Toxicological Study of Aqueous and Ethanolic Extracts from *Pueraria mirifica* Airy Shaw & Suvatabandhu on Male Rats

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Abstract

This research was carried out to investigate toxic effects of aqueous and ethanolic extracts from *Pueraria mirifica* Airy Shaw & Suvatabandhu on male rats. Both extracts at the doses of 400, 600 and 800 mg/kg bw were orally given to male rats for 4 weeks. The kidney and liver functions, histopathological and hematological alterations, including micronucleus formation were evaluated. The effect on rat body weight was also observed. The results showed that both extracts of all doses used had no effects on the kidney and liver functions according to the normal values of blood urea nitrogen (BUN), creatinine (Crea), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). However, histological alteration of both organs were observed in some rats treated with both extracts. Hematologically, total white blood cell count was not different from that of controls and differential cell count was normal, although lymphocytes and neutrophils in some treated rats was slightly different from those of control group. However, rats in all treated groups showed significantly lower packed red cell volume (PCV) than controls ($p < 0.01$) and their micronuclei in polychromatid erythrocytes (PCE) were significantly higher than those of controls ($p < 0.05$). These results revealed that both aqueous and ethanol extracts of *P. mirifica* affected the red blood cell formation and also acted as a mutagenic agent. Furthermore, all treated rats had significantly lower body weight gain as compared to control ($p < 0.01$). It could be clearly concluded that the extracts from *P. mirifica* at the doses used in this study tend to be toxic to rats. Nevertheless, its mutagenic effect is not in the same level as cyclophosphamide, a genuine mutagen.

INTRODUCTION

A Thai medicinal textbook on *Pueraria mirifica* mentioned that it had a rejuvenating effect on elderly. Due to its estrogenic properties (Kaweewat and Smitasiri, 1991), the applications of this plant in cosmetics and food additives are popular and their commercial sales are considerably growing. Presently Thai women and transvestites consume it widely for breast enlargement. Despite the popular use of *P. mirifica* in Thailand, there is no accurate knowledge about its side effect. With some reports on its toxicities (Aritajat et al., 2001; Manoruang, 1996; Tragoonboon et al., 1987), the safety use of this plant remains ambiguous. Since the consumption of *P. mirifica* without scientific support and reliable evidence could cause serious problems, the tests for its toxicities and side effects are important.

This research, thus, aims to investigate the toxic and mutagenic effects of *P. mirifica* in the forms of aqueous and ethanolic extracts on male rats by micronucleus test of bone marrow. The effects on the blood chemistry as well as the kidney and liver functions were evaluated.

MATERIALS AND METHODS

Animal Preparation

Male rats (*Rattus norvegicus*), approximately 6 weeks of ages and weighing between 250-270 g. were used in the present investigation. The animals were purchased...
from the National Laboratory Animal Centre, Salaya, Nakhorn Pathom. They were allowed to acclimatize in the departmental animal facility for at least one week prior to the day of experiment. They had access to water and standard diet (C.P. 082). The study room was maintained of approximately 25 ± 2°C. The photoperiod was 12-h light and dark.

**Plant Extraction**

*Pueraria mirifica* (tubers) was collected from Chiang Mai province, Thailand. The tubers were sliced, dried at 60°C and then ground to fine powder. Aqueous and ethanolic extract were prepared by soxhlet extraction and evaporated by rotary evaporation. The crude extracts were kept in dry place and used after preparing required dose in distilled water.

**Experimental Design**

Two sets of experimental groups were performed; aqueous and ethanolic extract treatments. Three groups of rats (7 each) were treated orally with 400, 600 and 800 g/kg.bw of each extracts for 30 days. Additional control group received only distilled water. Body weights of rats were recorded weekly during the entire period of experiment. At the end of treatment period the following items were evaluated.

1. **Blood Chemistry.** Alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine (Crea) were measured by automate (Synchron CSX, Beckman) with the cooperation of Clinical Chemistry Department, Faculty of Associated Medical Science, Chiang Mai University.

