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Study of Toxicity of *Curcuma xanthorrhiza* and *Averhoa bilimbi* in Rats (*Rattus norvegicus* Berkenhout) Kidney

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Acute toxicity, Averrhoa blimbi, Curcuma xanthorriza, Histological, Kidney, Rat

ABSTRACT

Biodiversity of medicinal plants such as *Curcuma xanthorrhiza* and *Averhoa bilimbi* are plants that are known to reduce blood glucose levels. The purpose of this study was to determine LD_{50} acute toxicity from a combination of *Curcuma xanthorrhiza* rhizome juice and *Averhoa bilimbi* of rat (Rattus norvegicus Berkenhout) male Wistar. The research method refers to the OECD 425 (2008), consisting of: limit test used a dose of 5000 mg/kg, followed by the main test using a Completely Randomized Design (CRD) with dose between 6500-30000 mg/kg was administered orally for 14 days. The parameters observed of animal mortality, kidney histological. The result showed that no mortality animal test on dose between 6500-25000 mg/kg, the value of LD_{50} is 29.944.821 mg/kgBW classified as Practically nontoxic. Histological studies of kidney that is no effect on the glomerular diameter, but there was an increase in the percentage of fat degeneration and necrosis with increasing doses. The conclusions of this study showed that the combination of juice was practically nontoxic.

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INTRODUCTION

Indonesia has very large biodiversity of medicinal plants, so it is called a megadiversity country. Medicinal plants that have been used since ancient times include *Curcuma xanthorrhiza* and *Averrhoa bilimbi* as traditional medicines that have benefits including: antioxidant, hepatoprotective, anticancer, gastroprotective, antihyperglycemic, immunomodulatory, and anti-inflammatory [1-6]. The combination of *C. xanthorrhiza* rhizome extract and A. bilimbi fruit is known to have antidiabetic activity and effectively reduce blood glucose levels [7-9]. Rhizome section of *C. xanthorrhiza* which is widely used because it contains phenol-derived compounds (curcuminoids), xanthorrhizol essential oils i.e., alkaloids, flavonoids, phenolics, triterpenoids, and glycosides [10;6]. *Averrhoa bilimbi* is a cultivation plant and medicinal plant that has many benefits including cough, high blood pressure, antidiarrheal, antidiabetic, antibacterial, and anti-inflammatory properties and can treat sciatica, rheumatism, mumps, thrush, and high blood pressure. The efficacy is caused by the content of compounds such as saponins, flavonoids, and polyphenols [11-14].

The use of traditional medicine has many benefits but is often used without a doctor's prescription and there are no rules in its use. Lack of supervision of these traditional medicines can have adverse effects on the body and can damage organs, including the kidneys. The kidney is an important organ for the body that plays a role in regulating homeostasis, maintaining salt, glucose, protein, water, and several essential substances, as well as filtering blood and removing substances that are not needed by the body and excreted in the form of urine. The kidneys are susceptible to the effects of various toxic substances contained in blood plasma. The volume of blood flow the kidneys receive from the heart in one minute is approximately 25%, which results in the organ being exposed to the most toxins. Toxic effects on the kidneys can be shown one of them in the form of cell death which then causes kidney damage. There are several cases of kidney damage due to the use of traditional medicines [15-19;12].

The content of xanthorizol in *C. xanthorrhiza* is known to trigger cell death in normal cells of bovine kidneys [20;6]. Consumption of *A. bilimbi* fruit juice for 4-5 days continuously in high doses can also cause acute kidney failure due to the high oxalate content. Kidney organ damage due to exposure to toxic substances can be seen from the glomerular diameter, cells undergoing fat degeneration, and necrosis [21].

