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Sub-Chronic Toxicity Test of Purple Leaf Extract on the Kidney Histopathology of Male White Rats

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Abstract: Traditional herbal medicine has become increasingly popular as an alternative to conventional medical treatments. Although the plant offers numerous health benefits, caution should be exercised regarding its long-term use. Long-term use of traditional herbal remedies can have detrimental effects on the kidneys. This study was designed to assess the histological changes in the kidneys of male rats following 28 days of purple sage leaf extract administration and to identify potential harmful effects. The study utilized 20 male Wistar rats and utilized a randomized group design for the experimental methodology. Rats were categorized into four distinct groups, consisting of one control group and three treatment groups. The control group received solely sodium chloride, whereas the treatment group 1 (P1) received 500 mg/kg BB of purple sage ethanol extract. Treatment group 2 received up to 2000 mg/kg BB of purple sage ethanol extract, while treatment group 3 received up to 5000 mg/kg BB. The oral examination was conducted for a duration of 28 days. The treatment groups showed an increase in weight and organ index of the right kidney. The findings suggest a potential for toxicity resulting from the administration of purple sage leaf extract over a duration of 28 days.

Keywords: Kidney; Purple leaf extract; Subchronic toxicity

Introduction

Pre-service chemistry teachers' preconceptions are Recently, there has been an increase in interest in alternative medicine, especially traditional practices that use medicinal plants (Tandi et al., 2017; Walean et al., 2018). World Health Organization (1993) defines traditional herbs drugs are naturally occurring herbal substances that undergo little or no industrial processing, used in a curative or topical way for the prevention and treatment of diseases (Osagie-eweka et al., 2021). Traditional herbal medicines and their preparations have been widely used for thousands of years in both developing and developed countries due to their origin and presumed non-toxic effects. Because it is more accessible, cheaper, and widely trusted, traditional medicine has wide support from the community. To ensure the short-term and long-term safety of many traditional medicines, more research is needed as the level of toxicity of many plants is still unknown. Toxicity testing is a method to evaluate the safety of drugs. Three types of toxicity safety studies need to be carried out: acute, subchronic, and chronic (Hasti et al., 2022; Tahseen et al., 2023).

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According to Dewi et al. (2024), purple leaf extract contains alkaloids, flavonoids, saponin, and tannin as a secondary metabolite. The phytochemical components found in purple plants, namely G. pictum (L.) Griff, includes alkaloids, flavonoids, saponins, and tannins. According to Makkiyah et al. (2023), purple plants have a series of pharmacological actions, including targeting cancer, bacteria, antioxidants, hemorrhoids, kidneys, a-glucosidase, and photoprotection. Although the health benefits of purple leaves have received a lot of attention, it is important to know if they are safe to consume. To assess a chemical, toxicity data must first be collected and compiled (F. Makkiyah et al., 2022; Maliza et al., 2021).

The pharmacological effects of purple plant extracts, including its antibacterial and antidiabetic properties, have been the subject of several studies. According to Juniarti et al. (2021), Streptococcus mutans can be killed with purple leaf extract with a concentration of 3.12%. Studies on the effects of purple leaf extract on blood glucose levels and urea creatinine levels in male white rats showed promising results. In a study conducted by Patala et al. (2021), researchers found that doses of purple leaf extract of 150, 200, and 250 mg/kg BB respectively were effective in reducing blood glucose levels and urea creatinine levels (Kenta, 2019; F. Makkiyah et al., 2021).

An acute toxicity study conducted by Dewi et al. (2024) showed that purple leaves are included in the very dangerous category, with LD50 values ranging from >500-2000 mg/kgBB. Subchronic toxicity testing can then be performed to determine the consequences of repeated exposure to the chemical at levels that are not lethal or relevant to humans. Histopathology and macropathology evaluation is part of subchronic toxicity testing (BPOM RI, 2020).

