Safety assessment of hydroethanolic rambutan rind extract: Acute and sub-chronic toxicity studies

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This study evaluated the safety of rambutan rind extract (RRE) in male Wistar rats. While acute toxicity was evaluated by feeding the rats with single doses of RRE (1000, 2000, 3000, 4000, and 5000 mg/kg) and its sub-chronic toxicity was observed in rats orally administered with RRE (500, 1000, and 2000 mg/kg) daily for 30 days. In acute toxicity study, the LD₅₀ was found to be greater than 5000 mg/kg of RRE. In sub-chronic toxicity study, no mortality and sign of toxicity was found up to 1000 mg/kg/day of RRE. At 2000 mg/kg/day dose, the mortality rate was 12.5%. Significant decreases in body weight gain and food consumption were found in both acute and sub-chronic toxicity studies. In acute toxicity study, all the studied doses of RRE did not alter serum levels of triglyceride (TG), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). In sub-chronic toxicity study, all studied doses of RRE significantly decreased plasma levels of TG and blood urea nitrogen, but did not alter plasma levels of AST and ALT. TC levels did not show any significant change in both the studies. The obtained results provide basic information for in vivo experimental studies of the pharmacological potentiality of RRE.

Keywords: Acute toxicity, Nephelium lappaceum, Rambutan, Sub-chronic toxicity

Rambutan (Nephelium lappaceum L. Fam. Sapindaceae) is a tropical fruit tree common to Southeast Asia¹. The rambutan fruit possesses various medicinal properties such as astringent, stomachic and febrifuge, and can be used for the treatment of diarrhea, dysentery and fever². The fruit rind of rambutan has been shown to contain substances possessing antioxidant activity such as ascorbic acid and phenolic compounds (anthocyanins, flavonoids, tannins, ellagic acid, corilagin, and geraniin)³⁶. It also has antibacterial⁴⁴, anti-herpes simplex virus type 1⁷, antiproliferative⁸, anti-hyperglycemic⁹¹⁰, and fatty acid synthase inhibiting activities¹¹. Though potential health benefits of the rambutan rind have been elucidated, very little information regarding its toxic effects is available. Therefore, we investigated the safety of the hydroethanolic rambutan rind extract by studying its behavioural and pharmaco-toxicological effects after acute and sub-chronic oral administration in male Wistar rats.

Materials and Methods

Plant material—Rambutan (Nephelium lappaceum L.) fruits were obtained from local market in Nakhon Ratchasima province, authenticated at the Forest Herbarium, Department of National Parks, Wildlife and Plant conservation, and a voucher specimen (BKF 185498) was deposited in the Herbarium.

Preparation of plant extract—The rind of rambutan was washed with copious amounts of water and dried at room temperature for 2–4 days. The dried rind was powdered using an electric mill. The powder was macerated with 85% hydroethanolic solution at room temperature for 7 days. The obtained suspension was filtered and concentrated using a rotary evaporator and then converted into crude extract by freeze dryer. The yield of the hydroethanolic rambutan rind extract (RRE) was 18% (w/w). The obtained crude extract was stored at −20 °C until further use.

Animals—Adult male Wistar rats (200-300 g) were obtained from Institutional Animal Care Unit, Suranaree University of Technology (SUT). They were maintained at standard laboratory conditions (12:12 h dark-light cycle, ambient temperature
20 ± 1 °C) with free access to food and water. All studies were conducted with permission from the SUT Animal Care and Use Committee.

**Acute toxicity study**—Acute toxicity test was performed according to the Organization of Economic Co-operation and Development (OECD) guidelines\(^1\). Rats were randomly divided into 6 groups of 8 rats each, fasted overnight and then given single graded oral doses of 1000, 2000, 3000, 4000, and 5000 mg/kg of the RRE dissolved in double deionized distilled (DDD) water at a dosing volume of 10 mL/kg while the control group received DDD water (10 mL/kg). All rats were observed for mortality and signs of toxicity for the first, second, third, and fourth hours and thereafter once daily for 14 days. Daily food consumption and weekly body weight changes for each rat were measured throughout the study.