For further development as a phytopharmaca, it is necessary to study the combination of these plants whose effects are mainly on the kidney. The preliminary step for screening natural products for pharmacological activity of compounds to evaluated the toxicity. Toxicity can cause various types of toxic effects on organisms. Toxicity tests are needed to determine the safety level of a drug that is still safe for consumption and to detect the toxic effects caused in a short time after oral administration of the test dosage [22]. The use of a combination of *C. xanthorrhiza* rhizome extract and *A. bilimbi* fruit as a traditional medicine should be ensured to be safe to consume, so it is necessary to know the level of toxicity, one of which is by acute toxicity tests. Acute toxicity can be analyzed quantitatively with a median lethal dose (LD_{50}), a dose that causes the death of 50% of the test animal population. Based on the LD_{50} value of a chemical compound is practically non-toxic if it has an LD_{50} value> 15000 mg/kgBW [23]. Several studies stated that LD_{50} ethanol extract of *C. xanthorrhiza* rhizome did not show any toxic effects in mice at 5 g/kgBW [24], and another report that the extract combination of legundi leaves and Javanese turmeric rhizomes (1:1) is also not toxic, with apparent LD_{50} of 17.1 g/kg in acute toxicity study, as well as in subacute toxicity study [25].

MATERIAL AND METHOD

Extraction of plant materials

C. xanthorrhiza used in the form of a nine-month-old rhizome came from the Research Institute for Medicinal Plants Manoko Lembang, while *A. bilimbi* fruit was obtained from the Jatinangor Unpad campus environment with a category of green fruit that was not too ripe. Extraction of combination of juice C. xanthorrhiza and A.bilimbi begins with weighing the weight of each plant with a ratio of 1: 1 according to the dose used and then put into mortar and crushed for 5 minutes until the juice from both plants is obtained, then filtered using gauze and a small sieve to obtain pure juice. Furthermore, 3ml of aquabides was taken to dissolve the pure juice.

Experimental animals

Test animals used were rats (*R. norvegicus* Berkenhout.) Male Wistar strain aged 8-10 weeks, with a bodyweight range of 160-190 grams with a variation of 2.57% and acclimatized for 7 days to adapt the laboratory environment with a temperature 26^{o}C and humidity of 60-70%. During acclimatization, observations of general conditions such as body weight, food given in the form of pellets

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as much as 20 grams/head/day and drinking water were given ad libitum. Cages are cleaned 2 times a week by washing and then drying.

Acute toxicity testing of this study refers to [26]. guidelines, consisting of a limited test and the main test. The limit test stage used a dose of 5000 mg/kgBW which aims to determine the minimum / maximum dose limit on the main test. This stage was divided into two treatments with 5 replications in each treatment, namely, control (aqua) and a combination of *C. xanthorrhiza* and *A. bilimbi* juice. The treatment is given once with an observation time of 14 days. The main test stage is carried out if there is no mortality of the test animal at the limit test stage with a dose referring to [26], and the treatment is the same as the limit test stage namely, control (aqua) and combination of *C. xanthorrhiza* with *A. bilimbi* juice. The main test dose is increased if in the previous treatment there was no mortality of the test animal within 48 hours with a fixed observation time of 14 days. The number of treatments was adjusted until 50% of mortality test animal were found and LD50 values were identified. The method used in the main test is an experimental method with a Completely Randomized Design (CRD). On day 15 rats were sacrificed by dislocation of the neck and then kidney organs were isolated which were then made into histological incisions using the paraffin method and HE stained.

Parameters observed

The observed parameters in this study were include the number of mortality, histological kidney. The mortality of tested animals were then analyzed using Probit to obtain LD_{50} (see Table 1). Determination in toxicity level was done according to the criteria as described in Table 1 [23].

Table 1. Toxicity of compound.