Although the health benefits of purple leaves have received a lot of attention, it is important to know if purple leaves are safe to consume in the long term. One of the massive dangers of the use of herbal components is harm to frame organs (Poh-yen et al., 2018). The organ that regularly studies unwanted outcomes because of drug use is the kidneys. The kidneys are the organs most affected by harmful substances, because this organ receives 25-30% of the blood for purification, so the probability of turning into disease as a filtration level is very high. This is because most of the drug is excreted through urine (F. A. Budiman et al., 2021; H. Budiman et al., 2017). This has been demonstrated in instances of acute kidney harm in China, round 15.3% of which have been due to the management of conventional medicine (Carabelly et al., 2021). Based on the provisions of BPOM, when developing drugs or traditional medicines, in addition to proving their effectiveness, the toxicity of laboratory animals is also evaluated to ensure safety during human use, both acute and long-term (subchronic) for testing. Toxicity testing in animals is useful in detecting possible biochemical, physiological and pathological reactions before human use (Ningsih et al., 2017).

Method

The This study used an experimental method with a randomized block design, using 20 male white rats divided into 4 groups. In this study, 1 control group and 3 test groups were used. In the control group they were only given Na-CMC, in treatment group 1 (P_1) they were given purple leaf ethanol extract at a dose of 500mg/kgBB. Then treatment group 2 (P_2) was given purple leaf ethanol extract at a dose of 2.000 mg/kgBB and treatment group 3 (P_3) was given purple leaf ethanol extract at a dose of 5.000 mg/kgBB. The test preparation was administered orally, for 28 days (BPOM, 2022).

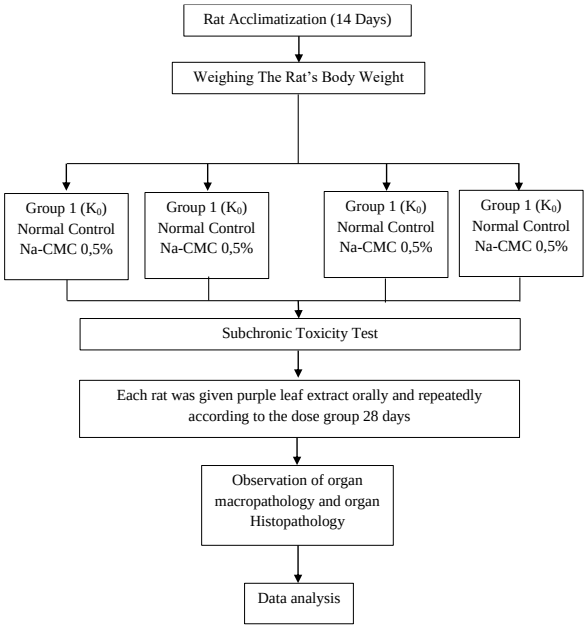

Figure 1. Research work scheme

This research was approved by the ethics committee of Tadulako University with ethical approval letter no 10581/UN28.1.30/KL/2023. This research was conducted in June-August 2023, researchers from the Bandung Biopat Laboratory, the STIFA Pelita Mas Palu pharmacology laboratory, and the phytochemical-pharmacognosis laboratory conducted the study.

Research Procedure

Purple Leaf Extract Suspension preparation

After calculations, each suspension was made by weighing purple leaf extract. After that, each extract is mixed with 0.5% Na CMC until the amount reaches 25

ml. The mixture is then stirred until completely homogeneous (Dewi, 2020).

Preparation of Test Animals

White rats Mus et al. (2023) were used as test subjects in this study. Rats must meet the following criteria: 3–4 months old, weigh 150–250 grams, have white fur, be in good condition, are male, and have been acclimatized for 14 days. There are four groups of test subjects in this study. There were five mice in each experimental group. What the test group got as a treatment was: control group, was given Na-CMC preparation at 0.5%. Treatment group 1, given purple leaf ethanol extract at a dose of 500 mg/kg. Treatment group 2, given purple leaf ethanol extract at 2,000 mg/kg and treatment group 3, given purple leaf ethanol extract at 5,000 mg/kg.

Subchronic Toxicity Test

The preparation of each treatment group was given to test animals many times for 28 days. When the time for handing over the preparation to the test animals was almost up, each animal was necropsied and its organs were examined by neuropathological and histological methods (Rachmawati et al., 2018). Histopathological and neuropathological findings were included in the analysis (BPOM RI, 2020).

The test animals were weighed daily using a scale to determine their weight. On days 0, 7, 14, 21, and 28, the average body weight of rats was determined by adding up their weights and then dividing them by the number of rats in each group (Kuncarli et al., 2014).

