

The Macroscopic Measurement Of The Liver And Kidney Of Male Mice (*Rattus Norvegicus Berkenhout*) Treated With *Neptunia Plena* Lour Leaf Extract

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Abstract

*The Supan Supan Laki plant (*N. plena* Lour) has been used traditionally by a group of people in Pal Batu Village, Paminggir District, North Hulu Sungai Regency as a herbal medicine to increase male sexual activity. Based on previous research, this plant has been proven to have a positive effect in increasing fertility in test animals. However, until now, there is no certainty regarding the safety of using this plant. This study aims to examine the effects and safety of consuming Supan Supan Laki extract on the macroscopic appearance of the liver and kidneys in mice. This research was carried out, there were four treatment groups in this study, namely Na-CMC 0.5%; 87.5mg/KgBW; 175mg/KgBW; and 350mg/KgBW, given for 28 days using a Completely Randomized Design (CRD) trial design. On the 29th day, surgery was performed to observe the liver and kidney macroscopically. Measurement of color, surface and relative organ weight parameters of the kidneys and liver of mice. The results of the study showed that administration of Supan Supan Laki leaf extract caused significant changes in the macroscopic appearance of the liver and kidney organs. In the research analysis using the One Way Anova test, the results of the test showed that the liver ($p>0.05$) did not have a significant level of variation, while the kidney ($p<0.05$) had a significant level of variation so that it could be concluded that the effect of *N.plena* Lour leaf extract have different effects on the relative weight of the Liver and kidney organs.*

Keywords:

N. plena Lour Plants
 Macroscopic Organs
 Male Mice (*Rattus norvegicus Berkenhout*)

Abstrak

Tanaman Supan Supan Laki (*N. plena* Lour) telah digunakan secara tradisional oleh sekelompok masyarakat di Desa Pal Batu, Kecamatan Paminggir, Kabupaten Hulu Sungai Utara untuk obat herbal dalam meningkatkan aktivitas seksual pria. Berdasarkan penelitian sebelumnya, tanaman ini telah terbukti memiliki efek positif dalam meningkatkan kesuburan pada hewan uji. Namun, hingga saat ini, belum ada kepastian mengenai keamanan penggunaan tanaman ini. Penelitian ini bertujuan untuk melihat pengaruh yang ditimbulkan serta keamanan dari konsumsi ekstrak Supan Supan Laki terhadap gambaran makroskopis organ hati dan ginjal pada mencit. Penelitian ini yang dilakukan terdapat empat kelompok perlakuan dalam penelitian ini, yaitu Na-CMC 0,5%; 87,5mg/KgBB; 175mg/KgBB; dan 350mg/KgBB, yang diberikan selama 28 hari menggunakan desain percobaan Rancangan Acak Lengkap (RAL). Pada hari ke 29 dilakukan pembedahan untuk mengamati makroskopis organ hati dan ginjal. Pengukuran parameter warna, permukaan dan berat organ relatif organ ginjal dan hati mencit. Hasil penelitian menunjukkan bahwa pemberian ekstrak daun Supan Supan Laki menimbulkan perubahan yang signifikan pada gambaran makroskopis organ hati dan ginjal. Pada analisis penelitian menggunakan uji One Way Anova hasil pengujian tersebut menunjukkan pada organ hati ($p>0.05$) tidak memiliki tingkat variasi signifikan, sedangkan pada organ ginjal ($p<0.05$) memiliki tingkat variasi yang signifikan sehingga dapat disimpulkan pengaruh ekstrak daun *N.plena* Lour memberikan pengaruh yang berbeda terhadap berat relatif organ jantung dan ginjal.

Kata Kunci : Makroskopis Organ, Tanaman *N. plena* Lour, Tikus Jantan (*Rattus norvegicus Berkenhout*)

PENDAHULUAN

Medicinal plants are plants that have pharmacological and restorative impacts that are beneficial to human well-being. Conventional healing systems are used and preferred throughout the world depending on the choice of religious conviction, superstitions, social elements such as socio-economic reasons, and family-based therapy systems since time immemorial using several terrestrial, aquatic, and semi-aquatic plants (Ozturk & Hakeem, 2019). Although people around the world are interested in

knowing the role of aquatic plants in herbal medicine systems, research on this subject is still rare especially on the aquatic plant *Neptunia Plena* L. This aquatic plant is native to Mexico, Central America and the Northern part of South America but can be grown in Tropical Asia. The synonyms for the supan supan plant are *Mimosa punctate* Lour (1502), *M. plena* Lour (1753), *Desmanthus plenus* (Lour.) wild (1806), *D. punctatus* Willd (1806), *M. lycopodioides* Desf (1807), *Acacia lycopodioides* Des (1814), *D. polyphyllus* DC (1825), *A. punctate* Desf (1829), *Mimosa adenanthera* Roxb (1832), *Neptunia polyphylla* (DC) Benth (1840), *N. surinamensis* Stued (1843), *Desmanthus comosus* A. Kaya (1845) (Csurhes, 2016).