2. **Histopathological Study.** Liver and kidney were removed and 7 µm thick sections were prepared by the routine histological method. Hematoxyline and eosin were used for tissue staining. All the slides were evaluated under light microscopes.

3. **Hematological Tests.** Packed red cell volume (PVC), total white blood cell (WBC) and differential white blood were detected.

4. **Micronucleus Test** (Dacie and Lewis, 1984). The bone marrow of femur was wash out with repeated larvage with 1 ml of fetal bovine serum. The sample was shaken and centrifuged at 1000 rpm for 5 minutes. The supernatant was carefully removed and precipitant was homogeneously mixed and use for bone marrow smear. Dried smears were fixed with absolute methanol for 5 minutes and dried at room temperature. After staining with Wright’s stain, polychromatic erythrocytes (PCE) were examined under light microscope for micronuclei. The micronucleated polychromatic erythrocytes per 1000 cell were counted and presented in percent. Additional positive control group was performed by intraperitoneal injecting of cyclophosphamide (30 mg/kg) for 7 days.

**RESULTS AND DISCUSSION**

**Blood Chemistry and Histopathology**

Levels of ALT, AST, BUN and Crea were not altered as compared to control group (Table 1) and were in normal level (Sharp and La Regina, 1998). These indicate that the functions of liver and kidney were not affected. The histopathology of liver and kidney was also normal in most of rats in all group. Nevertheless, histological changes found in some rats treated with aqueous extract of *P. mirifica* at the dose of 800 mg/kg.bw i.e. the irregular arrangement of hepatic sinusoid and the thickness of epithelial lining in Bowman’s capsule (figure not shown) gave us an awareness that using this plant in high doses may lead to the abnormality of liver and kidney functions.

**Hematological Tests**

The total white blood cell count in all treated groups were not different from that of controls (Table 2). Although the differential white blood cell counts showed the significant increase in number of lymphocytes and the decreased number of neutrophils and monocytes in some group treated with both extracts, all kinds of white blood cells

166
were in the normal level (Table 3). The changes in number of some kinds of white blood cells might be caused by the infection of bacteria at the mean time (Brown, 1980). The packed cell volume in all treated groups, however, were significantly lower than that of control group ($p \leq 0.01$) (Fig. 1). These hematological results suggest that both extracts had an effect on circulating red blood cells or hematopoiesis, but not on leucopoiesis. Decrease of RBC, neutrophilic segmented cells and hemoglobin were reported in rats receiving powder of this plant (Pongdam et al., 1987).

**Micronucleus Test**

Both aqueous and ethanolic extracts in all doses used could induce chromosomal damage in rat’s PCE. The frequencies of micronuclei found in all group were significantly higher than those of controls (Fig. 2). These results indicate the high probability of *P. mirifica* to be a genotoxic substance. The increase of micronucleus formation induced by both extracts was found to be dose-dependent. The cumulative efficiency of ethanolic extract from *P. mirifica* in inducing micronucleous formation in mice was reported previously (Aritajat, 2001). The higher percent of micronuclei detected in aqueous extract treated groups when compared to ethanolic extract treated groups indicated the higher level of toxic constituents in aqueous extract. This result coincides with the report of miroestrol, the toxic substance which detected in higher amount in aqueous extract than in ethanolic extract (Smtsiri et al., 1987). In further studies, special attention must be drawn on the aqueous extract of this plant. The average frequency of micronucleus formation in control group was 0.44% (Fig. 2). This value is not much higher than the spontaneous frequency of micronuclei in laboratory rat (0.12-0.41%) reported by Wild (1988). This may be able to confirm the standard condition of our experiment. From the results of micronucleus test, it is likely that *P. mirifica* may contain substances which inhibits polymerization of tubulin in the production of microtubules (Chuahan et al., 2000). The abnormality of sperm morphology induced by *P. mirifica* (Saenphet et al., 2003) which led to the infertility of male rat (Kaweewat and Smitasiri, 1992) might be resulted from chromosomal damage of sperm. The ability of both extracts in all doses used to induce micronucleus formation, however, was significantly lower than that of cyclophosphamide, a genuine mutagenic agent.

