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Research Article

# Acute Oral Toxicity Evaluation of *Biophytum umbraculum* Welw. Ethanolic Extract

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**ABSTRACT**

*Biophytum umbraculum* Welw. (Syn. *Biophytum petersianum*) is a traditional Papuan plant known for its antioxidant and stamina-enhancing properties *Biophytum umbraculum* Welw. contains diverse phytochemicals, which contribute to its protective effects against oxidative stress and its potential to support reproductive health. This study aims to evaluate the acute oral toxicity of *Biophytum umbraculum* Welw. (Syn. *Biophytum petersianum*) Ethanol extract in mice (*Mus musculus* Balb/c). A fixed-dose method was used with treatment groups receiving single oral doses of 300, 2000, and 5000 mg/kg body weight (BW), alongside a negative control group receiving 0.5% Na CMC. Clinical signs, mortality, body weight, and organ indices were monitored for 14 days following administration.. No abnormal clinical signs observed except mild, transient grooming, which is considered a normal behavioral response in mice in preliminary test. In the main test and limit test, only ptosis (2000 mg/kgBW) and piloerection (5000 mg/kgBW) were found. All groups showed consistent increases in body weight. No significant organ toxicity observed ($p>0.05$), except spleen ($p<0.05$).The ethanol extract of *B. umbraculum* is practically non-toxic at doses up to 5000 mg/kg body weight. It is safe for further pharmacological investigation and potential development as an herbal therapeutic agent.

**Keywords:** acute toxicity; *Biophytum umbraculum*; body weight ; fixed dose; *Mus musculus*

## INTRODUCTION

Indonesia is a megabiodiversity country with abundant biological resources, including various medicinal plants that communities have used for generations as traditional medicine. In addition, it is believed that medicines made from natural ingredients have almost no side effects. Rural communities have the skills to use plants as medicine because they have local knowledge (Novianti, 2017). One plant that has long been used is *Biophytum umbraculum* Welw., which belongs to the Oxalidaceae family. This shrub is commonly found in the Kebar District at an altitude of 500–600 meters above sea level, Manokwari Regency, West Papua. For a long time, people have used this plant as a traditional medicine through simple processing for various health needs (Azlina, 2023).

Analysis of the phytochemical profile of B. *umbraculum* indicated that this plant contains diverse secondary metabolites, including flavonoids, tannins, saponins, and steroids, compounds that are believed to play a key role in its biological functions (Rahmawati, 2020). Flavonoids, especially quercetin, can function as phytoestrogens, able to bind to estrogen receptors. Steroids and saponins function as precursors to steroid hormone biosynthesis, which aids reproductive function (Nurhikmah et al., 2023).

Acute toxicity tests evaluate the toxic effects after a single dose is administered to test animals within a short period (OECD, 2017). The standard limit test (dose limit) used is 2000 mg/kgBW, according to OECD test guidelines 420, 423, and 425. However, to ensure the safety category of the extract, the test can be

conducted up to 5000 mg/kgBW in some situations. This high dose is only justified scientifically, such as if toxicity symptoms do not appear at a dose of 2000 mg/kgBW (OECD, 2017).

In a study conducted by Sambodo et al. (2019), n-hexane extract of kebar grass in Wistar rats did not cause death at a dose of 5,000 mg/kg body weight. Therefore, the $LD_{50}$ value is estimated to be more than 5,000 mg/kg and is classified as low toxicity (GHS category 5). In a study by Fana (2023), ethanol extract from 70 % kebar grass was found to cause no toxic symptoms or death at a dose of 2,000 mg/kg BW. These results indicate that kebar grass has a low acute toxicity level and is relatively safe for use at the reported test dose limit.

Based on these findings, this study evaluated the acute toxicity of 96 % ethanol extract of kebar grass at test doses of up to 5,000 mg/kg BW in mice. The use of 96 % ethanol solvent is expected to extract secondary metabolite content more effectively. Therefore, the objective of this study was to evaluate the acute toxicity of the 96 % ethanol extract of kebar grass by observing clinical signs, mortality, changes in body weight, and macroscopic conditions of organs, including the liver, heart, kidneys, lungs, and spleen.

## METHODS

### Tools

The tools used in this study were glassware (Pyrex), analytical scales (Kenko KK-Lab), test animal scales, a 1mL syringe (OneMed), an oral probe (OneMed), a water bath, and a mortar and pestle.