Organ index is a sensitive indicator in detecting organ damage which occurs due to exposure to chemical compounds (Lazic et al., 2020). Overview of changes in body organs, Both enlargement and shrinkage of organs are one of the main indicators to observe the toxic effects of a test preparation (Ayun et al., 2021; Carolina et al., 2017). The kidney organ is the focus of the examination. The organ index is calculated by comparing the weight of the organ with the total body weight. After that, the organ indices of the control group and the test dose group were compared (Whidyastuti et al., 2019).

Histopathological preparations of kidney tissue were made at the Biopath Laboratory Bandung using the Hematoxylin-Eosin staining method. Hematoxylin and eosin are dyes that are often used to color tissue so that it is easier to observe with a microscope (Rosmala et al., 2017). The principle of this coloring is that the acidic cell nucleus will attract alkaline substances so that it turns blue. The alkaline cytoplasm will attract acidic substances so that it turns red. Histopathological observations in this study used a microscope with 400x magnification (Jannah et al., 2022). Scoring is carried out based on Sudira et al. (2019), namely score 0 no changes, score 1 focal (light), score 2 multifocal (moderate), score 3 diffuse (severe). The data obtained both at each dose given, tabulated, and subsequently analyzed using One-Way ANOVA and Kruskal Wallis statistical Test.

Result and Discussion

The results of measuring body weight of male white rats can be seen in Figure 1, shows that the study conducted on the effect of oral administration of purple leaf ethanol extract for 28 days on the body weight of test animals did not find significant changes in body weight. The average weekly body weight of Wistar rats fed 0.5% Na-CMC decreased. Furthermore, the average weight of Wistar rats in the test group of 500 mg/kgBB decreased in the first week, then increased in weeks 2, 3, and 4. In addition, in weeks 1 and 2, the average weight of Wistar rats in the test group was 500 mg/kgBB test animals in the group given a formulation of 2,000 mg/kgBB decreased but increased in weeks 3 and 4. Then in the 5,000 mg/kgBB group, the average weight of the test animals decreased in week 1, then increased in week 2. The average weight of the test animals remained unchanged in the third week and then decreased in the third week, fourth week. Both the experimental group and the control group showed similar levels of weight loss. According to Ubang et al. (2022), the results showed that external variables including food, sunlight, exercise, temperature, and environment had a greater impact on weight loss in male white rats than purple leaf extract.

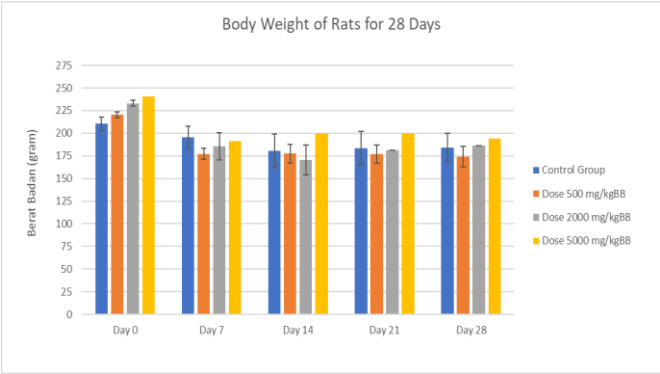

Figure 2. Body weight of rats for 28 days

The results of measuring the left and right kidney organ indices of male white rats can be seen in figure 3 and 4. Figure 2 shows that the index of the left kidney organ of male white rats did not experience significant changes between treatment groups, including control treatment and dose ($p>0.05$). So, after 28 days of dosing, the index of the left kidney organ is not affected by the preparation of the test. The index of the right kidney organ in the dose group increased significantly compared to the control group, as seen in Figure 5. Therefore, it can be concluded that the administration of

test preparations in the dose group increases the organ index. If the inflammation in the kidneys is more severe than usual, this can lead to an increase in the index of the kidney organs. A substantial increase in the index of the kidney organs, which causes glomerular filtration disorders, changes in kidney blood flow, and tubule dysfunction, is an indication of toxic effects on the kidney organs (Kyolo et al., 2019). Only by examining the results more deeply can we determine whether the organ indicated by the organ index data is completely damaged or repaired as a result of the effect of the compound on the sample. Therefore, histopathology and other criteria are needed to see changes in organ function (Metiefeng et al., 2023; Safira et al., 2023).