**Body Weight**

Suppression of body weight gains were found in All treated rats (Fig. 3). The reduction began in the first week and continued throughout the remaining days of the treatment period. The food consumptions in all treated rats were not different from those of controls (data not shown). The decrease in body weight might be due to the inhibition of digestive enzymes or the decreased efficiency in converting the absorbed nutrients to new body substance as reported in tannins (Carmona, 1996; Chung et al., 1998).

**CONCLUSION**

Blood chemistry results revealed that aqueous and ethanolic extracts of *P. mirifica* at the doses of 400, 600 and 800 mg/kg,bw did not show a clear adverse effect on liver and kidney functions. Nevertheless, histopathological changes of liver and kidney in some treated rats together with the reduction of body weights gave us a caution for the abnormality of rat’s metabolism which might be caused by those extracts. Hematological results indicated the normal condition of leucopoiesis, but not erythropoiesis. Results from micronucleous test suggested that both extracts in all doses used could be classified as mutagenic agents.

**ACKNOWLEDGEMENT**

Thanks to Faculty of Science for financial support.

**Literature Cited**

Aritajat, S., Kaweewa, K. and Manoruang, W. 2001. Toxicological study of some
Table 1. The blood chemistry of male rats treated with aqueous and ethanolic extracts of *P. mirifica* for 30 days as compared to controls. Means and standard deviations are given.

<table>
<thead>
<tr>
<th>Group</th>
<th>BUN (mg/dl)</th>
<th>Crea (mg/dl)</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.00±4.36a</td>
<td>0.60±0.12a</td>
<td>133.00±25.39a</td>
<td>29.80±5.07a</td>
</tr>
<tr>
<td>A 400 mg/kg</td>
<td>19.20±2.68a</td>
<td>0.54±0.05a</td>
<td>112.00±22.93a</td>
<td>27.20±4.92a</td>
</tr>
<tr>
<td>A 600 mg/kg</td>
<td>24.00±2.24a</td>
<td>0.64±0.05a</td>
<td>126.00±49.84a</td>
<td>29.80±6.02a</td>
</tr>
<tr>
<td>A 800 mg/kg</td>
<td>21.17±3.12a</td>
<td>0.65±0.08a</td>
<td>139.33±27.33a</td>
<td>37.60±2.97a</td>
</tr>
<tr>
<td>E 400 mg/kg</td>
<td>26.40±4.77a</td>
<td>0.62±0.13a</td>
<td>85.80±15.53b</td>
<td>40.00±10.70b</td>
</tr>
<tr>
<td>E 600 mg/kg</td>
<td>23.60±6.20a</td>
<td>0.56±0.55a</td>
<td>116.20±44.49a</td>
<td>31.20±7.46a</td>
</tr>
<tr>
<td>E 800 mg/kg</td>
<td>21.80±3.11a</td>
<td>0.58±0.45a</td>
<td>108.40±19.35a</td>
<td>33.80±11.50a</td>
</tr>
<tr>
<td>Standard (Sharp and La Regina, 1998)</td>
<td>11-23</td>
<td>0.4-1.4</td>
<td>50-150</td>
<td>10-40</td>
</tr>
</tbody>
</table>

a,b = significant differences at p≤0.05
Abbrevations: A= aqueous extract, E= ethanolic extract

Table 2. Total white blood cell counts of male rats treated with aqueous and ethanolic extracts of *P. mirifica* for 30 days as compared to controls. Means and standard deviations are given.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total White Blood Cell Count in aqueous extract (cell/mm³)</th>
<th>Total White Blood Cell Count in ethanolic extract (cell/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2668.4±1121.49</td>
<td>2668.4±1121.49</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>2632.00±957.62</td>
<td>2581.1±938.85</td>
</tr>
<tr>
<td>600 mg/kg</td>
<td>2532.96±1034.98</td>
<td>2306.80±1014.69</td>
</tr>
<tr>
<td>800 mg/kg</td>
<td>2532.41±1475.24</td>
<td>2482.80±1446.32</td>
</tr>
</tbody>
</table>

There were no significant differences.
Table 3. Differential white blood cell counts of male rats treated with aqueous and ethanolic extracts of *P. mirifica* for 30 days as compared to controls. Means and standard deviations are given.