No	Class	LD_{50} (mg/kgBW)
1	*Super toxic*	≤1
2	*Extremely toxic*	1-50
3	*Highly toxic*	50-500
4	*Moderately toxic*	500-5.000
5	*Slightly toxic*	5.000-15.000
6	*Practically nontoxic*	>15.000

Histological experiment preparation

Histological incision of rat kidney was examined under each microscope. The examination is carried out at 100x magnification then followed by 400x magnification with five different viewing fields. Histological kidney changes observed included glomerular diameter, cells in the kidney tubules undergoing fat degeneration, and necrosis. The percentage of damage was calculated as follows:

$$\text{Cell damage (\%)} = \frac{\text{Number of cells damaged}}{\text{Total number of cells}} \times 100\%$$

Data analysis

Results were expressed as mean ± standard deviation (S.D). Statistical significance was analysed using one-way ANOVA followed by Duncan multiple range test. P values <0.05 were considered significant.

RESULTS AND DISCUSSION

LD_{50} Value of Combination of *C. xanthorrhiza* and *A. bilimbi* Juice

The results of the limit test combination of *C. xanthorrhiza* juice and *A. bilimbi* dose of 5000 mg/kgBW showed no mortality was observed in *R. norvegicus* after 14 days of observation. Based on this result, it could be continued with the main test consisting of the control treatment and the combination of juice was started a dose of 6500 mg/kgBW. The result from the main test was presented in Table 2.

Table 2. Acute toxicity of extract on *R. norvegicus* derived from the main test.

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Dose of extract (mg/kgBW)	Number of tested animals (ind.)	Number of mortality (ind.)
0 (control)	5	0
6500	5	0
7200	5	0
8300	5	0
9100	5	0
12000	5	0
12600	5	0
17500	5	0
22500	5	0
25000	5	0
30000	5	2

The data in table 2 shows that the mortality of the tested animals occurred in the treatment P_{10} with the death of test animals> 50% which is 2 animals out of a total of 5 rats, so it is estimated that LD_{50} values are in the dose range of 25000 mg/kgBW to 30000 mg/kgBW. Probit analysis results show that the estimated LD_{50} value is 29,944,821 mg/kgBW, the value is classified as practically non-toxic (>15.000) [23]. In treatment dose 6500-25000, there were no mortality and no toxic symptoms were seen such as decreased motor activity and convulsions, whereas dose 30000 there were toxic symptoms and mortality that occurred on the first day and second day of observation. These toxic symptoms indicate that the treatment affects hemodynamics, the nervous system, and other body systems, causing organ failure and leading to death [16;27]. Referring to table 1 with LD_{50} 29,944,821 mg/kgBW classified as practically non-toxic, therefore it can be concluded that the combination of *C. xanthorrhiza* rhizome and *A. bilimbi* fruits is safe for consumption and can be developed as herbal raw materials.

Histological Observations of Kidney Rats (*R. norvegicus Berkenhout*)

The rhizome of *C. xanthorrhiza* and *A. bilimbi* are known to have various medicinal properties, but if improper use can cause toxic effects, especially on organs so that they need to be tested preclinically and clinically to be truly safe to use [22]. In the toxicokinetic or pharmacokinetic phase, the journey of chemical or toxic substances will go through the stages of absorption, distribution, and excretion in which the kidney organ plays an important role in the excretion process because it is the main pathway for disposal of metabolic waste including toxic substances [28].

Histological observations of the kidneys are performed to determine whether there is damage to kidney tissue caused by exposure to test compounds. The results of observations of kidney histological preparations were observed structures such as glomerulus and cells undergoing fat degeneration and necrosis. Kidney histological in the form of glomerular diameter in Figure 1., in the form of fat degeneration diameter and necrosis in Figure 2. The data of kidney Histological including glomerular diameter, fat degeneration, and necrosis were presented in Table 3.