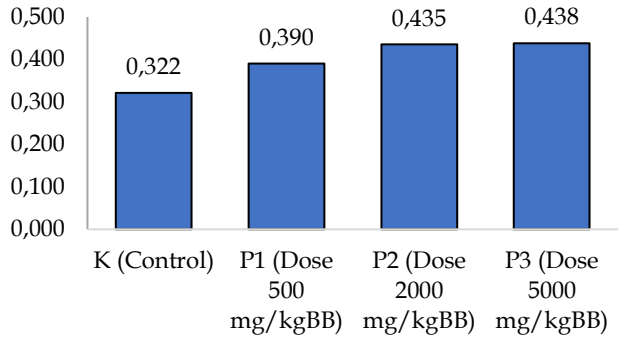

Figure 3. Results of the left kidney organ index measurement

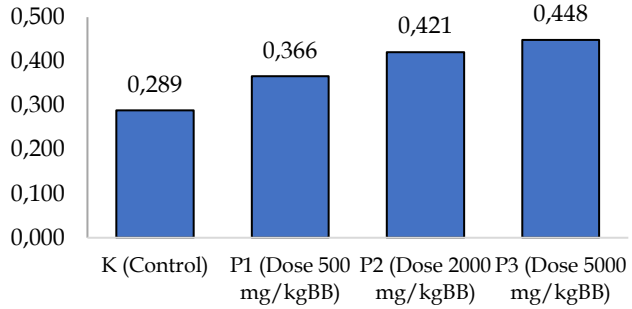

Figure 4. Results of the right kidney organ index measurement

Figure 4 shows the histological findings of the kidney organ exposed to the test preparation for 28 days. Testing showed that the kidneys of the control group, both left and right, had normal tubules consisting of cells with eosinophilic cytoplasm and basophilic nucleus, and the glomerulus was within normal parameters. Furthermore, in the 500 mg/KgBB treatment group, necrosis occurred in the cells of the atrophic tubule layer, and the glomeruli and basophilic nuclei appeared somewhat pale. Cell necrosis occurs when harmful chemicals enter the kidneys through the bloodstream and give rise to pathological changes that ultimately result in cell death (Gelis et al., 2020; Riwanto et al., 2020). When hepatocytes enlarge and die, that is the beginning of an inflammatory response in the liver called necrosis (Ringgi et al., 2023). Photos of atrophic tubules with necrosis of their coating cells were taken in treatment group 2 which was given a dose of 2000 mg/KgBB. Cells that underwent necrosis had glomeruli within normal limits, the basophilic nucleus was relatively pale, and the cytoplasm was rather pale. At a dose of 5000 mg/KgBB, the fourth treatment group had atrophic tubules with necrosis affecting more than two-thirds of the field of view. Necrotic cells have glomeruli, pale cytoplasm, and a smaller nucleus or none at all. In the third stage of necrosis which occurs at a dose of 5000 mg/kgBB, the cell nucleus changes so that it loses most of its color and appears pale or unnoticeable (Ringgi et al., 2023; Tang et al., 2017).

