

# Subchronic Toxicity Test of Purple Leaves Ethanol Extract (PLEE) on the Histopathological Picture of the Lymph of Wistar Rats and Antioxidant Activity

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**Abstract:** This study aims to determine the toxic effects of purple leaf ethanol extract on lymph organs at doses of 500; 2.000; and 5.000 mg/kgBB administered for 28 days and to determine the antioxidant activity of purple leaf *Graptophyllum pictum* (L.) This study is an experimental study with a random group design, using 20 test animals divided into 4 groups consisting of two treatment groups, namely in the normal control group given Na-CMC 0.5% and the experimental group given PLEE (k1: dose 500 mg/kgBB, k2: dose 2.000 mg/kgBB and k3: dose 5.000 mg/kgBB hematoxylin-eosin staining (HE) using a computer-connected Motic BA210 microscope with 40X magnification in 5 fields of view and antioxidant test using DPPH method with UV-Vis Spectrophotometer at 516 nm wavelength with concentration 20, 40, 60, 80, and 100 ppm. The results showed that leaf ethanol extract was toxic at a dose of 2.000 mg/kgBB – 5.000 mg/kgBB against the diameter of lymphatic organ pulp, organ index and organ weight, there were symptoms of toxicity and purple leaf ethanol extract had strong antioxidant activity with 66.26 µg/mL.

**Keywords:** Antioxidant activity; Histopathological lymph; Purple leaves; Subchronic toxicity

## Introduction

Traditional medicine is very easy to accept by the community and this medicine is cheaper and easier to get. However, there are still many plants whose toxicity level is unknown, so further research is needed so that certain traditional medicines are safe to consume both in the short- and long-term Drug safety assessment can be carried out by toxicity tests. Tests for toxicity safety that must be carried out include acute toxicity, subchronic toxicity, and chronic toxicity tests (Hasti et al., 2022). One of the plants that has medicinal properties is the purple leaf (*Graptophyllum pictum* (L.) Griff.) (Retnaningsih et al., 2019). Purple leaves (*Graptophyllum pictum* (L.) Griff)

are known to have pharmacological effects to overcome various health problems (Umboro et al., 2022).

The purple leaves were classified as moderately toxic with an LD50 value of > 500-2000 mg/kgBB. Then it can be continued with a subchronic toxicity test to see the effect of repeated exposure to substances at non-lethal doses or doses that are likely to be given to humans Subchronic toxicity testing includes macropathological and histopathological observations. The benefits of purple leaves in the health sector have been widely reported, but the safety of purple leaves for consumption is very important. To evaluate a chemical substance, it is necessary to recognize its danger by collecting and compiling toxicity data (Maliza et al., 2021) the purpose of this study is the subchronic toxic

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effect (Irfayanti et al., 2023). Lymph is the largest lymphoid organ that has an important role in the immune system (Setiawan et al., 2021). Measurement of white pulp diameter is a quantitative observation that can be used to determine the effect of the toxicity of a material observed from how many lymphocyte cells die and observe the rate of lymphocyte cell proliferation (Anindya et al., 2019; Putra et al., 2023).

Purple leaves have many known benefits, one of which can potentially ward off free radicals (Indrawati et al., 2022; Pratama & Busman, 2020). This is because purple leaves have phenolic compounds that act as antioxidant compounds to ward off free radicals. The powerful antioxidants in purple leaves are caused by the high flavonoids in purple leaves (Hasanah et al., 2024). The leaves of the purple plant have been known to have antioxidant properties (Salim, 2021).

Antioxidant compounds are substances that can absorb or neutralize free molecules so that they can prevent degenerative diseases such as heart disease, and cancer. Antioxidants are able to donate electrons to inhibit free radical chain reactions that can damage the body (Selfiani et al., 2023). Consuming adequate amounts of antioxidants can improve immunological status and inhibit the onset of degenerative diseases (Hidayah et al., 2021). Antioxidants play an important role in preventing cell damage thereby inhibiting common pathways for cancer, aging and various diseases (Nisa et al., 2019). To prevent cell damage, several extracts, oils, herbal formulations have been shown to be important antioxidant agents (Rahman et al., 2023). *G. pictum* leaves, which have been shown to have anti-inflammatory properties (Yasintha & Makkiyah, 2024) and antioxidants (Priyanto et al., 2024).