<table>
<thead>
<tr>
<th>Group</th>
<th>% Lymphocyte ± SD</th>
<th>% Neutrophils ± SD</th>
<th>% Monocyte ± SD</th>
<th>% Eosinophils ± SD</th>
<th>% Basophils ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>67.71 ± 5.47a</td>
<td>24.86 ± 5.81a</td>
<td>5.14 ± 1.95a</td>
<td>1.71 ± 0.76a</td>
<td>0.57 ± 0.53a</td>
</tr>
<tr>
<td>A 400 mg/kg</td>
<td>79.71 ± 3.90b</td>
<td>15.71 ± 2.93b</td>
<td>4.57 ± 2.07a</td>
<td>0.00 ± 0.00a</td>
<td>0.00 ± 0.00a</td>
</tr>
<tr>
<td>A 600 mg/kg</td>
<td>80.86 ± 6.07b</td>
<td>14.14 ± 6.31b</td>
<td>4.14 ± 1.21a</td>
<td>0.57 ± 0.79a</td>
<td>0.29 ± 0.49a</td>
</tr>
<tr>
<td>A 800 mg/kg</td>
<td>76.14 ± 11.75a</td>
<td>19.57 ± 11.22a</td>
<td>3.43 ± 2.70a</td>
<td>0.71 ± 0.76a</td>
<td>0.14 ± 0.00a</td>
</tr>
<tr>
<td>E 400 mg/kg</td>
<td>78.86 ± 7.27b</td>
<td>16.71 ± 5.94b</td>
<td>1.14 ± 1.07b</td>
<td>0.71 ± 1.50a</td>
<td>0.29 ± 0.49a</td>
</tr>
<tr>
<td>E 600 mg/kg</td>
<td>72.29 ± 11.50a</td>
<td>17.00 ± 4.36b</td>
<td>1.86 ± 0.69b</td>
<td>0.86 ± 0.69a</td>
<td>0.29 ± 0.49a</td>
</tr>
<tr>
<td>E 800 mg/kg</td>
<td>71.14 ± 8.57a</td>
<td>24.14 ± 5.90a</td>
<td>1.57 ± 0.79b</td>
<td>1.00 ± 1.15a</td>
<td>0.14 ± 0.38a</td>
</tr>
<tr>
<td>Standard</td>
<td>65-83</td>
<td>13-26</td>
<td>0-4</td>
<td>0-4</td>
<td>0-1</td>
</tr>
</tbody>
</table>

Standard (Sharp and La Regina, 1998)

a,b = significant differences at p ≤ 0.05

**Figures**

![Hematocrit Graph](image_url)

Fig. 1. Packed red cell volume (% hematocrit) of male rats treated with aqueous and ethanolic extracts of *P. mirifica* for 30 days as compared to controls. Means and standard deviations are given. ** P ≤ 0.01. Abbrevations: A = aqueous extract, E = ethanolic extract.
Fig. 2. Frequencies of micronuclei formation in male rats treated with aqueous and ethanolic extracts of *P. mirifica* for 30 days as compared to normal control and positive control (cyclophosphamide). Means and standard deviations are given. **P** ≤ 0.05. Abbrevations: A = aqueous extract, E = ethanolic extract.

Fig. 3. Frequencies of micronuclei formation in male rats treated with aqueous and ethanolic extracts of *P. mirifica* for 30 days as compared to normal control and positive control (cyclophosphamide). Means and standard deviations are given. Abbrevations: A = aqueous extract, E = ethanolic extract.