In southern Kalimantan this plant is used as a herbal medicine, especially in the Pal Batu Village area, Paminggir District, Hulu Sungai Utara Regency and the natives call it "Supan-supan Laki". This herbal medicine is believed by the natives to increase male sexual activity. However, plants used for herbal medicine have a variety of chemical compounds, which are not fully known for their therapeutic effects and accidentally, including mild and toxic effects (Dewoto, 2007). So it is crucial to conduct a safe and effective dose test in order to ensure the safety of its use as a medicine. phytochemical screening of *N. plena* Lour. leaves showed the presence of compound classes of alkaloids, saponins, steroids, flavonoids that can increase testosterone hormone. One of the functions of testosterone is to increase spermatogenesis. This study used a *Completely Randomized Design* (CRD) with a negative control treatment (NaCMC 0.5%), and the groups were treated with ethanol extracts of *N. plena* leaves each at a dose of 87.5; 175; and 350 mg kg⁻¹ body weight mice. Male mice was treated orally 2 mL 200-250 g⁻¹ body weight for 14 days. On day 15, all mice were sacrificed by neck dislocation, dissected, testicular organs were taken, weighed, and microanatomy preparations were made using paraffin and *Hematoxylin-Eosin* staining. The parameters can be observed from the spermatogenesis activity, such as testicles weight, testicles seminiferous tubules diameter, and the number of spermatogenic cell layers. The result showed that *N. plena* leaves had the potential to increase the spermatogenesis activity of male mice (Rusmiati et al., 2020).

The test that can be done is to give the test substance regularly, such as once a day or five times a week. This repeated administration aims to reflect the consumption pattern of supan-supan leaf extract by humans. This is important because therapy using herbal medicines often takes place over a long period of time, which can lead to the accumulation of toxic effects. Toxicity testing involves revealing the species-, organ-, and dose-specific toxic effects of the product under test. The toxicity of a substance can be observed by (a) analysis of accidental exposure to the substance, (b) in vitro studies using cells or cell lines, and (c) in vivo experiments involving exposure to experimental animals (Parasuraman, 2011). Evaluation of examinations involving body organs on toxicity effects such as the liver and kidneys that are most affected by certain chemicals including toxic substances (Bassan et al., 2022). In the Liver, toxic substances undergo transformation into non-toxic forms and become water-soluble, facilitating the excretion process. The presence of toxic substances in the Liver can cause problems with liver cells. The kidneys, as the main organ in filtering and excreting, have an important role in filtering and removing the remains of the body's metabolism including toxic substances that enter the body through the formation of urine (Lu, 2010).

This study aims to evaluate the safety impact of using ethanol extract from Supan-supan Laki (*N. plena* Lour) leaves through macroscopic analysis of liver and kidney. In addition, this study is expected to provide insight into the safety of using *N. plena* Lour leaves, which have previously been shown to improve the quality of spermatogenesis in mice.

METODE

In making the extraction of *N. plena* Lour leaves, young leaves were selected and then cleaned with running water to remove dirt. The leaf, lets were separated from the leaflets by dissection and then dried until the leaves were crushed when grasped. The dried leaf samples were then pulverized with a blender and filtered with a 60mesh sieve to obtain a homogeneous fine powder. Powdered leaves of *N. plena* Lour weighed as much as 25 grams. Then wrapped and extracted using 96% ethanol solvent by soklet method. The extract was then separated from the solvent with a rotary vacuum at a temperature

of 50 °C and thickened on a water bath with a temperature of no more than 50 °C until the weight remained. fixed weight. Calculation of extract yield was done with the following calculation as follows:

$$\text{Extract yield} = \frac{\text{weight of extract obtained (gr)}}{\text{weight of extract (gr)}} \times 100\% \quad (1)$$

Preparation of 0.5% Na-CMC solution was made by weighing 500 mg of Na-CMC. NaCMC was put into a glass beaker and then dissolved with hot distilled water of 70 mL. As much as 70 mL, stirred until homogeneous. then put into a 100 mL volumetric flask and added with 100 mL volumetric flask and added akudes to the limit mark. Na-CMC solution, solution in the measuring flask was then stirred until homogeneous.