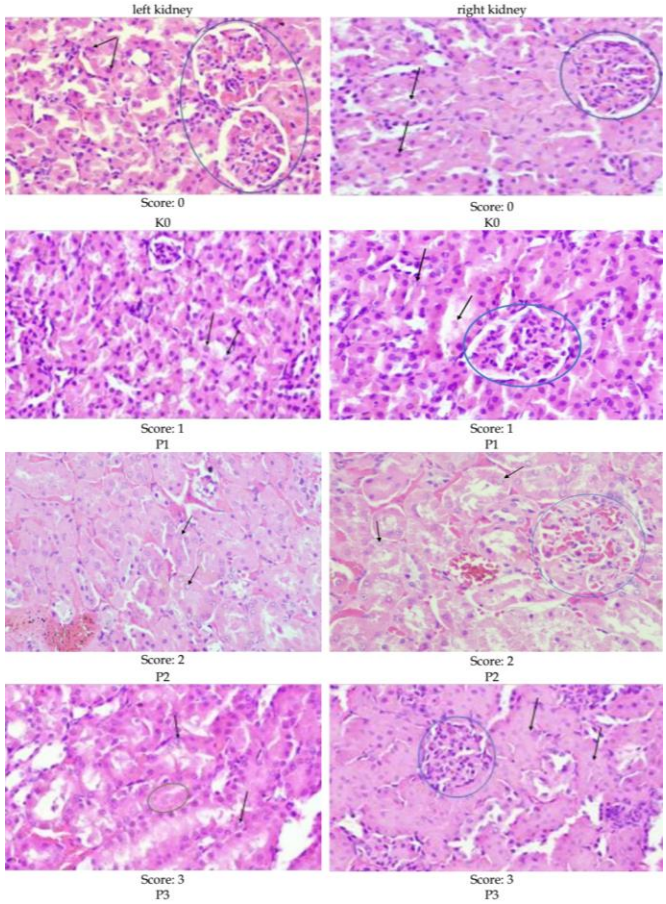

Figure 5. Histopathological picture of the right and left kidneys of the test group rats after being treated for 28 days at 400x magnification with HE staining and Cells experiencing necrosis characterized by a rather pale cytoplasm, basophilic but slightly pale nucleus (→), there is a glomerulus within normal limits (O)

Conclusion

The kidney histopathology of male white rats was examined through a subchronic toxicity test of purple

leaf ethanol extract for 28 days. The rats were divided into three treatment groups: control, treatment 1 (500 mg/kgBB), and treatment 3 (5000 mg/kgBB). The results showed the subchronic toxic effects of the extract, which is characterized by an increase in organ index and the occurrence of necrosis in the histopathological picture of the kidneys of male white rats

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Author Contribution
Conceptualization, I.D., and N. P.D.; methodology, I.D, N.P.D., M., W.W., and J.T.; validation, N.P.D.; data analysis, M., and M.R.; investigation, I.D.; resources, J.T.; data curation, S.A.,and M.R.; writing—original draft preparation, I.D.; writing—review and editing, I.D., M., and N.P.D.; supervision, N.P.D. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare there is no conflict of interest.

References

Ayun, A. Q., Faridah, D. N., Yuliana, N. D., & Andriyanto, A. (2021). Pengujian Toksisitas Akut LD50 Infusa Benalu Teh (Scurrula sp.) dengan Menggunakan Mencit (Mus musculus). *Acta VETERINARIA Indonesiana*, *9*(1), 53–63. https://doi.org/10.29244/avi.9.1.53-63

BPOM. (2022). *Peraturan Badan Pengawas Obat Dan Makanan Nomor 10 Tahun 2022 Tentang Pedoman Uji Toksisitas Praklinik Secara In Vivo*. Retrieved from https://jdih.pom.go.id/view/slide/eb8cbc35bbda4f81bd124ca7eb4842f1/1379/10/2022

BPOM RI. (2020). *Peraturan Badan Pengawas Obat Dan Makanan Tentang Pedoman Uji Toksisitas Praklinik Secara in Vivo*. Retrieved from https://jdih.pom.go.id/view/slide/eb8cbc35bbda4f81bd124ca7eb4842f1/1379/10/2022

Budiman, F. A., & Hidayat, F. (2021). Uji Toksisitas Akut Ekstrak Etanol Umbi Bit (Beta Vulgaris L.) Dengan Metode Bslt (Brine Shrimp Lethality Test). *Jurnal Health Sains*, *2*(3), 310–315. https://doi.org/10.46799/jhs.v2i3.129

Budiman, H., & Aliza, D. (2017). Histopathological changes of rat (Rattus norvegicus) kidney injected with formalin. *Jurnal Ilmiah Mahasiswa Veteriner*, *01*(3), 424–431. https://doi.org/10.21157/jim%20vet..v1i3.3372

Carabelly, A. N., Sinaga, U., & Dewi, N. (2021). Gambaran hipertrofi glomerulus dan degenerasi hidropik ginjal tikus model diabetes pada pemberian ikan toman. *E-Prodenta Journal of Dentistry*, *5*(1), 360–368. http://dx.doi.org/10.21776/ub.eprodenta.2021.005.01.1