## Method

This research has been approved by the ethics committee of Tadulako University with an ethical approval letter number 10581/UN28.1.30/KL/2023. This research was carried out in June-August 2023, and in May 2024 it was carried out in the instrument analysis laboratory, phytochemistry-pharmacognosy laboratory, pharmacology laboratory of STIFA Pelita Mas Palu and at the Biopath Laboratory.

This study was carried out using an experimental method, using 20 male wistar rats and divided into 4 groups, consisting of 1 control group and 3 test groups. In the control group, only Na-CMC was given, in treatment group 1 (k1) purple leaf ethanol extract was given a dose of 500 mg/kgBB. then in treatment group 2 (k2) purple leaf ethanol extract was given a dose of 2.000 mg/kgBB and in treatment group 3 (k3) purple leaf ethanol extract was given a dose of 5.000 mg/kgBB. The

administration of test preparations is carried out orally, for 28 days.

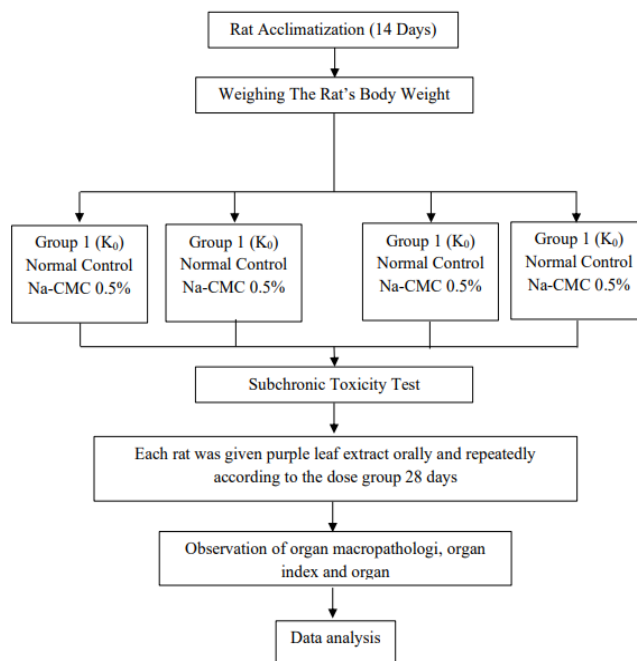


Figure 1. Research working methods

### Research Procedure

#### Preparation of Purple Leaf Ethanol Extract

The manufacture of PLEE is carried out using maceration techniques (Handoyo, 2020) by weighing 2.400 grams of purple leaf simplicia powder is then extracted using 8 L of 96% ethanol solvent for 3 x 24 hours at room temperature while stirring occasionally (Dewi et al., 2024). The extract is then filtered using filter paper and filtrate is obtained. Furthermore, the filtrate is concentrated using a Rotary Vacuum Evaporator at a temperature of 60°C and continued with evaporation using a waterbath until a thick extract is obtained (Ulfa et al., 2020). The extract yield is calculated using the formula (Amalia et al., 2023).

$$\text{Rendement} = \frac{\text{Weight sample (g)}}{\text{Extract weight (g)}} \times 100\% \quad (1)$$

#### Preparation of Test Animals

The test animals used by male white Wistar rats with the criteria are 3-4 months old, weight 150-250 grams, white fur color, active and healthy rats, male sex acclimatized for 14 days (Lahamendu et al., 2019). The Rats were divided into 4 groups, 1 normal group and 3 test groups. Animals were given preparations according to the treatment group repeatedly for 28 days, then the preparation was given to the test animals, all animals were necropsied, and then observations were made on the organs in macropathology. Histopathological examination was carried out (Bemidinezhad et al., 2023).

Test animals were given oral preparations according to the treatment group, the administration of preparations was carried out every day, for 28 days. Then observations are carried out for 2 hours after the preparation is given. Observations made are image and histopathological observations with the diameter of the pulp of lymphatic organs (Nugrahaningsih et al., 2023).

Observations are made on the lymphatic organs. The weight of the organ is weighed and compared with the weight of the body to obtain the organ index. The organ index of the test dose group was then compared with the organ index of the control group. Formula for calculating organ index (Whidyastuti, 2019).

$$\text{Organ} = \frac{\text{Organ weight (gram)}}{\text{Rat weight (gram)}} \times 100\% \quad (2)$$

Lymph histology was made by embedding and hematoxylin-eosin (HE) staining. The indicator used in this study was the diameter of the white pulp. Histological imaging was carried out using a Motic BA210 microscope connected to a computer with a magnification of 40X at 5 fields of view. Then, the diameter of the white pulp was measured using Motic Image Plus 2.0 software. The measurement of the diameter of the white pulp is carried out by adding the maximum diameter of the transverse and the maximum diameter perpendicular then divided in half (Moura et al., 2022).