### Materials

The materials used in this study were an ethanol extract of *B. umbraculum*, distilled water (CV. Surya Artathama), 0.9 % NaCl physiological solution (B. Braun), 10 % neutral buffered formalin (Paraform indopath), CMC-Na powder (Sigma), and 22 female mice (*Mus musculus*).

### Ethical Considerations for Test Animals

Animal testing before treatment should be conducted in accordance with ethical standards. The animals tested have received ethical approval from the ethics committee of the Faculty of Mathematics and Natural Sciences, Pakuan University, with number 019/KEPHP-UNPAK/07-2025.

### Preparation of Test Solutions

Suspension of 0.5 % sodium carboxymethyl cellulose (Na-CMC) was prepared by weighing 0.5 g of Na-CMC powder and gradually sprinkling it into a mortar containing 20 parts of hot distilled water. The mixture was allowed to stand for 15 minutes to swell, followed by adding 10 parts of distilled water and triturated until homogeneous. The suspension volume was then adjusted to 100 mL with distilled water and stirred until a uniform mass was obtained. For the extract preparation, the required amount of *Biophytum umbraculum* extract was weighed, dissolved in 25 mL of the Na-CMC suspension, and administered orally to mice in a single dose using an oral gavage.

### Preparation of Test Animals

The experimental animals used in this study were 22 female balb/c mice aged 8-12 weeks, weighing 20-30 g, healthy and mature, female mice that had never given birth and were not pregnant. This study used female animals because they tend to be more sensitive than males (BPOM, 2022). Acclimatization is done to adjust test animals to changes in their new environment (Dewi et al., 2017). The body weight of the mice was measured to assess the homogeneity of the test animals, and the Coefficient of Variation (CV) was determined. Animals were considered uniform when the CV was less than 15%. Before starting the experiment, the mice underwent a 7-day acclimatization period (BPOM, 2022).

### Acute Oral Toxicity

Acute toxicity testing was conducted per the guidelines of BPOM (2022) regarding in vivo nonclinical toxicity testing. The fixed dose method was chosen because it is more ethical and efficient. The experiment was conducted on 22 female mice divided into four treatment groups: one negative control group with 0.5% Na-CMC and three dose treatment groups. The acute toxicity test was conducted in stages, starting with a preliminary test, then the main and limit tests. One mice was used in the 300 mg/kgBW and 2000 mg/kgBW dose groups in the preliminary test phase. Next, in the main test, five mice were used in the 300 mg/kgBW, 2000 mg/kgBW, and control (0.5% Na-CMC) groups. In the limit test, five mice were used in the 5000 mg/kgBW group. Before administration and testing, mice were acclimatized for 5-7 days. Before treatment, mice were fasted for 3-4 hours to prevent food interaction with the active ingredients of the *B. umbraculum* extract, which could affect the manifestation of toxicity (Yesi et al., 2021). After fasting, the mice were weighed and given kebar grass ethanol extract and 0.5% Na-CMC in a single dose of 1 mL using a feeding tube according to the dosage variation used. After treatment, food could be given again after 1-2 hours.

### Experimental Animal Observation

#### *Clinical Symptoms and Mortality*

The animals were observed for at least the first 30 minutes after administration of the test preparation,

periodically every 4 hours during the first 24 hours, and once a day thereafter for 14 days. Clinical symptom parameters included motor activity, grooming, convulsions, lethargy, hanging, tremors, piloerection, lacrimation, straub, urination, defecation, ptosis, corneal reflex, pineal reflex, and death for 14 days.

***Body Weight of Mice***

Weight observation shall be performed daily for 14 days after test preparation. Measurements are made by weighing the weight of a mouse using an animal scale. Weight measurements for analysis are made before and after treatment, once every seven days on days 0, 7, and 14 after treatment.

***Absolute and Relative Weight of the Mice's Organs***

The surviving test animals were euthanized by $CO_2$ exposure in a chamber and dissected at day 15. After dissection, the mouse organs were separated from the attached fatty tissue and weighed using digital scales to obtain their absolute weight. The measured organs are the heart, kidney, liver, lung, and spleen. Subsequently, the relative weight of the organs was measured from the organs' absolute weight and the mice's weight on the last day of observation. (Rahman et al., 2016). The % relative organ weight (%ROW) is calculated using equation 1.

$$\%\ \mathrm{ROW} = \frac{absolute\ organ\ weight}{body\ weight} \times 100\% \qquad (1)$$

**Data Collection and Analysis**

The toxicity observation data are presented in descriptive tables for each treatment group. Analysis of the test animals' body weight began with *Shapiro-Wilk* normality testing and *Levene's test* for variance homogeneity. If the assumptions were met ($p>0.05$), the data were analyzed using *Repeated Measure* ANOVA to evaluate the effect of observation time and treatment between groups, followed by a *Post-Hoc Bonferroni* test if there were significant differences ($p<0.05$). For organ weight, normality was tested using the *Shapiro-Wilk*, followed by *One-Way Analysis of Variance* (ANOVA) at a 95 % confidence level, followed by *Post-Hoc Tukey*-HSD test. Results are presented in the form of mean ± standard deviation (SD), and the analysis was performed using SPSS software version 29.