At the end of the observation period, all rats were fasted overnight and then anesthetized with pentobarbital sodium (Nembutal, Ceva Sante Animale, Libourne, France) at a dose of 60 mg/kg (ip). Blood samples were collected via cardiac puncture into non-heparinized tubes and centrifuged at 5,000xg for 10 min. The serum was separated and frozen at −20 °C until further biochemical analysis. The serum biochemical parameters were measured using automatic chemistry analyzer Hitachi® 911 (Hitachi Ltd., Japan) and parameters assayed were triglyceride (TG), total cholesterol (TC), glucose, aspartate aminotransferase (AST), and alanine aminotransferase (ALT).

**Sub-chronic toxicity study**—Sub-chronic toxicity test was performed as per the modified method of Maphosa *et al.*\(^1\)\(^3\) and Organization for Economic Co-operation and Development (OECD) guidelines\(^1\)\(^4\). Male rats were randomly divided into 4 groups of 8 rats each. Rats were orally administered with 500, 1000, and 2000 mg/kg of the RRE dissolved in DDD water at a dosing volume of 2 mL/kg daily while the control group received DDD water (2 mL/kg) for 30 days. The doses of the RRE were selected based on the results of the acute toxicity study. All groups were observed for mortality and signs of toxicity for the first, second, third, and fourth hours and thereafter, once daily for 30 days. Rats were maintained on an *ad libitum* diet and tap water throughout the study. Daily food consumption and weekly body weight changes for each rat were measured throughout the study.

On day 31, all rats were fasted overnight and then anesthetized with pentobarbital sodium (60 mg/kg, ip). Blood samples were collected via cardiac puncture into ethylenediamine tetraacetic acid (EDTA) tubes and heparinized tubes. Blood with EDTA was used immediately for determination of hematological parameters: red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentrations (MCHC), red blood cell distribution width (RDW), platelet count, white blood cells (WBC), neutrophil, lymphocyte, monocyte, eosinophil and basophil. Hematological analysis was performed using automatic hematological analyzer Coulter® HmX (Beckman Coulter Inc., Ireland). Heparinized blood was centrifuged at 2000 RCF for 5 min, the obtained plasma was stored at −20 °C until further biochemical analysis. The plasma biochemical parameters [TG, TC, glucose, AST, ALT, creatinine, and blood urea nitrogen (BUN)] were measured as done in acute toxicity study.

Immediately after blood collection in both acute and sub-chronic toxicity studies, the organs (heart, liver, kidneys, and spleen) were quickly dissected, removed, carefully examined, and weighed individually. The organ weights were calculated into relative organ weight or ROW (g/100 g of body weight)\(^1\)\(^5\). Liver was then fixed in 10% buffered formalin solution for 7 days and processed for histopathological examination.

**Statistical analysis**—Results were expressed as mean ± standard error of mean (SE). Data were analyzed using program SigmaStat Version 3.5 (Systat Software, Inc., USA.). Weight gain and food consumption were analyzed using two-way repeated measures ANOVA while relative organ weight or ROW (g/100 g of body weight)\(^1\)\(^5\) were considered statistically significant. Post hoc testing was performed for inter-group comparisons. *P* values less than 0.05 (*P*<0.05) were considered statistically significant.

**Results**

**Acute toxicity study**—Single oral administration of the RRE in male rats at the doses of 1000, 2000, 3000 and 4000 mg/kg did not cause mortality but for the dose of 5000 mg/kg which showed mortality rate of 12.5% (one death out of eight rats). Also, dosage up to 3000 mg/kg did not show any toxic sign. However, some toxic effects were found with rats received more
The body weight gain of male rats on day 7 and day 14 after single oral administration of the RRE was significantly decreased in all groups treated with the RRE compared to the control group (Table 1). Average food consumption during first week showed significant decrease for all the groups except 5000 mg/kg RRE while during second week only groups treated with 1000 and 4000 mg/kg RRE showed significant decrease (Table 1).

Relative organ weights (ROW) of the spleen and kidneys in male rats treated with single doses of the RRE up to 5000 mg/kg were not different from the control group. The ROW of the heart was significantly less in the groups treated with 1000, 2000, and 5000 mg/kg RRE while that of liver was less in all groups treated with the RRE (Table 2). No histopathological change in the liver was found in the control group and all RRE treated groups.