Carolina, M., Michel, D. P., Márcia, N., Barcellos, S., De, P. M., Vieira, A., Magalhães, T., Oliveira, M., & Maria, V. (2017). Acute and sub chronic toxicity study of aqueous extract from the leaves and branches of Campomanesia velutina (Cambess) O . Berg. *Journal of Ethnopharmacology*, *201*(February), 17–25. https://doi.org/10.1016/j.jep.2017.02.043

Dewi, N. P., Miranti, M., Magfirah, M., Kurnia Kasim, A., & Dipayana, I. M. (2024). Determination of Total Levels of Secondary Metabolites and Oral Acute Toxicity Testing of Purple Leaf Ethanol Extract (PLEE) in Wistar Rats. *Journal of Public Health and Pharmacy*, *4*(2), 123–131. https://doi.org/10.56338/jphp.v4i2.5131

Gelis, T. N., Etriwati, Erwin, Nazaruddin, Zainuddin, & Muttaqien. (2020). Histopatologi Ginjal Tikus Putih (Rattus norvegicus) Setelah Implan Wire Material Logam. *Jurnal Ilmiah Mahasiswa Veteriner*, *4*(September), 101–106. https://doi.org/10.21157/jim%20vet..v4i4.15716

Hasti, S., Renita, L., & Santi, F. (2022). Sub-Chronic Toxicity of Syzygium Myrtifolium Walp on Liver and Kidney Function. *Jurnal Ilmu Kefarmasian*, *20*(1), 30–37. https://doi.org/10.35814/jifi.v20i1.1103

Jannah, D. R., & Budijastuti, W. (2022). Gambaran Histopatologi Toksisitas Ginjal Tikus Jantan (Rattus norvegicus) yang diberi Sirup Umbi Yakon (Smallanthus sonchifolius). *LenteraBio : Berkala Ilmiah Biologi*, *11*(2), 238–246. https://doi.org/10.26740/lenterabio.v11n2.p238-246

Juniarti, D. E., Kusumaningsih, T., Juliastuti, W. S., Soetojo, A., & Wungsu, N. D. (2021). Phytochemical Analysis and Antibacterial Activity of Purple Leaf Extract [Graptophyllum pictum (L.) Griff] Against Streptococcus mutans. *Acta Medica Philippina*, *55(8)*, 802–806. https://doi.org/10.47895/amp.v55i8.2125

Kenta, Y. S. (2019). Uji Efek Ekstrak Daun Ungu Terhadap Kadar Kreatinin Dan Ureum Tikus Putih Jantan Yang Diinduksi Streptozotocin. *Jurnal Mandala Pharmacon Indonesia*, *5*(2). https://doi.org/10.35311/jmpi.v5i02.47

Kuncarli, I., & Djunarko, I. (2014). Uji Toksisitas Subkronis Infusa Daun Sirih Merah (Piper crocatum Ruiz & Pav) Pada Tikus: Studi Terhadap Gambaran Mikroskopis Jantung Dan Kadar Sgot

Darah. *Journal of Pharmaceutical Science and Community*, *11*(2), 86–95. https://doi.org/10.24071/jpsc.00105

Kyolo, S. K., Odda, J., Lubega, A., & Bbosa, G. S. (2019). Blood Chemistry and Major Body Organ Induced-Toxicity by Locally-Made Traditional OMGKRP Karuho Poison in Wistar Albino Rats. *Scientific Research Publishing*, 272–291. https://doi.org/10.4236/nm.2019.103021

Lazic, S. E., Semenova, E., & Williams, D. P. (2020). Determining organ weight toxicity with Bayesian causal models: Improving on the analysis of relative organ weights. *Scientific Reports*, *10*(1), 1–12. https://doi.org/10.1038/s41598-020-63465-y

Makkiyah, F. A., Rahmi, E. P., Susantiningsih, T., Marliani, N., Arista, R. A., & Nurcholis, W. (2023). Optimization of Graptophyllum pictum leaves extraction using a simplex centroid design focused on extracting flavonoids with antioxidant activity. *Journal of Applied Pharmaceutical Science*, *13*(05), 214–221. https://doi.org/10.7324/JAPS.2023.117043

