250 mg/kgBW; (P2) ironwood bark extract 2.750 mg/kgBW; (P3) ironwood bark extract 4,750 mg/kgBW. Using an *Olympus CX43* light microscope (400x magnification, HE; yellow arrow: necrosis)

Table 4. The results of data analysis mean percentage of histopathological appearance of necrosis and its standard deviation

Group	Necrosis± SD	Score
C	0.5 ± 0.34[a]	1
P1	0.4 ± 0.28[a]	1
P2	0.9 ± 0.34[a]	1
P3	1.15 ± 0.55[a]	1

Description: Superscript letters in the same column indicate no significant difference (p>0.05).

The average percentage of histopathological appearance of necrosis were initially tested for normality first and obtained group K p = 0.195, group P1 p = 0.161, group P2 p = 0.195, and group P3 p = 0.650. Based on the results of the test, it was concluded that the data was normally distributed because the p value of the entire group >0.05. The data of the histopathological appearance of necrosis were declared homogeneous because the value of p = 0.271 (p > 0.05). Furthermore, the average percentage data of histopathological appearance of necrosis was tested using One Way ANOVA which resulted in p = 0.065 (p > 0.05) meaning that there were no significant differences between groups so they were not continued to the Post Hoc Bonferroni test.

DISCUSSION

Histopathological appearance of bleeding were found in the control group with an average percentage of 0.7 ± 0.94. The damage score based on the percentage obtained is a score of 1 with a mild degree of damage or <25%.[9,14] Based on the theory that aquades are harmless to rats, it is unlikely that the bleeding in the control group was caused by the addition of aquades in the kidneys of mice.[14] The existence of bleeding appearance in this control group is still mild and is included within reasonable limits and in line with several studies such as research by Jannah (2022), Sudira (2019), and Faradisa (2018) which also

found a histopathological appearance of bleeding with a mild degree of damage in the control group that was only given aquades.[9,14,15]

The results showed that the P1 group had an average percentage of bleeding images of 0.5 ± 0.41, the P2 group 1.4 ± 0.74, and the P3 group 1.7 ± 0.62. Histopathological changes in bleeding were found in groups P1, P2, and P3 in minimal amounts with focal lesions categorized in a score of 1 or mild (<25%).[9.14] The results of statistical analysis found that there were no significant differences between group P1 to group K, group P2 to group K, and group P3 to group K. According to Makiyah (2021), toxic potential can be seen from the significant differences between the treatment group and the control group.[16] So that ironwood bark extract doses of 1250 mg/kgBW, 2,750 mg/kgBW, and 4750 mg/kgBW were concluded no potentially toxic to kidney organs based on histopathological appearance of bleeding.[16]

The histopathological appearance of bleeding in the P1 group decreased when compared to the control group. This decrease in the average percentage of bleeding is in line with Faradisa (2018) research which also found a histopathological appearance of bleeding in the treated group was lower than the control.[15] The decrease in bleeding percentage due to the administration of ironwood bark extract at a dose of 1,250 mg/kgBW in this group is believed to have an antioxidant effect because it contains flavonoid compounds 30.48 mg CE / g, phenolics 31.28 mg CE / g, and proanthocyanidin 183.3 mg CE / g.[5] These three compounds are compounds antioxidant that strengthen, anticipate damage to blood vessels, and trigger vasoconstriction so as to prevent bleeding.[17]

Antioxidants have four mechanisms to prevent tissue damage such as bleeding as a result of oxidative stress by decreasing Reactive Oxygen Species (ROS).[18,19] First, antioxidants stimulate the formation of endogenous antioxidant enzymes of the body.[19,20] Such enzymes are glutathianion peroxidase (GPX), catalase, and superoxide dismutase (SOD).[6,20] Second, it inhibits ROS formation enzymes such as microsomal monooxygenase, glutathione s-transferase, mitochondrial succinoxidase, and NADH oxidase. [20] Third, antioxidant compounds cananchelate Cu^{+} and $Fe2^{+}$ metal ions which aims to reduce the catalytic activity of metals thereby reducing the formation of ROS.[20] The fourth mechanism is that antioxidant compounds such as flavonoids, phenolics, and proanthocyanidins or condensed tannins are able to donate H atoms to superoxide, peroxyl, alkoxyl, and hydroxyl radicals that will produce aroxyl radical compounds (Fl-O*).[7] Aroxyl adical R when reacting with other radicals will produce stable kuinone thus preventing oxidative stress.[7]