Serum biochemical parameters of male rats on day 14 after single oral administration of RRE are shown in Table 3. There were no significant difference in serum TG, TC, AST and ALT levels but for serum glucose which showed significant decrease in the groups treated with 3000 and 4000 mg/kg RRE.

Sub-chronic toxicity study—Similar to acute toxicity study, none of the treated groups but for 2000 mg/kg RRE treated group, showed any mortality nor toxic signs. Group treated with 2000 mg/kg RRE showed mortality (12.5% or one death out of 8 rats) and signs of toxicity (hypo-activity in 4 out of 8 rats).

The body weight gain and average food consumption were significantly decreased in male rats for all the studied doses of RRE throughout the study period (Table 4). There were no significant changes in all ROW of liver, kidneys, and spleen of RRE treated groups but for the heart in 2000 mg/kg RRE treated group (Table 2). Histopathological examination of the liver of the control group and all RRE treated group did not reveal any morphological difference. Rats treated sub-chronically with 500, 1000, and 2000 mg/kg of the RRE did not cause any adverse effect on histoarchitecture of hepatocytes.

The plasma biochemical parameter levels of glucose, TC, AST, ALT, and creatinine in treated rats were not different from that of control. However, significant decreases in plasma TG and BUN were shown in all treated groups (Table 5). The hematological parameter levels of RBC, Hb, Hct, MCV, MCH, RDW, WBC, monocyte and basophil in RRE treated rats were not different from that of the control (Table 6). Significant decrease in the MCHC level was found at a dose of 500 mg/kg RRE and in the lymphocyte percentage at a dose of 2000 mg/kg RRE. The RRE also caused significant increases in the platelet count at doses of 1000 and 2000 mg/kg, neutrophil at a dose of 2000 mg/kg and eosinophil at doses of 500 and 1000 mg/kg (Table 6).

Discussion

Acute and sub-chronic oral toxicity evaluation of the RRE could generate a safety data in animal models for extrapolation to human toxicity profiles. The present results demonstrated that the RRE may be considered relatively safe for usage in cosmetic, nutraceutical and pharmaceutical applications. Therapeutic dosage of the RRE for clinical use should be determined. Significant potential pharmacological properties of the RRE were reported due to its

### Table 1—Changes in body weight gain and average weekly food consumption of male Wistar rats after single oral administration of the RRE.

[Values are mean ± SE from 8 animals in each group, except for the 5000 mg/kg RRE group; where n = 7 animals]

<table>
<thead>
<tr>
<th>Dose of RRE (mg/kg)</th>
<th>Control</th>
<th>1000</th>
<th>2000</th>
<th>3000</th>
<th>4000</th>
<th>5000</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Week 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body wt gain (g) from day 0</td>
<td>25.00 ± 2.86</td>
<td>13.13 ± 3.02*</td>
<td>11.88 ± 2.46*</td>
<td>11.43± 2.82*</td>
<td>11.67 ± 4.40*</td>
<td>11.43 ± 2.82*</td>
</tr>
<tr>
<td>Food consumption (g/rat/day)</td>
<td>17.15 ± 0.32</td>
<td>13.67 ± 0.81*</td>
<td>15.96 ± 0.44</td>
<td>15.04 ± 0.51*</td>
<td>13.19 ± 0.88*</td>
<td>15.39 ± 1.29</td>
</tr>
<tr>
<td><strong>Week 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body wt gain (g) from day 0</td>
<td>38.75 ± 3.75</td>
<td>24.38 ± 2.76*</td>
<td>24.38± 3.70*</td>
<td>20.00 ± 2.86*</td>
<td>17.50 ± 5.62*</td>
<td>28.57 ± 4.96*</td>
</tr>
<tr>
<td>Food consumption (g/rat/day)</td>
<td>16.21 ± 0.37</td>
<td>13.62 ± 0.42*</td>
<td>15.15 ± 0.22</td>
<td>14.76 ± 0.25</td>
<td>13.62 ± 0.41*</td>
<td>15.17 ± 1.32</td>
</tr>
</tbody>
</table>