#### Preparation of Purple Leaf Ethanol Extract

PLEE to the solvent at concentrations of 20, 40, 60, 80, and 100 ppm (Ranti et al., 2021). Each of these concentrations was pipetted as much as 3 ml and dissolved with 1 ml of 100  $\mu$ M DPPH solution (Setiawan et al., 2021). The mixture was then incubated at 30°C for 30 minutes in the dark. Then, the absorbance was measured using a UV-Vis spectrophotometer at a maximum wavelength of 516 nm (Maulydya et al., 2023). Antioxidant activity was expressed in units of % inhibition. This value was obtained using the following formula (Permatananda et al., 2023).

The antioxidant activity of the extract obtained was then calculated by calculating the percentage of DPPH absorption inhibition (Islam et al., 2023; Wartono et al., 2021).

$$\text{Inhibition (\%)} = \frac{\text{Abs. Control} - \text{Abs. Sample}}{\text{Abs. Control}} \times 100\% \quad (3)$$

The data obtained from the histopathology of the lymph of wistar rats at each dose given, the data were tabulated and subsequently analyzed by the Kruskal-wallis nonparametric statistical test. If there was a significant difference ( $P < 0.05$ ) followed by the Mann-

Whitney test. And in the antioxidant activity test. After the comparative data of solution absorption is described, a linear regression equation  $y = a + bx$  is produced. Using a UV-Vis spectrophotometer, identification data in the form of maximum wavelength and spectrum is collected. To analyze the antioxidant data, the percentage (%) of antioxidant activity for each absorption sample was calculated from the absorption data. The IC50 value is then calculated using a non-linear regression equation.

## Results and Discussion

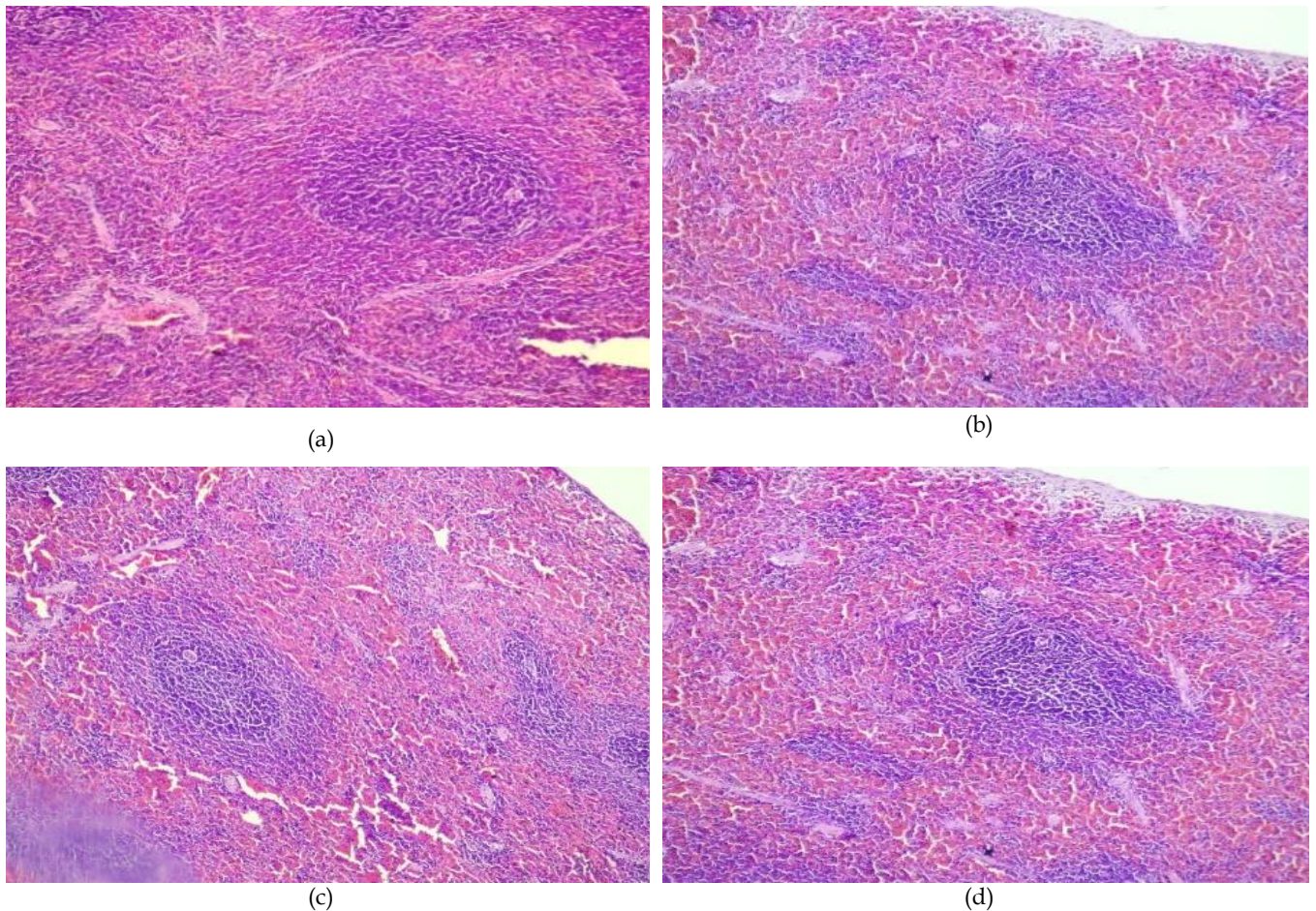
In this study, purple leaf test material was used from the Pasangkayu district, West Sulawesi. The identification was carried out to ensure the correctness of the test material used, identification of purple leaves was carried out at the Biological Resources UPT, Tadulako University, Sulawesi. The identification results prove that the purple leaf used in the research is indeed the *Graptophyllum pictum* (L) species. Griff, which belongs to the *Acanthaceae* family. 2.400 grams of purple leaf powder were macerated in 3 maceration vessels and used 8 liters of 96% ethanol solvent. The resulting thick extract was 188 grams with an extract yield of 7.8%. Toxicity tests are all tests carried out to detect the toxic effects of a substance on a biological system to obtain typical dose-response data from the test preparation. Subchronic toxicity test research is carried out with the aim of detecting toxic effects that appear after administering the test preparation with repeated doses for 28 or 90 days.

