



# Sub-Acute Toxicity Test of Red Betel Leaves (*Piper crocatum*) on Liver Function in Male White Mice (*Mus musculus*) Using SGPT Test

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## Abstract

**Background:** This research aims to investigate the sub-acute toxicity of red betel leaves (*Piper crocatum*) on liver function in male white mice (*Mus musculus*) using a serum glutamic-pyruvic transaminase (SGPT) test on a clinical photometer instrument. **Methods:** This research aims to investigate the sub-acute toxicity of red betel leaves (*Piper crocatum*) on liver function in male white mice (*Mus musculus*) using a test serum Glutamic Pyruvic Transaminase (SGPT) assay on a clinical photometer instrument. In this study, an experimental method was employed using a randomized block design, with 72 mice serving as research subjects and divided into eight treatment groups. Group 1 was given 0.5% Na CMC, group 2 as a positive control; group 3 was given a comparison of metformin; group 4 was given an extract with a dose of 100 mg/KgBB, group 5 was given the N-Hexane fraction with a dose of 100 mg/KgBB, group 6 was given the Ethyl Acetate fraction with a dose of 100 mg/KgBB, group 7 was given the N-Butanol fraction with a dose of 100 mg/KgBB, group 8 was given the Residual Water Fraction with a dose of 100 mg/KgBB, the treatment was carried out for 2, 4, 6 days, in the SGPT test used was serum. **Results:** The administration of extracts and fractions to animals did not cause Subacute Toxicity effects on the livers of male white mice. Looking at the SGPT (Serum Glutamic Pyruvic Transaminase) parameters, the activity of which was still within normal conditions, namely 25-200  $\mu$ /l. **Conclusions:** did not show any Subacute Toxicity effects as seen from the activity of the SGPT enzyme in the blood of male white mice (*Mus musculus*).

**Keywords:** Ethyl acetate Fraction; n-butanol Fraction; n-hexane Fraction; Residual Water Fraction; SGPT Test

## Introduction

The toxicity test is a method to observe the pharmacological activity of a compound. The principle of the toxicity test is to assess bioactive components that are toxic when administered in high doses and can be a drug when administered in low doses. Foreign substances or compounds in the environment can be absorbed into the body through diffusion and directly affect the body's balance (Aufia et al., 2018). Toxicity testing is also a pharmacological test conducted to determine the level of toxicity or safety of using a substance or ingredient in treatment (Rachman et al., 2021).

Sub-acute toxicity testing can provide information on signs of toxicity, such as changes in activity, body weight, and organ weight, as well as changes in liver function, as observed through SGPT (Serum Glutamic Pyruvic Transaminase) parameters (Sudimartini et al., 2021). The liver is an organ that plays a role in metabolic and excretory functions in the body. One of the signs of liver damage is hepatocyte necrosis, which releases several enzymes from the hepatocyte cytoplasm to the extracellular space (Sudimartini et al.,



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2021).

Red betel is a plant native to Peru, which has since spread to several regions worldwide, including Indonesia. Red betel is a shrub tendrils and joints (Kohar & Kartini, 2016). Red betel contains flavonoids, alkaloids, polyphenolic compounds, tannins, and essential oils. The above compounds are known to have antibacterial properties (Rachmawaty et al., 2009).

In previous research, specifically the Antibacterial Activity Test of Red Betel Leaves (*Piper crocatum*) Against the Growth of *Propionibacterium acnes*, the antibacterial activity test was conducted using the disc diffusion method. The test concentration for the Minimum Inhibitory Concentration was determined at the lowest concentration of the Inhibitory Power Diameter value. The results of the Inhibitory Power Diameter test showed inhibitory activity against *Propionibacterium acnes* bacteria at concentrations ranging from the lowest to the highest, namely 9.53 mm (10% concentration), 10.36 mm (15% concentration), 10.50 mm (20% concentration), and 10.90 mm (25% concentration). The presence of inhibitory activity against *Propionibacterium acnes* suggests that red betel leaves contain several compounds that play an essential role as antibacterials, such as alkaloids, flavonoids, and tannins. The method used in the Minimum Inhibitory Concentration test is the solid dilution method. The negative control used was 100% dimethyl sulfoxide (DMSO). Minimum Inhibitory Concentration test results against *Propionibacterium acnes* showed no bacterial growth at a concentration of 10%. This indicates that at that concentration, the extract can inhibit the growth of *Propionibacterium acnes* bacteria (Vilya & Rabitha, 2019).