Makkiyah, F., Rahmi, E. P., Revina, R., Susantiningsih, T., & Setyaningsih, Y. (2021). Graptophyllum pictum (l.) griff. (syn: Justicia picta linn.) and its effectiveness: A well-known indonesian plant. *Pharmacognosy Journal*, *13*(3), 835–838. https://doi.org/10.5530/pj.2021.13.106

Makkiyah, F., Rahmi, E. P., & Setyaningsih, Y. (2022). Phytochemical Test and Acute Safety Evaluation of Oral Purple Leaves (Graptophyllum Pictum L. Griff) Extract in Rats. *Pharmacognosy Journal*, *14*(5), 649–654. https://doi.org/10.5530/pj.2022.14.148

Maliza, R., Tofrizal, A., & Setiawan, H. (2021). Uji Toksisitas Subkronis Ekstrak Metanol Kulit Buah Kopi Arabika (Coffea arabica l.) pada Ginjal Mencit (mus musculus l.) Galur BALB / c Uji Toksisitas Subkronis Ekstrak Metanol Kulit Buah Kopi Arabika (Coffea Arabica L.) pada Ginjal Mencit. *Semin Nas Bioteknol VI*, 137-46. Retrieved from https://rb.gy/82adch

Metiefeng, N. T., Tamfu, A. N., Tagatsing, M. F., Tabopda, T. K., Kucukaydin, S., Mbane, M. N., Atchade, A. D. T., Talla, E., Henoumont, C., Laurent, S., Anouar, E. H., & Dinica, R. M. (2023). In Vitro and In Silico Evaluation of Anticholinesterase and Antidiabetic Effects of Furanolabdanes and Other Constituents. *Molecules*. https://doi.org/10.3390/molecules28124802

Mus, S., Wahyuddin, N., Rahimah, S., & Melani, E. (2023). Uji toksisitas akut ekstrak etanol daun sembukan (paederia foetida l.) Terhadap kadar blood urea nitrogen dan kreatinin mencit (Mus Musculus). *Majalah Farmasi Dan Farmakologi*, *34*, 34–36. https://doi.org/10.20956/mff.Special

Ningsih, S., Agustini, K., Nizar, & Damayanti, R. (2017). Sub-Chronic Toxicity Test of Uncaria gambir and Caesalpinia Sappan Combined Extract. *Jurnal Kefarmasian Indonesia*, *7*(1), 34–45. https://doi.org/10.22435/jki.v7i1.5690.34-45 Jurnal

Osagie-eweka, S. D. E., Orhue, N. E. J., Omogbai, E. K. I., & Amaechina, F. C. (2021). Oral acute and sub-chronic toxicity assessment of aqueous leaf extract of Simarouba glauca DC (Paradise tree). *Toxicology Reports*, *8*, 239–247. https://doi.org/10.1016/j.toxrep.2021.01.008

Pratiwi, M., Tandi, J., & Wirawan, W. (2019). Uji Efek Ekstrak Daun Tempuyung Terhadap Kadar Glukosa Darah Tikus Putih Jantan Diinduksi Streptozotocin. *Farmakologika: Jurnal Farmasi, 16*(02), 165-176. Retrieved from https://www.jfarma.org/index.php/farmakologika/article/view/132

Poh-yen, K., Lay-jing, S., & Hanani, F. (2018). In vitro evaluation of photoprotective potential of the different solvent extracts of Graptophyllum pictum leaves. *Journal of Applied Pharmaceutical Science,* *8*(1), 147-151. https://doi.org/10.7324/JAPS.2018.8122

Rachmawati, E., & Umayah Ulfa, E. (2018). Uji Toksisitas Subkronik Ekstrak Kayu Kuning (Arcangelisia flava Merr) terhadap Hepar dan Ginjal Subchronic Toxicity Test of Yellow Root Extract (Arcangelisia flava Merr) on Hepar and Renal. *Global Medical and Health Communication*, *6*(July 2017), 1–6. http://dx.doi.org/10.29313/gmhc.v6i1.2203