*P< 0.05 significantly different from control group using two-way repeated ANOVA with Duncan`s method.
The RRE and geraniin isolated from RRE possess alpha-glucosidase, alpha-amylase and aldol reductase inhibitory activities, suggesting therapeutic potential for the treatment of diabetes and obesity. In the present study, single oral doses at 3000 and 4000 mg/kg of the RRE demonstrated hypoglycemic activity, and 30-day repeated oral doses at 500, 1000, and 2000 mg/kg exhibited hypotriglyceridemic and body weight reducing effects. Moreover, the RRE at an oral dose of 100 and 200 mg/kg could prevent tissue damage and inflammatory conditions in collagen-induced arthritis in dark Agouti rats. The results obtained from the acute toxicity studies revealed that the tolerated dose of the RRE was approximately 4000 mg/kg, or 40- and 20-fold higher than the recommended therapeutic dose against collagen-induced arthritis in rats. Even at this high dose, only hypoactivity was observed.

The oral acute toxicity study in male Wistar rats indicated the no-observed-adverse-effect level (NOAEL) and the lowest-observed-adverse-effect level (LOAEL) of the RRE to be 3000 and 4000 mg/kg, respectively. Based on the OECD classification, the evaluated median lethal dose (LD₅₀) of the RRE was then greater than 5000 mg/kg (po). This result was in accordance with previous data from

### Table 2—Relative organ weight (ROW) of male Wistar rats after acute and 30 days repeated oral administrations of the RRE

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>500</th>
<th>1000</th>
<th>2000</th>
<th>3000</th>
<th>4000</th>
<th>5000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>0.35 ± 0.01</td>
<td>-</td>
<td>0.32 ± 0.01</td>
<td>0.31 ± 0.01</td>
<td>0.33 ± 0.01</td>
<td>0.33 ± 0.01</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>Repeated (30 days)</td>
<td>0.45 ± 0.03</td>
<td>0.45 ± 0.01</td>
<td>0.49 ± 0.03</td>
<td>0.55 ± 0.03</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>3.44 ± 0.07</td>
<td>-</td>
<td>3.07 ± 0.09</td>
<td>3.13 ± 0.12</td>
<td>3.02 ± 0.07</td>
<td>2.96 ± 0.10</td>
<td>3.06 ± 0.07</td>
</tr>
<tr>
<td>Repeated (30 days)</td>
<td>4.13 ± 0.39</td>
<td>3.24 ± 0.09</td>
<td>4.11 ± 0.40</td>
<td>3.70 ± 0.36</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kidneys</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>0.64 ± 0.01</td>
<td>-</td>
<td>0.64 ± 0.01</td>
<td>0.60 ± 0.01</td>
<td>0.61 ± 0.01</td>
<td>0.62 ± 0.02</td>
<td>0.61 ± 0.01</td>
</tr>
<tr>
<td>Repeated (30 days)</td>
<td>0.80 ± 0.05</td>
<td>0.75 ± 0.02</td>
<td>0.83 ± 0.06</td>
<td>0.85 ± 0.06</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>0.22 ± 0.01</td>
<td>-</td>
<td>0.23 ± 0.02</td>
<td>0.23 ± 0.01</td>
<td>0.23 ± 0.01</td>
<td>0.24 ± 0.02</td>
<td>0.22 ± 0.01</td>
</tr>
<tr>
<td>Repeated (30 days)</td>
<td>0.25 ± 0.02</td>
<td>0.23 ± 0.01</td>
<td>0.27 ± 0.03</td>
<td>0.26 ± 0.02</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*P < 0.05 significantly different from control group using one-way ANOVA with Duncan’s method.