## RESULTS AND DISCUSSION

This study aims to evaluate the acute oral toxicity of the ethanol extract of *B. umbraculum* in mice (*Mus musculus*). Acute oral toxicity testing is conducted to determine the toxic effects of test substances following oral administration, either as a single dose or repeated doses, within 24 hours (BPOM, 2022). The treatment groups consisted of a negative control (Na-CMC), a dose of 300 mg/kgBW, a dose of 2000 mg/kgBW, and a dose of 5000 mg/kgBW. The negative control group was included to provide a comparison between test animals that did not receive the extract and those that did. The evaluation of toxic signs was based on visual observation, which is inherently subjective; therefore, comparisons were made with the behavioral patterns of animals during the acclimatization phase or those in the negative control group (Fithria et al., 2018).

***Observation of Clinical Symptoms and Mortality***

Clinical symptoms were monitored during the preliminary, main, and limit test phases, shown in Table 1. At doses of 300 mg/kg BW, doses of 2000 mg/kgBW (preliminary test) and negative control na-cmc, dose of 300 mg/kgBW (main test), no abnormal clinical signs observed except mild, transient grooming, which is considered a normal behavioral response in mice were found after 14 days of administration. At doses of 2000 mg/kgBW (main test) and 5000 mg/kgBW (limit test), ptosis and piloerection appeared within 30 minutes after administration. However, these symptoms did not result in clinically significant alterations. No further clinical signs were detected during the 14-day observation period.

**Table 1.** Results of Clinical Symptom Observations

| Treatment Group | Dose (mg/kgBW) | Time of Incident | Clinical symptoms |
|---|---|---|---|
| Preliminary Test | Dose 300 | 14 days after administration | No abnormal clinical signs observed |
| | Dose 2000 | 14 days after administration | No abnormal clinical signs observed |
| Main Test | Negative control Na-CMC | 14 days after administration | No abnormal clinical signs observed |
| | Dose 300 | 14 days after administration | No abnormal clinical signs observed |
| | Dose 2000 | ½ hour after administration | ptosis |
| Limit Test | Dose 5000 | ½ hour after administration | piloerection, ptosis |

Grooming frequency decreasing indicates pressure on the central nervous system, while an increase in frequency indicates stimulation of the central nervous system (Amal et al., 2022). The 5000 mg/kgBW dose limit test showed signs of piloerection, and ptosis ½ hour after administration, but no significant changes were observed. Ptosis is a symptom in which the test animal closes its eyes, as if sleepy, with the eyelids half or completely closed. This symptom may be caused by the sedative effect of the test drug administered to the test animal (Amal et al., 2022). Piloerection refers to the standing up of hairs on a mouse's body due to its sensitivity to touch (Majid et al., 2023). Grooming does not automatically reflect toxicity, but can arise due to stress or anxiety. If it occurs only at the beginning, is short, and is not followed by other clinical signs, then this behavior is more likely a normal reaction to the environment than a toxic effect (Liu et al., 2021).

The mortality was recorded in any group, including control, at doses of 300 mg/kgBW, 2000 mg/kgBW, and 5000 mg/kgBW. The ethanol extract was well tolerated up to 5000 mg/kgBW, with no observed lethal effects. According to OECD (2001) criteria and the classification of Loomis and Hayes (1996), compounds with oral $LD_{50}$ exceeding 5000 mg/kgBW are considered practically non-toxic.

***Observation of Mice's Body Weight***

The results of the mice weight observations based on the mean ± standard deviation (SD) can be seen in **Table 2**. Observations show that there was an increase in weight after administration of the test preparation. According to the Repeated-Measures ANOVA, treatment groups did not differ significantly ($p>0.05$). In contrast, body weight varied significantly across observation periods ($p<0.05$), with a consistent increase over time.