The study was conducted using a completely randomized design (CRD) involving 24 male Wistar line mice aged 6-8 weeks with body weight ranging from 100 to 200 grams. They were divided into four dose groups (I, II, III and IV): Na-CMC 0.5%, the group given leaf extract 87.5 mg/kg BW dose, 175 mg/kg BW dose, and 350 mg/kg BW dose. The *N. plena* leaf extract was administered to the mice for 28 days, with the exception that they were first fasted to enhance absorption before administration of the extract. On day 29, all mice were tested with ketamine and given neck dislocation. Then, liver and kidney organs were removed for macroscopic analysis.

Prior to organ harvesting, mice were allowed to fast for 12 hours before removal. The mice were anesthetized using ether placed on a cotton swab in a desiccator. Afterwards, the mice were removed and placed in a supine position on a paraffin bath. Dissection was performed from the abdomen to the thorax. Liver and kidney organs were removed and cleaned using NaCl solution (Anggraeni et al., 2017). Then the liver and kidney organs were observed for color and surface texture. Normal organs have a brownish red color, while abnormal ones have a paler color and a mottled surface. Measurement of the relative weight of organs was carried out by weighing the organs after drying with absorbent paper, then directly weighed to obtain the absolute weight. The method of measuring the relative weight of organs refers to the guidelines from BPOM (2014) then Data relative organs weight were analyzed statistically using SPSS v.21 Anova analysis, which can be obtained by the following formulation:

$$\text{Relative organs weight} = \frac{\text{absolute organ weight}}{\text{body weight}} \quad (2)$$

HASIL DAN PEMBAHASAN

The results showed that the administration of *N. plena Lour* leaf extract to male wistar mice resulted in changes in liver and kidney organs along with an increase in the dose of *N. plena Lour* leaf extract administration based on macroscopic measurements of male mice with dosing for 1 week once which was observed for color, surface, and relative organ weight. The results of macroscopic observations can be seen in **Figure 1**.

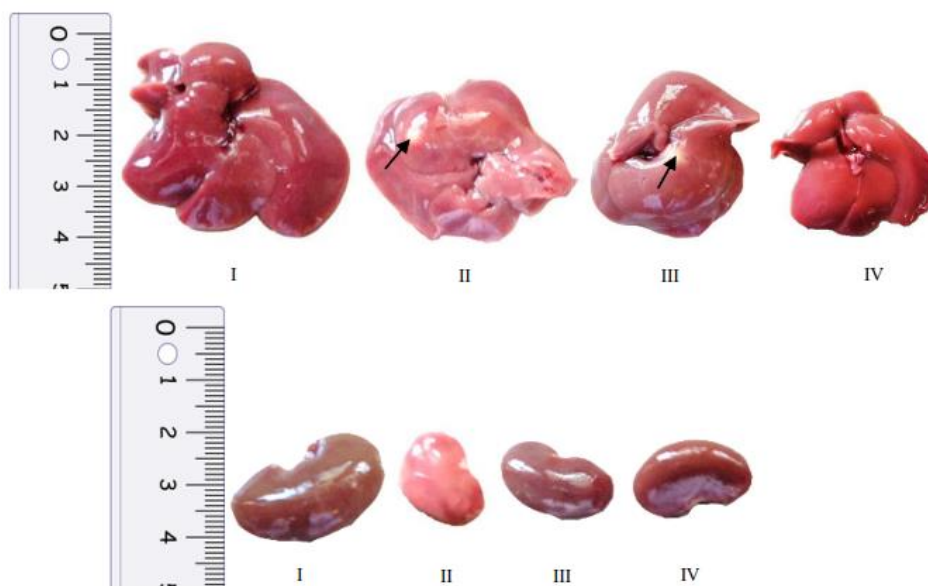


Figure 1. Macroscopic Liver and Kidney Organs. Group I (Na-CMC 0.5%), Group II (87.5 mg/kgWB), Group III (175% mg/kgWB), Group IV (350% mg/kgWB).

