Acute and Subchronic Toxicity Assessment of 70% Ethanol Extract of Leaves of Gendarussa (Justicia gendarussa Burm. f.) in vivo

Bambang Pragoso1, Mahluhatus Solehah2, Luthfiatun Kanina3, Hania Plumeriastuti4

ABSTRACT

Background: In Indonesia, Justicia gendarussa Burm. f. have been used in traditional medicine for being anti-inflammatory, antihypertensive, anti-bacterial, anti-fungal, and anti-fertility. Nowadays, the use of J. gendarussa Burm. f. has increased due to the availability of 70% ethanol extract of leaves of J. gendarussa has been developed as an alternative for male anti-fertility. The aim of this study was to determine the acute and sub-chronic toxicity of 70% ethanol extract of leaves of J. gendarussa in vivo were performed in the present study in order to evaluate its safety.

Methods: In the acute toxicity study, a single dose of 2000 mg/kg BW was orally administered to mice (n=10), which were monitored for 24 days. For sub-chronic toxicity study, rats were randomly divided into four groups (n=10). The control group received distilled water, while the experimental groups received a repeated dose of 40 (converting dose from human), 200 (5 times) and 1000 (25 times) mg/kg BW orally for 30 days. At the end of the experiment, blood samples were collected for hematological and biochemical evaluations. Gross pathology and histopathology of liver and kidneys were assessed.

Results: In the acute toxicity study, no mortality or non-observed adverse effect level (NOAEL) observed. In the sub-chronic toxicity study, no significant differences between control and all treated groups in most of the parameters examined, except for the thrombocyte, basophil, neutrophil, lymphocyte, and hematocrit. The biochemical like ALT and creatinine were no change, giving 200 mg/kg BB increase the level of AST, while increasing level of BUN were observed in all treated groups. Additionally, the body weight was not affected in the treated groups compared with the control group. No abnormalities of histopathological changes were observed in the liver and kidney at 200 and 1000 mg/kg BW and at 40 mg/kg BB.

Conclusion: This result suggest that a further research is needed to ensure its safety for clinical study.

INTRODUCTION

Justicia gendarussa Burm.f. (JG), family Acanthaceae, is indigenous to Malaysia, Indonesia and in several other countries in Asia such as Sri Lanka, India, and Malaysia. JG was used in Indian folk medicine for treating many diseases such as rheumatism, bronchitis, fever, eczema, and jaundice. JG has been known and used as a traditional male anti-fertility drug in Papua New Guinea. In vitro and in vivo antifertility tests of JG showed that the possible mechanism was through competitive and reversible inhibition of spermatozoa hyaluronidase. The anti-fertility effects might be caused by the C-glycosyl flavone group with an aglycon base structure. Apigenin and its glycoside vitexin in JG can be used for their anti-inflammatory and antitumor activities. Thus, JG herbal drug has the potential to be developed into a phyto- pharmaceutical product as a non-hormonal male contraceptive.

Acute and subacute toxicity assessment of J. gendarussa was performed using the principle of the Organisation for Economic Co-operation and Development (OECD) guidelines and other toxicological guidelines.

RESULTS

Table 1. Results of biochemical statistical data processing of mice treated with SPSS.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>40 mg/kg</th>
<th>200 mg/kg</th>
<th>1000 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/L)</td>
<td>1.08</td>
<td>1.06</td>
<td>1.05</td>
<td>1.04</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>1.00</td>
<td>1.02</td>
<td>1.03</td>
<td>1.04</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>1.00</td>
<td>1.02</td>
<td>1.03</td>
<td>1.04</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.00</td>
<td>0.02</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>0.18</td>
<td>0.17</td>
<td>0.16</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Table 2. The results of statistical data processing of mice treated with SPSS.

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</tr>
</tbody>
</table>

Table 3. Scoring degree of change in organs

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control</th>
<th>40 mg/kg</th>
<th>200 mg/kg</th>
<th>1000 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>0.20</td>
<td>0.22</td>
<td>0.24</td>
<td>0.26</td>
</tr>
<tr>
<td>Liver</td>
<td>0.18</td>
<td>0.19</td>
<td>0.20</td>
<td>0.21</td>
</tr>
<tr>
<td>Heart</td>
<td>0.12</td>
<td>0.13</td>
<td>0.14</td>
<td>0.15</td>
</tr>
</tbody>
</table>

DISCUSSION

The administration of 70% ethanol extract of J. gendarussa at a dose of 2000 mg/kg BW of mice was found to not cause death. There were no toxic symptoms, such as standing hair, yellow eyes, abnormal behavior (not staying in one place, not biting certain body parts). The following is the observation of body weight of mice in the acute toxicity test.

In the subchronic toxicity test of 90 days, 70% ethanol extract of leaves of J. gendarussa in mice did not find any mortality in experimental animals, behavioral observations and signs of clinical toxicity of ethanol extract 70% leaves J. gendarussa does not cause changes in behavior such as walking backwards, biting certain body parts, yellowing eyes, and standing hair.

The hematological analyses did not show significant differences between control and all treated groups in most of the parameters examined, except for the thrombocyte, basophil, neutrophil, lymphocyte, and hematocrit. The biochemical like ALT and creatinine were no change, giving 200 mg/kg BB increase the level of AST, while increasing level of BUN were observed in all treated groups.

METHODS AND MATERIALS

Materials: ethanol extract 70% gendarussa leaves, Na, citrate, 70% ethanol, CMC Na, aquadest, acetonitrile, methanol.

In this study, male Balb / C mice weighing between 150-350 grams and 8-12 weeks old were used for administering ethanol extracts of leaves of J. gendarussa. According to the body weight, the samples were divided into three groups of equal numbers, with each group having 10 mice.

Therefore, before being used as a medication, further research is needed to ensure the quality of the extract.

CONCLUSIONS

Using of 70% ethanol extract of leaves of J. gendarussa as therapeutic dose was safe but it needs attention at higher dose. This result suggests further research is needed to ensure its safety for clinical study.

REFERENCES


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ACKNOWLEDGEMENTS

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Figure 1: Hepar (control group)
Figure 2: Hepar (treatment 1 group)
Figure 3: Hepar (treatment 2 group)
Figure 4: Hepar (treatment 3 group)
Figure 5: kidney (treatment 1 group)
Figure 6: kidney (treatment 2 group)
Figure 7: kidney (treatment 2 group)
Figure 8: kidney (treatment 2 group)

Figure 9: Justicia gendarussa Burm./