Ringgi, T. N., Sitompul, Y. Y., Amalo, F. A., & Winarso, A. (2023). Gambaran Patologi Anatomi dan Histopatologi Organ Visceral Kambing Kacang. *Jurnal Veteriner*, *VI*(24). https://doi.org/10.35508/jvn.v6i2.8538

Riwanto, I., Nugroho, E. A., Susilaningsih, N., Prajoko, Y. W., Budiono, P., Prasetyo, S. A., & Azhar, A. (2020). Antioxidant and Anti-inflammatory effect of Graptophyllumpictum (L.) Griff extract Study on SOD and COX-2 serum of experimental hemorrhoids. *Medica Hospitalia : Journal of Clinical Medicine*, *7*(2), 422–426. https://doi.org/10.36408/mhjcm.v7i2.515

Rosmala, A., Khumaida, N., & Sukma, D. (2017). Alteration of Leaf Anatomy of Handeuleum (Graptophyllum pictum L . Griff) due to Gamma Irradiation. *HAYATI Journal of Biosciences*, *23*(3), 138–142. https://doi.org/10.1016/j.hjb.2016.12.003

Safira, M. N., Apridamayanti, P., Kurniawan, H., Fajriaty, I., Nugraha, F., Nani Nurbaeti, S., & Pratiwi, L. (2023). Pengaruh Pemberian Kombinasi Ekstrak Kulit Pisang dan Kulit Nanas terhadap

Indeks Organ Tikus Wistar. *Journal Syifa Sciences and Clinical Research*, *4*(1), 227–236. https://doi.org/10.37311/jsscr.v4i1.13910

Sudira, W., Merdana, M., Winaya, I. B. O., & Parnayasa, I. K. (2019). Perubahan Histopatologi Ginjal Tikus Putih yang diberikan Ekstrak Sarang Semut diinduksi Parasetamol Dosis Toksik. *Buletin Veteriner Udayana*, 136. https://doi.org/10.24843/bulvet.2019.v11.i02.p05

Tahseen, M. A., Bhatnagar, A., & Bhatnagar, A. (2023). Phytochemical and pharmacological investigations of Graptophyllum pictum leaves. *Journal of Pharmacognosy and Phytochemistry*, *12*(6), 252–266. https://doi.org/10.22271/phyto.2023.v12.i6c.14791

Tandi, J., Wulandari, A., & Asrifa. (2017). Effect of Ethanolic Extract of Gendola Merah (Basella alba L.) Leaves on Creatinine, Urea, and Description of Histological Tubular Kidney of Diabetic Male Rats induced by Streptozotocin. *Galenika Journal of Pharmacy*, *3*(2), 93–102. https://doi.org/10.22487/j24428744.2017.v3.i2.8813

Tang, R., Tian, R., Cai, J., Wu, J., & Shen, X. (2017). Acute and sub-chronic toxicity of Cajanus cajan leaf extracts. *Pharmaceutical Biology*, *55(1)*, 1740–1746. https://doi.org/10.1080/13880209.2017.1309556

Ubang, F., Siregar, V. O., & Herman, H. (2022). Efek Toksik Pemberian Ekstrak Etanol Daun Mekai (Albertisia papuana Becc.) Terhadap Mencit. *Proceeding of Mulawarman Pharmaceuticals Conferences*, *16*(November), 49–57. https://doi.org/10.25026/mpc.v16i1.672

Walean, M., Rumondor, R., & Maliangkay, H. P. (2018). Pengaruh pemberian ekstrak etanol kulit batang pakoba (syzygium sp.) Terhadap gambaran histopalogi ginjal tikus putih yang diinduksi etilen glikol. *E-Journal Unsrat*, *11*(1). https://doi.org/10.35799/cp.11.1.2018.27911

Whidyastuti, D., Nurbaeti, S. N., & Kurniawan, H. (2019). Pengaruh Pemberian Minyak Cincalok Terhadap Bobot Badan dan Indeks Organ Hati, Jantung, Ginjal, Paru-Paru, dan Limpa Tikus Putih Galur Wistar. *Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN*, *4*(1), 2–3. Retrieved from https://jurnal.untan.ac.id/index.php/jmfarmasi/article/view/40732

World Health Organization. (1993). *Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines Manila*. WHO Regional Office for the Western Pacific. Retrieved from https://www.who.int/publications/i/item/9290611103