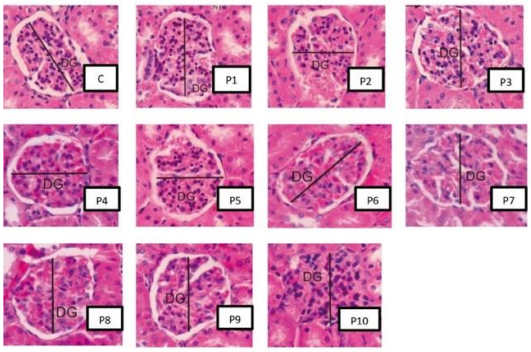

Fig.1 Histological of Rats Kidney HE. 400x

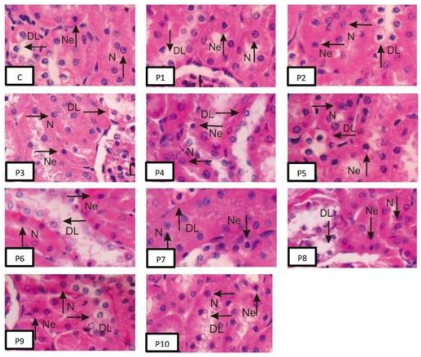

Fig. 2 Histological of Rats Kidney HE. 400x; DL (Fat Degeneration), Ne (Necrosis), N (Normal).

Table 3. Measurement of histological parameters the rats kidney.

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Dose of extract (mg/kgBW)	Glomerular diameter (µm)	Fat degeneration (%)	Necrosis (%)
0 (control)	53.19 ± 6.95	6.33 ± 0.35^{ab}	6.30 ± 0.46^{a}
6500 (P_1)	49.72 ± 4.76	6.10 ± 0.20^{a}	6.46 ± 0.21^{a}
7300 (P_2)	47.39 ± 4.77	6.73 ± 0.20^{b}	7.70 ± 0.10^{b}
8200 (P_3)	45.66 ± 3.59	7.53 ± 0.35^{c}	8.40 ± 0.30^{c}
9100 (P_4)	42.85 ± 2.45	8.06 ± 0.35^{d}	9.16 ± 0.25^{d}
12000 (P_5)	47.70 ± 2.99	8.70 ± 0.26^{e}	11.33 ± 0.40^{e}
12600 (P_6)	45.62 ± 2.18	9.26 ± 0.40^{f}	12.50 ± 0.36^{f}
17500 (P_7)	45.23 ± 3.98	9.73 ± 0,21^{f}	12.53 ± 0.23^{f}
22500 (P_8)	45.92 ± 12.21	10.33 ± 0.40^{g}	13.60 ± 0.10^{g}
25000 (P_9)	50.27 ± 14.21	11.36 ± 0.25^{h}	13.96 ± 0.15^{gh}
30000 (P_{10})	54.26 ± 2.49	12.00 ± 0.36^{i}	14.13 ± 0.30^{h}

The glomerular diameter in all treatment has fluctuating differences with increasing doses. ANAVA test results show that no significant effect of the treatment on glomerular diameter. In P_{10} it can be seen that there is a narrowing of the bowman space, this is due to the proliferation of bowman capsule cells resulting in glomerular adhesion (narrowing between the glomerulus and the bowman capsule) which can lead to kidney failure and can cause death. Toxic effects on glomerulus can be seen from the presence of glomerular swelling marked by increasing glomerular diameter. This occurs because of the interaction of toxic substances with capillaries that cause endothelial proliferation in response to inflammation resulting in capillary vasodilation and enlargement of glomerular capillary webbing [29-30]. This study shows that the combination of *C. xanthorrhiza* and *A. bilimbi* rhizome juice did not effect the glomerulus marked by no significant difference in the glomerular diameter, this is under the study of [31] that the combination of extracts can improve the histological kidney rat's diabetic.

Kidney tubules are vulnerable to toxic effects due to the process of secretion and reabsorption to concentrate on toxic substances, so toxic substances will accumulate in the kidney tubules and cause kidney damage including fat degeneration and necrosis. Interactions between toxic substances and cell membranes can cause damage to cell membranes which can then affect the regulation of ions that will disrupt cell metabolism [15].