The body weight of the test animal was measured one week after administration of the extract and again at the end of the test period (Amelia et al., 2024). Across all treatment groups, average body weight recorded on days 0, 7, and 14 consistently increased. The body weight of test animals can physiologically increase and decrease due to various factors, such as feed intake, activity, and daily metabolic conditions. *Shapiro-Wilk* normality testing indicated that body weight data were normally distributed ($p>0.05$, and Levene's test verified homogeneity of variance among groups ($p>0.05$). Repeated measures ANOVA detected a significant increase in body weight from day 0 to day 7 in all groups ($p<0.05$), as shown in Table 2. The increase in body weight of the mice indicated normal growth during the study and did not indicate any significant physiological disturbances. No significant differences in body weight were found between groups receiving the same treatment on individual days ($p>0.05$). The ethanol extracts of *B. umbraculum* at doses of 300, 2000, and 5000 mg/kgBW did not significantly affect body weight compared tonegative control group. These findings are consistent with OECD Guideline 423 (2001), which states that stable weight gain suggest absence of metabolic or toxic effects. The results support previous reports indicating that herbal extract in high doses do not induce significant weight loss and may be considered safe from a toxicological perspective (Smith et al., 2018; Rahman et al., 2020).

***Observation of the Absolute and Relative Weight of Mice's Organs***

Organ weights were measured at necropsy to assess potential toxic effects of the test compound. Organs weighed included the liver, heart, kidneys, stomach, lungs, and spleen. Both absolute organ weights and relative organ weights—calculated as (organ weight/body weight) × 100%—were evaluated to account for differences in body size among animals. The liver, as a central metabolic organ, is particularly sensitive to toxicants and commonly analyzed in toxicity studies.

**Table 2.** Average body weight of mice's

| Treatment Group | Average Body Weight of Mice (gram) ± SD (n=5) | | |
|---|---|---|---|
| | Day 0 | Day 7 | Day 14 |
| Negative control Na-CMC | $24.8 \pm 1.78^{a}$ | $26.8 \pm 2.48^{b}$ | $28.2 \pm 2.04^{c}$ |
| Dose 300 mg/kgBW | $26.0 \pm 2.00^{a}$ | $28.8 \pm 2.16^{b}$ | $31.4 \pm 3.78^{c}$ |
| Dose 2000 mg/kgBW | $24.4 \pm 1.14^{a}$ | $25.0 \pm 2.54^{b}$ | $25.2 \pm 3.27^{c}$ |
| Dose 5000 mg/kgBW | $25.0 \pm 1.22^{a}$ | $26.0 \pm 1.87^{b}$ | $27.0 \pm 2.54^{c}$ |

Note: The same superscript letter in the same column indicates no significant difference between treatment groups ($p>0.05$).

**Table 3.** Average absolute and relative weight data of the mice's organs

| Parameters | Treatment Group | | | |
|---|---|---|---|---|
| | Negative control Na-CMC | Dose 300 mg/kgBW | Dose 2000 mg/kgBW | Dose 5000 mg/kgBW |
| Absolute weight (g) | | | | |
| Liver | 1,37 ± 0,10[a] | 1,43 ± 0,21[a] | 1,57 ± 0,27[a] | 1,58 ± 0,32[a] |
| Heart | 0,14 ± 0,01[a] | 0,14 ± 0,01[a] | 0,13 ± 0,01[a] | 0,13 ± 0,03[a] |
| Spleen | 0,22 ± 0,10[a] | 0,20 ± 0,03[a] | 0,45 ± 0,26[b] | 0,36 ± 0,14[b] |
| Lung | 0,17 ± 0,02[a] | 0,22 ± 0,06[a] | 0,21 ± 0,04[a] | 0,20 ± 0,02[a] |
| Kidney | 0,15 ± 0,01[a] | 0,18 ± 0,04[a] | 0,16 ± 0,03[a] | 0,17 ± 0,03[a] |
| Relative weight (%) | | | | |
| Liver | 4,86 ± 0,26[a] | 4,57 ± 0,35[a] | 6,27 ± 0,50[b] | 5,81 ± 0,74[b] |
| Heart | 0,50 ± 0,09[a] | 0,46 ± 0,07[a] | 0,54 ± 0,06[a] | 0,47 ± 0,08[a] |
| Spleen | 0,80 ± 0,36[a] | 0,66 ± 0,15[a] | 1,73 ± 0,82[b] | 1,34 ± 0,50[b] |
| Lung | 0,61 ± 0,07[a] | 0,71± 0,15[ab] | 0,86 ± 0,11[a] | 0,77 ± 0,04[ab] |
| Kidney | 0,54 ± 0,07[a] | 0,58 ± 0,07[a] | 0,65 ± 0,09[a] | 0,63 ± 0,10[a] |

Note: Different superscript letters on the same row indicate significant differences between treatments ($p<0.05$).