The results recorded in **Figure 1** show that the liver and kidney organs from groups II and IV showed a pale red color with spots on the organ surface, while organs from group I had a brownish red color without spots on the organ surface. The study continued with the weighing process of the organs to evaluate the relative weight of the organs. Observations of the relative weights of the liver and kidney are presented in **Table 1**.

Table 1. The mean of relative organ weight measurements of kidney and liver organs of male mice after administration of *N. plena Lour* extract for 28 days.

Group	Percentage of Relative Organ Weight ^b (Mean±SD)	
	Liver	Kidney
I (Na-CMC 0.5%)	3.83±0.52	0.89±0.14 ^b
II (87.5 mg/kgWB)	3.27±0.59	0.66±0.05 ^a
III (175 mg/kgWB)	3.35±0.80	0.69±0.15 ^a
IV (350 mg/kgWB)	3.31±0.41	0.70±0.07 ^a

Note: Numbers followed by different letters (^{a,b}) indicate there is a significant difference between groups, while numbers followed by the same letter (^{a,a}) in the same column are not significantly different ($\alpha = 5\%$).

Observations of macroscopic features of the liver and kidneys included color, surface, and relative organ weights after administration of *N. plena Lour* leaf extract. Observation of the color of the liver and kidneys showed that after dosing the extract, the organs showed a pale red color with spots on the surface, while in the control group or group I, no spots were found, and the organs were brownish red. This is consistent with research conducted by (Meliawati, 2018) and (Sutomo et al., 2019) regarding the dosing of ethyl acetate extract from Kasturi fruit (*Mangifera casturi Koesterm*), which showed differences in color and surface between the control group and the experimental dose group. (Robinson & Kumar, 1992) explained that normal organs have a brownish red color and a smooth surface, while abnormal organs experience changes in color and surface.

Further observations were made by weighing the Liver and kidney to determine the percentage of relative organ weight. The average percentage of relative liver and kidney weights showed higher values in the control group compared to the extract dose group. The administration of *N. plena* Lour leaf extract produced smaller values of relative organ weights of the Liver and kidneys compared to the control. The administration of *Mimosa pigra* Linn. root extract may cause an increase in the value of relative organ weights of the Liver and kidneys (Afieroho & Shorinwa, 2016). The decrease in the percentage of relative organ weights may be due to the *ad-libitum* feeding and drinking, which increased the body weight of the test animals without causing a proportional increase in organ weights. (Sihombing & Sulistyowati, 2011) and (Herlinda, 1986) explained that the body weight of 1-month-old Wistar mice can increase up to 103% by feeding 54% carbohydrates. The decrease in the percentage of organ weight can also be caused by the effect of increasing age, which is not directly proportional to organ weight. This is consistent with a study by (Fukuda et al., 2004), who found a decrease in organ weights of Liver, kidney, lung, spleen, pancreas, testes, and ovaries with increasing age in three different strains of Liver raised under certain pathogen-free conditions, namely *Spontaneously Hypertensive Mice* (SHR/Izm), *Stroke-prone 9WKY/Izm*, and *Wistar Kyoto mice* (WKY/Izm).

The results of the percentage of relative organ weight were statistically analyzed with the normality and homogeneity tests. The *Kolmogorov-Smirnov* test results showed the value of significance values of relative organ weight of Liver and kidney were 0.507 and 0.618 which means the data is normally distributed. Furthermore, *Levene's* test was conducted test showed the significance value of Liver and kidney weight of 0.588 and 0.072, so that the data is homogeneously distributed.

The next test was carried out by testing the variance with the *One Ways Anova* table. The results obtained by the Anova test showed the significance value of organ weight organ weight > 0.05, while the significance of kidney organ weight < 0.05, which means that the organ weight of the Liver organ weight has no variation while kidney organ weight has variation.

KESIMPULAN

Based on the results of the study of Macroscopic Measurements of the Liver and Kidney of Male Mice (*Rattus norvegicus Berkenhout*) given *Neptunia plena* Lour Leaf extract, it shows that the results of statistical analysis of data on the relative organ weights of the Liver and kidneys have a normal and homogeneous distribution. *One Way Anova* test shows that there is no variation in organ weights in Liver organ weights, but kidney organ weights have significant variations. This indicates that the effect of *N. plena* Lour leaf extract gives a different effect on the relative weight of the Liver and kidney organs.

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