Effect of red *Piper betle* leaf (*Piper crocatum* Ruiz & Pav.) ethanolic extract on plasma biochemical and hematological parameters *in vivo*

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**ABSTRACT**

**Aim:** The objective of this study was to investigate the effect of oral administration of red *Piper betle* extract at doses of 500 mg/kg BW on plasma biochemical and hematological parameters in the Wistar strain of female rats after long-term use.

**Materials and Methods:** The ethanolic extract of the red *P. betle* extract was administered to Wistar rats for a period of 90 days. After 90 days of treatment, the biochemical and hematological parameters were observed. The effect of extracts on plasma biochemical was assessed by measuring the level of aspartate aminotransferase, alanine aminotransferase, and creatinine. Effects on hematological parameters were assessed on hemoglobin levels, red blood cell count (erythrocytes), white blood cell count (leukocytes), platelets, and hematocrit values.

**Results and Discussions:** The result showed that the levels of liver enzymes and creatinine levels significantly decreased. Meanwhile, the number of erythrocytes, leukocytes, hematocrit, and platelets of the test group tends to be lower than the control group, except hemoglobin. However, all parameters tested were still within the normal limit range.

**Conclusion:** The ethanolic extract of red *P. betle* leaves is selectively toxic to plasma biochemical and hematological parameters at the examined dose or lower.

**KEY WORD:** Biochemical, Extract, Hematological, *Piper crocatum*, Rat

**INTRODUCTION**

Red betel leaves (*Piper crocatum*) are one of the potential medicinal plants that are empirically known to have various medicinal properties to cure various types of diseases. Based on the research, it was known that the ethanol extracts of red *Piper betle* leaves presented the presence of flavonoids, polyphenolics, steroids, quinones, and saponins. Other chemical substances contained in the red betel leaf are hydroxychavicol, kavikol, kavibetol, allylpyrocatechol, carvacrol, eugenol, esimene, simeole, kariofelen, estragole, terpinene, and phenyl propada. Carvacrol is disinfectant, antifungal, so it can be used as an antiseptic to eliminate bad breath and leucorrhea. The results of some studies also showed that the ethanol extract of red piper betel leaves has antibacterial and antifungal activity. In addition, this extract has also been studied to be a natural remedy for wounds, anti-inflammatory, antioxidants, and widely used to cure various diseases such as hypertension, diabetes, stroke, kidney failure, and hepatitis.

The potential of red betel as a multi-functional medicinal plant is highly broad that it needs to be improved in its use as a modern medicine agent. Every new chemical must be examined for its toxic properties before its wide-ranging use, both toxic chemicals and the safety of any chemicals that can enter the body. Since the assessment of xenobiotic traits cannot be performed in humans as is commonly practiced for drugs, therefore xenobiotic studies are conducted on experimental animals. Herbal products are usually considered as safe medicine due to their empirically use in various cultures for a long period of time. However, there are some reports about the cases of its adverse effect after herbal product administration. Thus, the traditional drug toxicity test was expected to reveal the safety associated with its intended use. This study focused on the effect of ethanolic extract of red *P. betle* leaves on hematological and biochemical parameters of rats.
MATERIALS AND METHODS

Plant Materials
The leaves of red Piper betel (P. crocatum Ruiz and Pav.) were obtained from Bogor, Indonesia, and determined in Plant Taxonomy Laboratory of Biology Major, Faculty of Mathematics and Natural Science Padjadjaran University, Bandung, Indonesia.

Chemical Materials
The chemicals used were distilled water (Brataco), ethanol 70%, ether, creatinine reagent (ST Reagensia), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) reagents (ST Reagensia), and Pulvis Gummi Arabicum (PGA).

Animal
Female Wistar rats were used for the toxicology study. The rats were aged about 8–12 weeks and weighing 170–185 g was supplied from School of Life Sciences and Technology, Bandung Institute of Technology, Bandung, Indonesia. The rats were acclimatized to laboratory conditions for 7 days before the experiments. The rats were kept at room temperature of 22 ± 3°C with a 12 h light and dark cycle. During acclimatization, the rats were fed and drank using pellet and water according to laboratory standards.

Extraction
Red betel leaves were thoroughly rinsed using tap water, then chopped, dried and ground to obtain simplicia powder. The simplicia powder of 372.8 g was then extracted by maceration for 3 × 24 h using 70% ethanol as a solvent. The macerates was collected every 24 h, and the evaporation process was performed to obtain concentrated extracts of constant weight.