There are various chemical compounds in red betel leaves that enable them to be used in the treatment of diseases such as diabetes mellitus. Based on the results of previous studies, patients with diabetes mellitus have random blood sugar levels of > 200 mg/dL before being given boiled red betel leaf water. The average GDS value obtained before being given boiled red betel leaf water is more than 200 mg/dL. The average GDS value obtained after consuming boiled red betel leaf water is less than 200 mg/dL. So, there is a difference in the GDS levels of diabetes Mellitus patients before and after being given boiled red betel leaf water Effective in reducing blood sugar levels in Diabetes Mellitus patients (Listiana, et al, 2015).

Therefore, this research focuses on the onSub-acute toxicity test of the results of fractionation of red betel leaves (*Piper crocatum*) on the liver of male white mice (*Mus musculus*) against the SGPT test using a clinical photometer instrument,

## Methods

This research was conducted at the Pharmacology and Biology Laboratory of Harapan Ibu Jambi Health College and the Jambi Provincial Health Laboratory Center, which was carried out from March to June 2023. This study employed an experimental method with a randomized block design, using mice as research subjects and examining the effect of red betel leaves on liver safety through sub-acute toxicity using the SGPT test. Seventy-two animals, with a body weight of 20-30 grams and aged 2-3 months, were used in this study. The experiment was divided into eight groups. The tools used in this study were analytical scales (Fujitsu), spuid (one med), oral sonde, bedside knife (allee), surgical scissors, rotary evaporator (BUCHI), centrifuge (EBA 200), photometer (DIALAB), microtube (axygen), surgical tweezers, separating funnel (pyrex), pins, cotton, test tubes (pyrex), tube racks, micropipettes (dragon lab), styrofoam, aluminum foil, watch glass, evaporator cup, dropper pipette. The materials used in this study were 72 male white mice, ethanol, glucose, n-hexane, ethyl acetate, n-butanol, distilled water, Ringer lactate (RL), and picric acid. The sample used in this study was 5 kg of red betel leaves. Determination of the Red Betel Leaf plant (*Piper crocatum*) was carried out at the Plant Systematics Laboratory, Faculty of Biology, Gadjah Mada University. The preparation of 900 grams of sample maceration was carried out by soaking the raw materials, which had been prepared beforehand, in the solvent. The sample was dried first by airing it and

avoiding direct sunlight. After being dried, the sample was chopped and then placed into a suitable solvent in a vessel, where it was left at room temperature for some time. The extract was prepared from dry, powdered leaves of the simplicia by maceration using a 70% Ethanol solvent. After carrying out the extraction with red betel leaf extract, it was then fractionated by liquid-liquid extraction (LLE) using a 1:10 solvent based on its degree of polarity. In this study, blood was used to test SGPT. In this study, statistical analysis was performed using the non-parametric Kruskal-Wallis test, followed by Dunn's test (also known as the Duncan test), to determine which treatment groups were significantly different from the others.

## Discussion

In this study, samples of Red Betel Leaves (*Piper crocatum*) were obtained in quantities of up to 5 kg and then dried in the air. After that, the leaves were chopped using a blender to make them smooth, and then the sample was extracted using the cold maceration method. This involved soaking the sample in 70% ethanol, and maceration was carried out for 3 x 24 hours until the color was clear.