ANAVA test results of the average percentage of fat degeneration show that have a significant effect ($p < 0.05$). Duncan's further test results showed that not significantly different on treatments P_1 and P_2 compared to controls ($p > 0.05$) so that the dose not cause kidney histological damage. Treatments P_3 to P_{10} showed marked differences compared to controls ($p < 0.05$) and experienced an increase in the percentage of fat degeneration and the percentage of necrosis with increasing doses. Thus, it shows that at high doses it can cause histological damage to the kidneys. This is in line with the study of [22], that the administration of single-dose ma-aa (*Chisocheton macrophyllus*) seed extract can cause fat degeneration and also cause necrosis in kidney histological. Fat degeneration occurs due to abnormal accumulation of triglycerides which is characterized by the presence of clear vacuoles in the cytoplasm [15]. Cell necrosis is characterized by cell changes such as concentrated nuclei (pianos), fragmented nuclei (karyorrhexis), and visible nuclei fading (karyolysis). Cells that experience necrosis (death) then these cells cannot function again so that necrosis can be said to be irreversible.

Xanthorizol contained in *C. xanthorrhiza* can also trigger kidney damage if used in high concentrations [20]. Chemical compounds such as saponins, tannins, and oxalates in *A. bilimbi* are thought to be toxic and cause cell damage. Saponins and tannins in high concentrations can interfere with cell metabolism and can inhibit oxygen transport resulting in ischemia which results in fat degeneration and cell necrosis [30]. Oxalates are known to be corrosive to cells so that if the levels of oxalate in the body are high then it can cause necrosis. Oxalates can also form calcium oxalate crystals in the body and can precipitate to form kidney stones [21].

Increasing the percentage of fatty degeneration and necrosis along with the increase in the dose indicates the response to the treatment is directly proportional ie the higher the dose of the treatment, the response (damage) is greater [16]. The treatment that caused histopathological damage in this study was classified as mild damage (<25%), even though P_{10} caused the death of test animals. Toxic effects on each organ can vary according to the workload of these organs and how likely they are to be exposed to toxic substances. Toxic effects in the form of histological damage such as fat degeneration and necrosis tend to be found in organs that play an important role in the metabolic process and disposal of toxic substances such as the liver and kidney [27]. The treatment that did not cause kidney histological rats damage is a dose of 6500 mg/kgBW. Obtained LD_{50} combination of *C. xanthorrhiza* and *A. bilimbi* rhizome

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that are classified as practically non-toxic in rats (*R. norvegicus* Berkenhout.) And some treatments do not cause damage to the kidney histological of rats at a dose of 6500 mg/kgBW.

C. xanthorrizha rhizomes has an active compound that potential as natural antioxidants including curcumin, desmethoxycurcumin, and bisdemethoxycurcumin. Curcuminoids are derivatives of diferuloylmethane i.e. demethoxydiferuloylmethane (curcumin) and monodesmethoxy diferuloylmethane (desmethoxycurcumin) compounds. Curcumin is a molecule with low levels of polyphenols but has a high biological activity, including the potential as an antioxidant. Curcuminoids have a phenolic group which is an important group in antioxidants. The antioxidant mechanism has two functions. Its main function is in the administration of hydrogen atoms. Antioxidant compounds (AH) can give hydrogen atoms quickly to radical lipids (R*, ROD*) or convert them to a more stable form, while antioxidant radical derivatives (A*) are more stable than lipid radicals. The secondary function of antioxidants which slows down the rate of autoxidation with various mechanisms beyond the termination of the radical autoxidation chain to a more stable form. Curcumin treatment significantly increased SOD activity, while MDA content and reactive oxygen species (ROS) production was reduced in kidney tissue, indicating reduced oxidative stress [1;32].

The antioxidant radicals (A*) formed in the reaction are relatively stable and do not have enough energy to react with other lipid molecules to form new lipid radicals. Antioxidant radicals can react to each other to form non-radical products. Inhibition of lipid oxide by antioxidants through more than one mechanism depends on the reaction conditions and the food system. There are four possible mechanisms of inhibition i.e. (a) giving hydrogen, (b) giving electrons, (c) complex formation between lipids and aromatic antioxidant rings, and (d) adding lipids to the aromatic antioxidant rings [14;1].