The results of the observation of the Diameter of Lymph Pulp of Rats can be seen in Table 1 showing the results of histopathological examination of lymphatic organs that have been carried out on the diameter of the pulp using the Kruskal Wallis data analysis test, the average value in the normal control group was obtained (K0) 135.35, control doses 500 mg/ kg BB (K1) (141.94), control doses 2.000 mg/ kg BB (K2) (126.59), dan control doses 5.000 mg/ kg BB (K3) (126.71) The results of the observation of the Diameter of lymph Pulp of Rats can be seen in Table 1 showing the results of histopathological examination of lymphatic organs that have been carried out on the diameter of the pulp using the Kruskal Wallis data analysis test, the average value in the normal control group was obtained become very large. Based on the analysis of the data, it can be found that purple leaves contain secondary metabolite compounds that can affect the increase of germinal centers in pulp diameter (Setiawan et al., 2021) such as flavonoids that have immunostimulant activity. This flavonoid can increase the number and activity of B cells where the flavonoid type quercetin can increase the

phagocytic activity of peritoneal macrophages (Rousdy et al., 2017).

**Table 1.** Results of Diameter of Pulp Lymph of Rats

Group	Diameter Pulp Lymph (µm)					Average ± SD	P
K0	147.54	152.45	105.94	147.82	123.02	135.35 ± 20.08	0.764
K1	114.58	160.69	154.40	134.50	145.51	141.94 ± 18.19	
K2	-	126.59	-	-	-	126.59 ± 0.00	
K3	-	-	126.711	-	-	126.71 ± 0.00	



**Figure 2.** Histopathology of diameter Pulp Lymph Nodes in the test group after treatment for 28 with 40X magnification on 5 fields of the hematoxylin-eosin (HE) staining day display: (a) (K0) Normal control; (b) (K1) Dose 5.00 mg/kgBB; (c) (K2) Dose 2.000 mg/kgBB; and (d) (K3) Dose 5.000 mg/kgBB

The organ index is a sensitive indicator in detecting organ damage that occurs due to exposure to chemical compounds. The picture of changes in the body's organs, both enlargement and organ removal, is one of the main indicators to observe the toxic effects of a test preparation. The purpose of the organ index comparison between the control group and the dose was to observe the effects of exposure after administering the test preparation for 28 consecutive days. Meanwhile, the purpose of comparing organ indices in the satellite group is to observe the reversibility effect and delayed effect of the test preparation (Safira et al., 2022).