Based on the results of the study, there was an increase in the histopathological appearance of bleeding in the P2 group dose of 2,750 mg/kgBW and the P3 group dose of 4,750 mg/kgBW. This is in line with Jannah (2022) research which also found an increase in bleeding appearance in treatment with increased doses. [14] The increase in bleeding is thought to be due to the theory that highconcentrations of lavonoids and phenolics convert into prooxidants.[7] Prooxidant action can occur when antioxidant compounds donate H atoms to ROS producing acroxyl radical compounds.[7] Side reaction of aroxyl radicals in the form of superoxide anion (O_2^{*}).[7] O_2* if it meets H_2O_2 through the Haber-Weiss reaction on condition that there is a metal ion as a catalyst that produces hydroxyl radicals (OH*).[7,21] Radical hydroxyl is the most reactive and dangerous radical which, if excessive amounts, will trigger oxidative stress and can attack and damage kidney cells.[7,21] Oxidative stress activates NOD-like receptor protein 3 (NLRP3) which triggers inflammation in the kidneys and causes bleeding.[10,11] During inflammation, various inflammatory mediators are released such as prostaglandins, histamine, and bradykinin.[22] These mediators affect vasodilation and increased capillary permeability causes blood cells to penetrate blood vessels so hemorrhage can occur.[22]

However, the formation of hydroxyl radicals that cause oxidative stress can be prevented by a protective mechanism from the kidney organ itself, namely by the presence of metallotionein protein which functions to detoxify excess metal ions in the kidneys.[23] In addition, the kidneys also found the enzyme catalase in abundance.[24] The enzyme serves to break down H_2O_2 into O_2 and H_2O.[24] The presence of metallotionein protein and catalase in the kidneys can prevent the Haber Weiss reaction so that hydroxyl radicals cannot be produced.[23,24] This is the reason why the bleeding histopathology rate found in the P2 and P3 groups is still relatively mild, and the damage also has no impact on kidney failure.[14] This is evidenced by the absence of fibrosis in the histopathological appearance of the kidneys of group P2 and group P3.[14] Therefore, in addition to the kidney protection mechanism itself, the antioxidant content of ironwood bark extract also works effectively to prevent severe bleeding in the kidneys.[5] Based on these circumstances and also supported by statistical test results that did not show significant differences between the P2 and P3 groups in the control group, ironwood bark extract dose 2,750 mg/kgBW and dose 4,750 mg/kgBW were declared not potentially toxic to kidney organs with parameters of bleeding histopathologic appearance.

The results showed that there was a picture of necrosis with an average percentage of 0.5 ± 0.34 in the control group. This picture is categorized as a score of 1 with a degree of minor damage or <25%.[14] The existence of this necrosis picture is still within reasonable limits because it is still mild and has also been found in several previous studies, such as Jannah (2022) research which found a histopathological appearance of necrosis with a mild degree of damage to the kidneys of control group wistar rats that were only given aquades and standard feed.[14]

Necrosis was found in the P1 group of 0.4 ± 0.28, the P2 group of 0.9 ± 0.34, and the P3 group of 1.15 ± 0.55. This picture of necrosis is found in minimal numbers with focal lesions categorized in a score of 1 or mild (<25%).[9,14] As for the three treatment groups, after statistical testing there was no significant difference when compared to the control group. Based on this, it can be said that there is no toxic potential of ironwood bark extract either at doses of 1,250 mg/kgBW, 2,750 mg/kgBW, or doses of 4,750 mg/kgBW against the kidney organs of wistar rats when viewed based on the parameters of the histopathological appearance of necrosis.[16]