Table 3 shows the results of the absolute and relative weights of mice organs. The absolute weights of the liver, heart, lungs, and kidneys did not differ between groups, except for the spleen, which differed significantly. Regarding relative weight, the liver at doses of 2000 and 5000 mg/kgBW increased significantly compared to the control and 300 mg/kgBW, while the heart and kidneys did not differ significantly. The spleen and lungs showed a tendency to change without significant differences.

Changes in organ weights, particularly relative weights, serve as sensitive indicators of organ-specific toxicity and aid in discerning direct compound effects from systemic changes in body weight. Organ weight data are considered essential parameters alongside histopathological and clinical chemistry measures in toxicology studies to comprehensively evaluate the safety profile of test substances.

Test animals were weighed and the organs were dissected and their weights measured, including the heart, liver, kidneys, stomach, lungs, and spleen, at the end of the 14-day observation period. The liver, recognized as a key metabolic organ frequently affected by toxicants, was of particular interest (Kluxen, 2019). Both absolute and relative organ weights were assessed, with relative organ weight calculated as the absolute organ weight divided by body weight, multiplied by 100 % (Mihmidati & Athiroh, 2017).

Normality tests (Shapiro-Wilk) confirmed that absolute organ weight distribution was normal ($p>0.05$), except for heart weight, which failed normality and homogeneity tests and was analyzed using non-parametric methods. Organ weights of the liver, lungs, and kidneys showed homogeneity, permitting analysis by One-Way Analysis of Variance (ANOVA), while heart weight data were subjected to the Kruskal-Wallis test followed by post-hoc *Mann-Whitney U* test. Relative organ weights of liver, heart, lungs, and kidneys passed homogeneity assumptions and were analyzed by One-Way ANOVA with Tukey-HSD post-hoc comparisons. Spleen weights, which were non normally distributed, were analyzed by Kruskal-Wallis tests with subsequent Mann-Whitney U post-hoc analysis.

The absolute weights of the liver, heart, lungs, and kidneys between control and treated groups at doses of 300, 2000, and 5000 mg/kgBW was not significant. However, groups treated with 2000 and 5000 mg/kgBW exhibited higher absolute weights compared to controls and the 300 mg/kgBW group. Relative liver weight increased significantly in the 2000 and 5000 mg/kg BW dose groups compared to controls and the low-dose group. A slight increase in liver weight that is still within the physiological range is considered a common adaptive response in acute exposure. Several studies have shown that mild hepatocellular hypertrophy can reflect metabolic enzyme induction or increased detoxification activity, without indicators of hepatic damage (Hall et al., 2012). Since no histopathological changes or significant clinical manifestations were found, the liver changes in this study tended to be adaptive rather than toxic. The increase in spleen weight observed in the high-dose group, both absolute and relative to body weight, potentially reflects an adaptive immunological or hematopoietic response rather than a direct toxic reaction. This interpretation is reinforced by the

absence of clinical or histopathological findings indicating tissue damage. The toxicology literature states that an increase in lymphoid organ weight is often associated with lymphoid cell hyperplasia or increased hematopoietic activity, which is a physiological response to xenobiotic exposure and does not always indicate adverse changes (Elmore, 2006). Thus, changes in spleen weight that are not accompanied by structural abnormalities can be categorized as non-adverse changes. Heart and kidney weights remained unchanged across all doses. Interestingly, lung weights were significantly elevated only at medium dose (2000 mg/kgBW), exceeding both the control and high-dose groups. These findings strengthen the general safety profile of the extract, but suggest the need for additional subchronic studies, including tissue histopathology, to fully ensure the safety of the organs.

## CONCLUSION

Ethanol extract of *Biophytum umbraculum* Welw. to female mice (*Mus musculus*) showed no signs of toxicity based on clinically observed behavioral changes. Although a slight increase in average body weight was observed, this change was not statistically significant. There were also no significant differences in organ weights across treatment groups In the limit test at the highest dose of 5000 mg/kg BW, no mortality or significant toxic symptoms were observed. These results indicate that the ethanol extract of *B. umbraculum* is relatively safe at doses of up to 5000 mg/kg BW, as it does not appear to induce acute toxic effects in female mice.

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