**Table 2.** Results of observations of the weight of the Lymph organ

Group	Lymph organ weight (grams)					Average ± SD	P
K0	0.48	0.56	0.76	0.81	0.73	0.66 ± 0.14	0.214
K1	0.31	0.64	0.39	0.45	0.60	0.47 ± 0.14	
K2	-	0.44	-	-	-	0.44 ± 0.00	
K3	-	-	0.51	-	-	0.51 ± 0.00	

The results of the Kruskal wallis statistical test in table 2 obtained a value of  $p > 0.05$  which shows that the average value is not significantly different. The results

showed that organ weight at a dose of 2.000 mg/kg BB could have a better effect on the Lymph organs of white rats compared to doses of 500 mg/kg BB and 5.000 mg/kg BB, this change showed lesions due to the influence of compounds contained in purple leaf ethanol extract (Hasibuan, 2015).

**Table 3.** Observation results of lymph organ index

Group	Lymph organ weight (grams)					Average ± SD	P
K0	0.28	0.32	0.37	0.42	0.38	0.35 ± 0.05	0.18
K1	0.16	0.37	0.22	0.28	0.33	0.27 ± 0.08	
K2	-	0.23	-	-	-	0.23 ± 0.00	
K3	-	-	0.26	-	-	0.26 ± 0.00	

In the average results of the organ index, the value was not significantly different from the  $p > 0.05$  value, which can be seen in Table 3, where the average results showed that the control dose of 2.000 mg/kgBB decreased, while at the doses of 500 and 5.000 mg/kgBB, this occurred after the administration of purple leaf ethanol extract for 28 days had a reversible toxic effect (Khalishah et al., 2021).

**Table 4.** The results of Quercetin IC50 Values

Concentration (ppm)	Absorbance			%inhibition		
	R1	R2	R3	R1	R2	R3
Control	0.56	0.56	0.56			
20	0.32	0.31	0.30	43.26	44.14	45.92
40	0.30	0.28	0.26	46.09	48.93	52.48
60	0.29	0.28	0.26	47.51	48.75	52.66
80	0.28	0.27	0.25	50.00	51.59	54.25
100	0.27	0.27	0.25	51.06	50.70	54.25
			IC50 (ppm)	84.73	74.83	39.23
			IC50 mean (ppm) ± SD		66.26 ± 36.11	

**Table 5.** The results of IC50 values of PLEE

Concentration (ppm)	Absorbance			%inhibition		
	R1	R2	R3	R1	R2	R3
Control	1.03	1.03	1.03			
20	0.95	0.69	0.62	8.00	32.78	39.82
40	0.81	0.63	0.56	21.60	39.15	45.41
60	0.64	0.50	0.45	38.09	51.39	56.60
80	0.58	0.46	0.42	43.97	55.44	58.92
100	0.56	0.45	0.33	45.61	56.22	67.31
			IC50 (ppm)	98.00	69.50	49.44
			IC50 mean (ppm) ± SD		72.31 ± 24.40	

Based on the results of the study in Table 4, purple leaf ethanol extract has antioxidant activity with an

average IC50 value of 66.26 µg/mL which is interpreted as a strong antioxidant in warding off free radicals. In Table 5 with comparative statistical analysis testing, quartz has antioxidant activity with an average value of IC50 of 72.31 µg/mL which shows the presence of strong antioxidants because quartz can produce phenoxyl radicals that are stabilized by the rationalization effect of the aromatic ring, so that it can ward off free radicals (Haeria, 2013).

### Conclusion

The results showed that leaf ethanol extract was toxic at a dose of 2.000–5.000 mg/kgBB against the diameter of lymph organ pulp, organ index and organ weight, there were symptoms of toxicity and purple leaf ethanol extract had strong antioxidant activity with 66.26 µg/mL.

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### Author Contribution

Conceptualization, D., and N.P.D.; methodology, M.; validation, D., and J.T.; data analysis, N.P.D., investigation, J.T.; resources, M and D.; data curation, Y.S and J.T.; writing – original draft preparation, D and N.P.D.; writing -review and editing, M.R.; supervision, M and J.T.; all author 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.

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