Secondary metabolite content in A. bilimbi in the form of saponins, tannins, flavonoids, glucoside, formic acid, citric acid, calcium oxalate and potassium. Flavonoids have high activity to prevent the body from attacking free radicals. It is known that the body's cells can be continuously damaged by free radicals that result from aerobic metabolism or induced by exogenous damage. The antioxidant effects of phenolic compounds on flavonoids are very strong in breaking the peroxyl chain, flavonoids inhibit the action of enzymes involved in the reaction of superoxide anion production, for example xanthine oxidase and protein kinase. Flavonoids also inhibit the action of cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione-S-transferase, mitochondrial succinoxidase, and NADH oxidase. A number of efficient flavonoid compounds in chelating trace metals such as free iron ions and free copper increase the formation of reactive oxygen species. Flavonoids (Fl-OH) have a low reduction potential value (0.23 - 0.75 V) so that it is easy to reduce superoxide radicals, peroxyl, alkoxyl, and hydroxyl. The fruits extracts of A. bilimbi have strong DPPH radical scavenging activity with IC_{50} value of 20.35 µg/ml. It also displayed remarkable total antioxidant capacity (417.093 ± 6.577 mg/g in an ascorbic acid equivalent) and has high level of total phenolic compounds that apparently explains the antioxidant activity. A. bilimbi juice enhanced the antioxidant activity both in blood and tissues of rats intoxicated or challenged with paracetamol [14;33;34;35].

The flavonoid compounds consumed have an additive effect on the clearance of free radicals by increasing the work function of endogenous antioxidants to participate in three different radical producing systems. Direct cleaning of free radicals by flavonoids can produce stable substances. The activity of flavonoid groups can stabilize reactive oxygen species by directly clearing superoxide, and some other flavonoids can cleanse peroxinitrit reactive oxygen. Epicatechin and routine are the most powerful radical cleansers, which are routine groups capable of inhibiting xanthine oxidase activity. [33;36].

Conclusion

The study results carried out that the treatment did not cause mortality animal test and the obtained LD_{50} combination of *C. xanthorrhiza* and *A. bilimbi* rhizome that are classified as practically non-toxic in rats (*R. norvegicus* Berkenhout.) and all treatments do not cause damage to the kidney histological of rats. This combination was safe to use and can be developed as herbal raw materials.

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References

[1] Rosidi A, Khomsan A., Setiawan B., Riyadi H., Briawan D. 2016. Antioxidant potential of temulawak (*Curcuma xanthorrhiza* roxb). Pakistan J Nutrition, 15 (6): 556-560. ISSN 1680-5194.