When compared with the control group, there was a decrease in the average percentage of necrosis in the P1 group of ironbark extract dose of 1,250 mg/kgBW. The decrease in the percentage of necrosis in the treatment group was lower than the control group was also found in a study conducted by Sudira (2019).[9] This is possible because the flavonoids, phenolics, and proanthocyanidins contained in ironbark extract function as an exogenous ntioxidant capable of inhibiting lipid peroxidation as one of the biomarkers of oxidative stress.[6,25] Lipid peroxidation due to increased ROS is the cause of cell membrane damage.[25] Damage to cell membranes can alter membrane potential and osmotic pressure of cells, ultimately leading to cell death in the form of necrosis.[25] Exogenous antioxidant compounds contained in ironwood bark extract can inhibit lipid peroxidation so that necrosis can be avoided.[25]

In the P2 group dose of 2,750 mg/kgBW and P3 dose 4,750 mg/kgBW, the histopathological appearance of necrosis was found to increase when compared to the control group. This increase in necrosis is in line with Jannah's research (2022) which also found an improvement in the necrosis picture in the treatment if the dose of extract was added.[14] This is thought to be due to the prooxidant activity of flavonoids and phenolics.[7] At high concentrations, phenolics and flavonoids can form the Haber-Weiss reaction which produces hydroxyl radicals that are reactive to the lipid bilayer of the kidney cell membrane and trigger lipid peroxidation and loss of membrane integrity, causing cell death in the form of necrosis.[13]

The necrosis appearance found in the P2 and P3 groups were still relatively mild and did not show significant differences when compared to the control group. This is possible because the antioxidant content of ironwood bark extract works effectively, besides the formation of hydroxyl radicals themselves can be prevented by the mechanism of kidney organ protection as a form of homeostasis.[23,24] The kidney organ has an abundance of metallotionein proteins and the enzyme catalase which together function to prevent the formation of hydroxyl radicals.[23,24] In the histopathological appearance of groups P2 and P3 also found no fibrosis, indicating that oxidative injury that can result in damage to tubular epithelial cells in severe conditions and acute tubular necrosis did not occur in this study group.[14] Based on this and supported by statistical analysis, it was concluded that ironbark extract doses of 2,750 mg/kgBW and 4,750 mg/kgBW were also not potentially toxic to the kidney organs of wistar rats when viewed based on the histopathological appearance of necrosis.[16]

Among the three doses treatment, after statistical analysis did not show significant differences and was considered non-toxic in the kidneys of wistar rats based on the parameters of histopathological appearance of bleeding and necrosis. [16] However, when viewed from the average percentage of bleeding and necrosis found, the dose of 1,250 mg/kgBW is the recommended dose to use because it has the lowest histopathological changes in bleeding and necrosis compared to doses of 2,750 mg /kgBW and 4,750 mg/kgBW.[14] In conclusion, there are no toxic effects from giving ironbark extract (*Eusideroxylon zwageri*) doses of 1,250 mg/kgBW, 2,750 mg/kgBW, and 4,750 mg/kg body weight orally based on histopathological appearance of bleeding and kidney necrosis of wistar rats.

REFERENCES

1. World Health Organization. WHO global report on traditional and complementary medicine 2019. Geneva: World Health Organization; 2019. 146.
2. Susanty, Bachmid F. Perbandingan metode ekstraksi maserasi dan refluks terhadap kadar fenolik dari ekstrak tongkol jagung (*Zea mays L.*). J Konversi. 2016;5(2):87–93.
3. Salwa IN, Firdaus IWAK, Azizah A. Uji toksisitas ekstrak kulit batang ulin (*Eusideroxylon zwageri*) terhadap sel fibroblas BHK-21 secara in vitro. Dentin J Kedokt Gigi. 2021;5(3):148–53.
4. Wila H, Yusro F, Mariani Y. Skrining fitokimia dan aktivitas antibakteri ekstrak kulit batang ulin (*Eusideroxylon zwageri*) terhadap *Escherichia coli* dan *Salmonella typhi*. J Tengkawang. 2018;8(1):38–49.