The result of the plant maceration process is a liquid extract, which is then separated using a rotary evaporator until a thick extract is obtained (Sa'adah & Nurhasnawati, (2017). Phytochemical screening aims to determine the compounds contained in the plants to be studied. In the phytochemical screening test, it was found that the red betel leaf plant (*Piper crocatum*) contains Alkaloids, Flavonoids, Saponins, and steroid compounds. In previous studies, Phytochemical Screening tests have been carried out to determine the content of compounds in the red betel leaf plant. The results of the phytochemical screening showed that the red betel leaf plant (*Piper crocatum*) contains Alkaloid, Flavonoid, and saponin compounds (Tandi et al., 2020).

The method chosen for fractionation in this study was the multistage liquid-liquid extraction method by Savitri et al. (2020). Two grams of dry extract were dissolved in 200 ml of water and then added to 100 ml of n-hexane solvent, which formed two phases. The upper phase is taken and collected as the n-hexane fraction. The lower phase is continued by adding 100 ml of ethyl acetate solvent to form 2 phases: the upper phase is taken and collected as the ethyl acetate fraction, and the lower phase is taken and collected and added with 100 ml of N-butanol and forms 2 phases, the upper phase is taken and collected as the N-butanol fraction, and the lower phase is the remaining water fractionation, after which each fractionation is concentrated using a rotary evaporator until thick.

In this study, 72 healthy male white mice (*Mus musculus*) aged 2-3 months, weighing 20-30 grams, were used. Before being treated, the mice were acclimated for 7 days by providing them with food and water regularly. This adjustment aims to prevent the animals from experiencing stress and has allowed them to adapt to the environment. Before being treated, the mice were first fasted for 18 hours.

In this study, the research animals were divided into eight groups, with each group consisting of 9 mice. Alloxan was given intraperitoneally at a dose of 150 mg/kg BW and given to all groups except the negative control group; alloxan was given 3 times until blood sugar levels increased; after blood sugar levels increased, continued with metformin as a comparison with a dose of 78 mg/kg BW in group 3 (comparator), administration of 100 mg/kg BW extract in group 4. Administration of N-Hexane fraction in group 5 with a dose of 100 mg/kgBW, administration of Ethyl Acetate fraction in group 6 with a dose of 100 mg/kgBW, administration of N-Butanol fraction in group 7 with a dose of 100 mg/kg BW and administration of residual water fraction in group 8 with a dose of 100 mg/kgBW, each administration of extract and fraction was given 2 times.

For the treatment carried out on the 2nd, 4th, and 6th day of each group, samples were taken by dislocating the neck vein to draw blood. The SGPT (Serum Glutamic Pyruvic Transaminase) test was performed using serum, with a serum concentration of 22 u/l.

In the treatment, each group was found to have a significant difference in treatment between groups and days 2, 4, 6 and there was no effect of the administration of the preparation and did not cause subacute toxicity effects on the liver, based on the relative weight data of the Liver Organ, the results showed that the weight of the liver organ did not differ between groups and days after administration of the preparation, from the results of this study showed that the administration of extracts and fractionation in animals did not cause Subacute Toxicity effects on the liver of Male White Mice by looking at the SGPT (Serum Glutamic Pyruvic Transaminase) parameters for its activity is still within the normal range of 25-200  $\mu$ /l (Radiastuti et al., 2021)

## Conclusions

Subacute administration of Red Betel Leaf extract and fraction (*Piper crocatum*) to male white mice (*Mus musculus*) with a dose of extract and fraction of 100 mg/kg BW with SGPT (Serum Glutamic Pyruvic Transaminase) testing did not show any Subacute Toxicity effects seen from the activity of the SGPT Enzyme in the blood of male white mice (*Mus musculus*). In liver function testing, no effect was observed in the laboratory results of the SGPT value test, which remained within normal limits, specifically 25-200 u/L.

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## Declaration statement

The authors reported no potential conflict of interest.

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