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ISSN 2320-9186
[2] Sabiha SC, Golam MU, Nazia M, Mokarram H, Raquibul HS. 2012. ***In-vitro*** antioxidant and cytotoxic potential od hydromethanolic extract of Averrhoa bilimbi L. fruits. Int J Pharm Sci Res. 3:2263–8.
[3] Precious LA, Coren JP, Celestine LA, Mary RM, Janina CE, Rheinmark LS, et al. 2012. Topical administration of Averrhoa bilimbi Linn. leaves crude extract prevents UVB-induced oxidative damage in albino mice. The STETH. 6:29–41
[4] Dnyaneshwar MN, Shekhar BY, Shaijesh SW, Juvekar R. 2010. Hepatoprotective effect of Averrhoa bilimbi Linn. against carbon tetrachloride-induced hepatic damage in rats. Pharmacologyonline. 3:1–6.
[5] Ali R, Hossain M, Runa JF. 2013. Preliminary cytotoxic activity of different extracts of Averrhoa bilimbi (fruits). Int Curr Pharm J. 2:83–4.
[6] Salleh NA., Ismail S., Ab Halim MR. 2016. Effects of *Curcuma xanthorrhiza* Extracts and their constituents on pPhase II drug-metabolizing enzymes activity. Pharmacognosy Res. 8(4):309-315. doi: 10.4103/0974-8490.188873. PMID: 27695274; PMCID: PMC5004525.
[7] Alipin K, Istiqamah N, Maryani A, Madihah (2019) The Potential of Combined *Curcuma xanthorrhiza* Rhizome and *Averrhoa blimbi* fruit extract on decreasing blood glucose levels, insulitis degree and liver structure repair of diabetic male Wistar rats streptozotocin induced. *J Diabetes Metab* 10:835. doi: 10.35248/2155-6156.19.10.835.
[8] Daud N, Hashim H, Samsulrizal N. 2013. Anticoagulant activity of Averrhoa bilimbi Linn. In normal and alloxan-induced diabetic rats. Open Conf Proc J. 4:21–6.
[9] Hasanuzzaman M, Ali MR, Hossain M, Kuri S, Islam MS. 2013. Evaluation of total phenolic content, free radical scavenging activity and phytochemical screening of different extracts of Averrhoa bilimbi (fruits) Int Curr Pharm J. 2:92–6
[10] Cahyani MN. 2014. Effect of ethanol extract of ginger rhizome (*Curcuma xanthorrhiza*) on blood glucose levels of Wistar rats (*Rattus norvegicus*) induced alloxan. J Mahasiswa PSPD FK Universitas Tanjungpura. 3(1): 1-17. [Indonesian]
[11] Bhaskar B, Shantaram M. 2013. Morphological and biochemical characteristics of Averrhoa fruits. Int J Pharmaceut Chem Biol Sci, 3 (3): 924-928.
[12] Ekor M. 2014. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Neurol. 4:177. DOI: 10.3389/fphar.2013.00177
[13] Nair S, George J, Kumar S, Gracious N. 2014. Acute oxalate nephropathy following the ingestion of Averrhoa bilimbi juice. Case Rep Nephrol vol. 2014, Article ID 240936. https://doi.org/10.1155/2014/240936
[14] Asna AN.; Noriham, A. 2014. Antioxidant activity and bioactive components of Oxalidaceae fruit extracts. Malays J Anal Sci. 18: 116-126.
[15] Gartner LP, Hiatt JL. 2014. Color textbook of histological 3ed. Saunders. Jakarta. [Indonesian]
[16] Schuppan D, Dayan A, Charlesworth FA. 2014. The contribution of acute toxicity testing to the evaluation of pharmaceuticals. Springer-Verlag, Berlin Heidelberg.
[17] Lee BM, Kacew S, Kim HS. 2017. Lu's basic toxicology: fundamentals, target organs, and risk assessment. Seventh Edition. CRC Press. London.
[18] Yuet Ping K, Ibrahim D, Chen Y, Sreeramanan S, Sasidharan S, 2013. Acute and subchronic toxicity study of *Euphorbia hirta* L. methanol extract in rats, BioMed Res Int, 1-14.
[19] Wonder KMA, George KA, Eric BG, 2011. Acute and sub-acute toxicity studies of the ethanolic extract of the aerial parts of *Hilleria latifolia* (Lam.) H. Walt. (Phytolaccaceae) in rodents, West African J Pharmacy, 22 (1):27-35