5. Kusuma IW, Rahmini, Ramadhan R, Rahmawati N, Suwasono RA, Sari NM. Phytochemicals and antidiabetic activity of *Eusideroxylon zwageri* stem bark collected from East Kalimantan, Indonesia. IOP Conf Ser Earth Environ Sci. 2018;144(1):1-7.
6. Anuj, Rewa K, Yadav A, Mishra, Srivatva S, Praba S. Antioxidants and its functions in human body - A Review. Res Environ Life Sci. 2016;9(11):1328–31.
7. Rudin NA, Hulaimah A, Rahmawati R. The potential utilization of young teak leaves (*Tectona grandis Linn . f .*) as basic ingredients on producing anti-cancer herbal tea. J Pendidik Biol Undiksha. 2022;9(1):10–23.
8. BPOM. Peraturan badan pengawasan obat dan makanan no 7 tahun 2014 tentang pedoman uji toksisitas nonklinis secara in vivo. Badan Pengawas Obat Dan Makanan RI. 2014;1–165.
9. Sudira W, Merdana M, Winaya IBO, Parnayasa IK. Perubahan histopatologi ginjal tikus putih yang diberikan ekstrak sarang semut diinduksi parasetamol dosis toksik. Bul Vet Udayana. 2019;11(2):136-42.
10. Ali AH, Ibrahim RA, Ahmed AF, Talaat EMK. Histological study of toxic effects of acrylamide on the liver and kidney of adult male albino rats. Malaysian J Med Res. 2020;31(3):345–50.
11. Biswas SK. Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? Oxid Med Cell Longev. 2016;2016:17–9.
12. Ratliff BW, Abdulmahdi W, Pawar R, Wolin MS. Oxidant mechanisms in renal injury and disease. Antioxidants Redox Signal. 2016;25(3):119–46.
13. Fujii J, Homma T, Osaki T. Superoxide radicals in the execution of cell death. Antioxidants. 2022;11(3):1-30
14. Jannah DR, Budijastuti W. Histopatological overview kidneys toxicity of a male rat (*Rattus norvegicus*) being given yakon tuber (*Smallanthis sonchifolius*). LenteraBio. 2022;11(2):238–46.
15. Faradisa N, Marfu'ah N, Amal S. Uji toksisitas sub akut infusa daun afrika (*Vernonina amygdalina Del.*) terhadap histopatologi ginjal mencit galur BALB/C. Pharm J Islam Pharm. 2018;2(1):1-7.
16. Makiyah SNN, Tasminatun S, Arsito PN, Fauziah KN, Nugrahanti DR, Putriani A. Subchronic toxicity of piperine in piper nigrum on the histology of the kidney, liver, and lungs of mice (*Mus musculus L.*). Bali Med J. 2021;10(3):1161–7.
17. Asmariati YD, Busman H, Susantiningsih T, Susanti. Protective effect of binahong leaf (*Anredera Cordifolia (Ten.) steenis*) extract against ethanol-induced proximal tubule renal histopathological features of sprague dawley strains white rats. Med J Lampung Univ. 2017;3(3):168–77.
18. Sulistiani RP, Rahayuningsih HM. Pengaruh ekstrak lompong mentah (*Colocasia esculenta L Schoot*) terhadap aktivitas fagositosis dan kadar nitrat oksida mencit BALB/C sebelum dan sesudah terinfeksi *Listeria monocytogenes*. J Nutr Coll. 2015;4(2):409–15.
19. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. Hindawi. 2013;2013:353–74.
20. Wahjuni S. Superoksida dismutase (SOD). Udayana University Press; 2015.16-30.
21. Maanari CP, Suryanto E, Pontoh J. Aktivitas penangkal radikal hidroksil fraksi flavonoid dari limbah tongkol jagung pada tikus wistar. J MIPA. 2014;3(2):134-8.
22. Ashina K, Tsubosaka Y, Nakamura T, Omori K, Kobayashi K, Hori M, et al. Histamine induces vascular hyperpermeability by increasing blood flow and endothelial barrier disruption in vivo. PLoS One. 2015;10(7):1–16.
23. Permana R, Andhikawati A. Metallotionein pada tanaman akuatik dan peranannya dalam akumulasi logam berat. J Akuatek. 2022;3(1):1–8.
24. Hong YA, Park CW. Catalytic antioxidants in the kidney. Antioxidants. 2021;10(1):1–22.
25. Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, Emwas AH, Jaremko M.. Important flavonoids and their role as a therapeutic agent. Molecules. 2020;25(5243):1–39.