Phytochemical Screening
The phytochemical tests to detect the presence of alkaloids, flavonoids, quinones, polyphenols, tannins, saponins, steroids, monoterpenoid, triterpenoid, and sesquiterpenoid were performed following the method described by Farnsworth. The analytical response of the test was the precipitate formation and color intensity after the addition of certain reagents.

Toxicity Study
Wistar female Wistar strains were divided into two groups, the control group and the test group. The control group was given a 10% PGA suspension. The test group was given an ethanol extract of red betel leaf dose 500 mg/kg BW in PGA 10% suspension. Administration of the test preparation was performed daily for 90 days, administered orally using syringes and oral sonde volume of 2 mL/200 g of the rat. After 90 days of treatment, the animals were sacrificed, and the blood samples were collected using heparinized tubes and observed for the biochemical and blood parameters. The serum was subjected to biochemical analysis. Blood samples were collected into ethylenediaminetetraacetic acid -tubes for hematological analysis.

Evaluation of Plasma Biochemical
The biochemical observations included the examination of enzyme levels associated with kidney and liver function. The observation of kidney function performed was creatinine while liver function included analysis of AST and ALT. For creatinine determination, a volume of 0.2 ml serum was pipetted into the test-tube and 3 ml of distill-deproteinization water was added to all the tubes and mixed followed by centrifugation for 2500 rpm for 5–10 min. Then, 1.5 ml standard and test solutions were taken and put into the new test tubes followed by adding 0.5 ml of 0.5 N sodium hydroxide and picric acid. They were mixed and allowed to stand at room temperature for 20 min. Absorbances were read at 520 nm.

For AST and ALT level determination, 1.0 ml of the reagent was pipetted into the tube marked standard, and 1.0 ml of distilled water was pipetted into a blank tube. A volume of 1 ml working reagent was pipette and mixed with 1.0 ml of the sample in the sample tube. They were mixed and incubated at 37°C for 1 min. Absorbances were taken at 340 nm.

Evaluation of Hematological Parameters
The hematological parameters were observed on several parameters such as hemoglobin levels, red blood cell count (erythrocytes), white blood cell count (leukocytes), platelets, and hematocrit values. The calculation of the amount of erythrocytes was done using a hemocytometer. Fresh blood was pinned to a mark of 0.5 (on a pipette), then diluted 200 times with a diluent solution (2.5% sodium citrate) and shaken until homogeneous. The mixture was dripped over the hemocytometer for about two drops on object glass and covered by a glass cover, then observed under a light microscope.

The calculation of the number of leukocytes was performed using a hemocytometer. Fresh blood was pinned to a mark of 0.5 (on a pipette), then diluted 20 times with a diluent solution (2.5% sodium citrate) and shaken until homogeneous. Then, the two drops of the mixture were dripped on the glass of the hemocytometer and covered by a glass cover, then observed under a light microscope.

In the calculation of the number of platelets and red blood cells were done using hemocytometer tool. Fresh blood was pinned up to the 1.0 mark (on a pipette), then diluted 100 times using 1% ammonium oxalate solution and shaken homogeneously. About 2–3 drops
of the mixture then dripped on the hemocytometer and covered by a glass cover, allowed to stand for 10 min in a closed Petri dish, hence the platelets settled and could be observed under light microscope.

For hematocrite calculation, the fresh blood that had been obtained dripped into a hematocrit capillary tube and then covered with a candle. Capillary pipes were placed in a micros centrifugation chamber in such a way that the position was balanced. The centrifugation was performed at high speed for 4 min, then the hematocrit value was determined by measuring the high ratio between blood (blood cells and plasma) with blood cells.

The hemoglobin estimation was performed by Sahli’s method. Sahli tubes were filled by 0.1 N hydrochloric acid up to 10% of the tube scale (2.2 g/dL scale), then 20 µL of blood was put into the tube using a pipette, stirred until homogeneous then diluted with 0.1 N HCL until the blend color was the same as the standard color. After the getting the same color, then the amount of hemoglobin was read on the tube scale (g/dL).15

Statistical Analysis
Differences between groups on blood parameters and biochemistry were analyzed using Student t-test with 5% confidence level.

RESULTS AND DISCUSSION
Extraction and Phytochemical Screening Results
Extraction process resulted rendemen extract in 13.24% from 372.8 g of red P. betle leaves simplicia. Phytochemical screening was performed to determine the presence of a secondary metabolite group contained in simplicia and extracts. Based on the results of phytochemical screening of simplicia and extract from red P. betle leaves, it contained of several secondary metabolites such as alkaloids, flavonoids, polyphenols, quinones, and saponins.