[20] Ismail N, Pihie AHL, Nallapan M. 2005. Xanthorrhizol induces apoptosis via the up-regulation of Bax and p53 in HeLa cells. Anticancer Res. 25: 2221-2228.
[21] Bakul G, Unni VN, Seethaleksmy NV, Mathew A, Rajesh R, Kurien G, Rajesh J, Jayaraj PM. 2013. Acute oxalate nephropathy due to '*Averrhoa bilimbi*' fruit juice ingestion. Indian J Nephrol. 23(4): 297-300
[22] BPOM. 2014. Guidelines for in vivo non-clinic toxicity testing.
[23] Lu, F.C. and S. Kacew. 2002. *Lu's Basic Toxicology: Fundamentals, Target Organs, and Risk Assessment.* Fourth Edition. Taylor & Francis. London.
[24] Devaraj S, Esfahani AS, Ismail S, Ramanathan S, Yam MF. 2010. Evaluation of the antinociceptive activity and acute oral toxicity of standardized ethanolic extract of the rhizome of *Curcuma xanthorrhiza* Roxb. Molecules. 15(4): 2925-2934. DOI: 10.3390/molecules15042925
[25] Ikawati Z, Yuniarti N. (2010) Acute toxicity study of extract combination of *Vitex trifolia* leaves and *Curcuma xanthorrhiza* rhizome on Wistar rats. International Congress of Phytopharm. St.Petersburg Rusia
[26] OECD 425. 2008. OECD guidelines for the testing of chemicals: acute oral toxicity- up and down procedure (UDP). Available at https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecdtg425.pdf.
[27] Desi H., Nurlelasari, Madihah, Deni D, Maharani R, Mayanti T, Supratman U. 2018. Evaluation of the acute toxicity of two extracts of *Chisocheton macrophyllus* seeds in female Wistar rats. Res J Chemist Environ. 22(1): 18-27.
[28] Benet LZ, Kroetz DL, Sheiner LB. 1996. Pharmacokinetics. the dynamics of drug absorption, distribution, and elimination. McGraw-Hill, New York. http://jdih.pom.go.id/showpdf.php?u=YxKvqqo9%2BafK92T2CZVIlFNZksPkYvR6K1nZxKoiF30%3D
[29] Moneim WA, Ghafeer H. 2007. The potential protective effect of natural honey against Cadmium-induced hepatotoxicity and nephrotoxicity. Mansoura J. Forensic Med Clin Toxicol. 15 (2): 75-95.
[30] Fahrimal Y, Rahmiwati, Aliza D. 2016. Histopathological picture of the kidney of male white rats (*Rattus norvegicus* Berkenhout) infected with *Trypanosoma evansi* and given serum leaf extract (*Wedelia bifora*). J Medika Veterinaria. 10 (2): 166-170
[31] Alipin K, Sari EP, Madihah, Setiawati T, Ratningsih N, D. Malini M. 2017. Kidney histological in streptozotocin-induced diabetic male Wistar rats treated with combined extraxt of temulawak rhizome and belimbing wuluh fruit. Nusantara Bioscience. 9(3): 312-317.
[32] Sun LN., Liu, XC., Chen, XJ., Guan, GJ., Liu, G. 2016. Curcumin attenuates high glucose-induced podocyte apoptosis by regulating functional connections between caveolin-1 phosphorylation and ROS. Acta Pharm. Sin. 37, 645-655.
[33] Othman FA., Nooraain H., Noriham A., Azizah AH., Mohamad FFA., Zainon MN., Normah I., Wan RMH., Nurul HR. 2014. Toxicity evaluation of *A. bilimbi* L. fruit extract on hematological and histopathological analysis in the animal model. Int J Pharma Sci Rev Res, 26 (2): 39-43.
[34] Thamizh SN., Santhi PS., Sanjayakumar YR., Venugopalan TN., Vasanthakumar KG., Swamy G. 2015. Hepatoprotective activity of *Averrhoa bilimbi* fruit in acetaminophen induced hepatotoxicity in wistar albino rats. J Chem Pharm Res. 7:535–40.
[35] Kaurinovic B. and Djendji V. 2019. Flavonoids and Phenolic Acids as Potential Natural Antioxidants. DOI: 10.5772/intechopen.83731
[36] Suluvoy JK., Berlin Grace VM. 2017. Phytochemical profile and free radical nitric oxide (NO) scavenging activity of *Averrhoa bilimbi* L. fruit extract. 3 Biotech 7, 85