### Table 3—Serum biochemical parameters on day 14 after single oral administration of the RRE in male Wistar rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>1000</th>
<th>2000</th>
<th>3000</th>
<th>4000</th>
<th>5000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>194.10 ± 8.84</td>
<td>189.64 ± 7.18</td>
<td>177.86 ± 11.73</td>
<td>147.500 ± 11.73</td>
<td>137.86 ± 7.25</td>
<td>182.14 ± 6.11</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>68.86 ± 9.87</td>
<td>67.81 ± 8.04</td>
<td>82.86 ± 8.89</td>
<td>52.86 ± 5.08</td>
<td>50.31 ± 6.34</td>
<td>63.93 ± 5.30</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>76.00 ± 3.87</td>
<td>75.63 ± 2.56</td>
<td>74.69 ± 3.96</td>
<td>70.42 ± 2.49</td>
<td>65.94 ± 2.42</td>
<td>78.21 ± 5.52</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>321.50 ± 21.72</td>
<td>275.00 ± 29.18</td>
<td>211.00 ± 17.54</td>
<td>255.50 ± 32.92</td>
<td>320.00 ± 37.69</td>
<td>246.00 ± 46.83</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>51.67 ± 15.85</td>
<td>42.14 ± 4.35</td>
<td>28.57 ± 6.77</td>
<td>47.08 ± 12.99</td>
<td>47.08 ± 8.59</td>
<td>33.21 ± 5.67</td>
</tr>
</tbody>
</table>

*P < 0.05 significantly different from control group using one-way ANOVA with Duncan’s method.
Subramaniam et al.\textsuperscript{17} who reported that the LD\textsubscript{50} of the ethanolic extract from the rambutan rind in male Sprague Dawley rats was more than 2000 mg/kg (po). In the sub-chronic toxicity study, the NOAEL of the 30-day repeated doses of the RRE found in this study was 1000 mg/kg/day which was lower than the NOAEL (2000 mg/kg/day) of the 28-day repeated doses of the ethanolic extract from the rambutan rind found in previous study\textsuperscript{17}. This discrepancy could be due to the difference in chemical constituents and their contents in the ethanolic and hydroethanolic extract from the rambutan rind.

The decreased body weight gain in both single oral doses and 30-day repeated oral doses of the RRE were in line with the decrease in average food consumed by the rats. These changes may be caused by behavioral signs of toxicity, stress, or physiological variation in rats such as metabolism\textsuperscript{18-20}. Total phenolic content and geraniin found in the RRE may have an impact on carbohydrate metabolism through the inhibition of alpha-amylase and alpha-glucosidase enzymes\textsuperscript{9-10}. Inhibition of these enzymes by the RRE may prevent or delay the digestion of carbohydrates into glucose and the absorption of glucose, potentially resulting in weight loss. Such alteration of carbohydrate metabolism may cause reduction in serum glucose levels in single oral doses of the RRE (3000 and 4000 mg/kg) treatment. Significant decrease in plasma TG levels found in 30-day repeated oral doses of the RRE treatment may be related to potentially low plasma glucose levels, which in turn may depress fat mobilization\textsuperscript{21}. These results reveal that the RRE may have a potential use in treating obesity and related diseases such as diabetes, atherosclerosis and cardiovascular diseases.

Although abnormal changes in some hematological parameters were found in sub-chronic toxicity study, they had no apparent toxicological significance. Significant decrease in MCHC, but relatively small, together with non-significant changes in MCH and MCV implied that the RRE may not affect the oxygen-carrying capacity of each red blood cell and of the whole blood since the RBC did not change\textsuperscript{22}. The increase in platelet count without notable effects in RBC and WBC counts could be attributed to the stimulatory effect of the RRE on thrombopoietin\textsuperscript{23}.
The changes in the ROW of heart and liver were however slight and the differences could be due to the variation in size of internal organs of the animals. The changes in the ROW of liver indicated that the RRE might have acute toxic potential on liver. However, it could be argued that these changes are not toxicologically significant as revealed by the non-hepatotoxicity of the extract such as the biochemical findings (ALT and AST levels) and histopathological study. Thus, the RRE has no potential toxicity to liver. Moreover, significant decrease in plasma levels of BUN, but still within the normal range, together with no significant change in plasma levels of creatinine after 30-day repeated oral doses of the RRE indicates that the RRE do not alter kidney function as well.

Conclusion

In conclusion, information about acute and sub-chronic toxicity of the RRE in rats is very important as a baseline before exploring further to develop the RRE as a new herbal medication. The present findings will encourage clinical and in vivo experimental studies of the pharmacological potential of the RRE.

Acknowledgement

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