Blood Biochemical Results
The blood biochemical tests are one of the most important tests of toxicology studies to understand the mechanisms of the disease process.16,17 In a toxicity study, the most important is the examination of the enzyme levels that play an important role in diagnosing disorders of the liver and kidneys. The observation of liver function was conducted for AST and ALT levels. AST and ALT are cytosolic marker enzymes reflecting hepatocellular necrosis as they are released into the blood after cell membrane damage.18 In this study, the AST and ALT levels of the test group were lower than the control group, and gave a significant difference compared to the control group because the AST and ALT values were <0.000. The results of liver organ function tests were performed in Table 1.

A significant reduction in AST and ALT levels in the plasma indicated that the extract caused a mild change in the liver but not caused liver injury. Because the liver injury is characterized as hepatocellular when there is the predominant elevation of the ALT, meanwhile AST is a mitochondria enzyme whose increased activity in plasma reflects severe tissue injuries.19,20 This data indicated that the red P. betle extract could be a potent herbal drug to improve and preserve liver function.

On the examination of kidney organ enzymes, the results obtained that the creatinine levels of the test group increased, but there was no significant difference compared to the control group because the value of \( P > 0.05 \) [Table 2]. This indicated that the red piper leaf extract did not affect and damage the function of kidney rats. Because a significant elevation of creatinine level signifies impaired kidney function or kidney disease. As the kidneys become impaired, the creatinine level in the blood will rise due to poor clearance of creatinine by the kidneys. Abnormally high levels of creatinine thus warn of possible malfunction or failure of the kidneys.

Hematological results
The analysis of blood parameters included erythrocytes, leukocytes, hematocrit, hemoglobin, and platelet number. The number of erythrocytes, leukocytes, hematocrit, and platelets of the test group tends to be lower than the control group, except hemoglobin [Table 3]. There was no alteration on hemoglobin count in treating animal compared with the control group. Based on statistical analysis, it was found that the difference of blood parameter analysis result from both groups showed no significant difference and still included in the normal range. A nonsignificant decrease in the count of erythrocytes in treating animals with no alteration in hemoglobin count showed that the extract would not lead to anemia and causes no toxic effect on erythrocytes. In this study, the leukocyte counts tend to be decreased than the control group. Even though, the decreased of leukocytes was no significant, but it needs further investigation if the dose of the red P. betle extract was

<table>
<thead>
<tr>
<th>Group</th>
<th>AST (UI/L)</th>
<th>P</th>
<th>ALT (UI/L)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>61.11±0.87</td>
<td>0.63±0.06</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>51.22±0.50</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( P<0.05 \) = significant, *No significant, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

<table>
<thead>
<tr>
<th>Group</th>
<th>Creatinine (mg/dl)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.57±0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Test</td>
<td>0.63±0.06</td>
<td>0.05</td>
</tr>
</tbody>
</table>

\( P<0.05 \) = significant, *No significant
higher than 500 mg/Kg BW. Because a decreased of leukocytes showed the suppression of leukocytes and their production from the bone marrow.\textsuperscript{21-24} The result of platelets count analysis pointed out that the extract gave no significant decline compared with the control group. The value of platelets number of rats after treatment for 90 days decreased, but it remains in normal range. However, it is important to be considered if the dose used more than this examined dose, reduced blood platelets affect the viscosity of blood, which is correlated positively with blood pressure.\textsuperscript{25,26}\ The treatment duration might play a role in the alteration in hemological parameters.

**CONCLUSION**

It could be concluded that the ethanolic extract of red *P. betle* leaves at doses examined had significant ($P < 0.05$) positive effect on the parameters investigated; hence, it is safe on its use as an herbal drug at an examined dose.

**REFERENCES**


**Table 3: Effect of red *P. betle* extract on hematological parameters**

<table>
<thead>
<tr>
<th>Blood parameter</th>
<th>Control</th>
<th>Group</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes ($\times 10^{12}$/mm$^3$)</td>
<td>7.90±0.37</td>
<td>6.77±0.09</td>
<td>0.004*</td>
</tr>
<tr>
<td>Leukocytes ($\times 10^{3}$/mm$^3$)</td>
<td>5.68±0.04</td>
<td>3.80±0.36</td>
<td>0.001*</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>44.33±0.58</td>
<td>40.67±1.15</td>
<td>0.008*</td>
</tr>
<tr>
<td>Hemoglobins (g/dl)</td>
<td>14.13±0.23</td>
<td>14.27±0.12</td>
<td>0.422</td>
</tr>
<tr>
<td>Platelets ($\times 10^{9}$/mm$^3$)</td>
<td>1.96±0.06</td>
<td>1.57±0.10</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

$P < 0.05$ = significant, *No significant, $P$. betle: Piper betle.