

MUTAGENICITY OF *PUERARIA MIRIFICA* AIRY SHAW & SUVATABANDHU AND ANTIMUTAGENICITY OF *THUNBERGIA LAURIFOLIA* LINN.

K Saenphet, P Kantaoop, S Saenphet and S Aritajat

Biology Department, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand

Abstract. *Thunbergia laurifolia* Linn has been reputed to have antitoxic effects for all toxic substances. In this present study, we evaluated its effect against the mutagenicity induced by aqueous extracts from *Pueraria mirifica* Airy Shaw & Suvatbandhu in male rats. The formation of micronuclei in polychromatic erythrocytes was induced by oral administration of an aqueous extract of *P. mirifica* at the doses of 400, 600, and 800 mg/kg to the rats for 30 days. The results were that the extracts of *P. mirifica* at doses of 600 and 800 mg/kg acted as a mutagenic agent by inducing higher frequencies of micronuclei as compared to the controls. For the antimutagenic test, *P. mirifica* extract at a dose of 600 mg/kg (minimal effective dose) was mixed with fresh and dried extracts of *T. laurifolia* in proportions of 7:3 and 1:1, respectively. The results of 4-week-treatment indicated that aqueous extracts of *T. laurifolia*, prepared by both fresh and dry methods, could significantly inhibit the induction of micronuclei as induced by *P. mirifica*. It could be concluded from the results that, under certain circumstances, *T. laurifolia* exhibits a significant antimutagenic activity. The use of *P. mirifica* and *T. laurifolia* as fusion herbal medicines is suggested.

INTRODUCTION

T. laurifolia Linn, or “Rang Juad” in Thai, is a climbing plant, which belongs to the Axanthaceae family. This plant has been recommended by Thai folk physicians for its antitoxic effect (Utogapachn, 1976). The antidotal activity of *T. laurifolia* against pesticides has been confirmed by scientific research (Tejasen and Thongtharb, 1980). The reduced levels of cholinesterase observed in people exposed to parathion, a commonly used insecticide in Thailand, were restored after receiving aqueous extracts of *T. laurifolia* (Usanawarong, 2000). Due to its popular use as a detoxifying herb, *T. laurifolia* was selected in this present study to evaluate its antimutagenic effect against *Pueraria mirifica* Airy Shaw and Suvatbandhu (Papilionaceae) that has been reported to induce micronucleus formation in bone marrow of mice (Aritajat *et al*, 2001) and rats (Sanchanta *et al*, 2003). *P. mirifica* is recommended for its estrogenic properties (Pope *et al*, 1958), and a Thai medical textbook has mentioned its rejuvenating effect on the elderly. The applications of this plant in cosmetics and supplement foods are popular, and their commercial sales are growing considerably. With the increased consumption of *P. mirifica* by Thai people and its possible toxicological effect being largely ignored, the importance of investigating the effect of some

protective herbs on mutation induce by this plant was warranted. Therefore, This present study was designed to assess the ability of the extract from *T. laurifolia* to modulate the mutagenic effects induced by extracts from *P. mirifica* in male rats using a micronucleus test.

MATERIALS AND METHODS

Animal preparation

Male Wistar rats (*Rattus norvegicus*), approximately 6 weeks of age and weighing between 200-250 g, were used in the present investigation. The animals were purchased from the National Laboratory Animal Center, Thailand. They were allowed to acclimatize in the departmental animal facility for one week prior to the day of the experiment. They had access to water and standard diet (CP 082). The study room was maintained at approximately 25 ± 2°C. The photoperiod was 12-hour light and dark.

Plant extraction

Pueraria mirifica tubers and *Thunbergia laurifolia* leaves were collected from Chiang Mai Province, Thailand. The tubers of *P. mirifica* were sliced, dried at 60°C, and then ground to fine powder. An aqueous extract was prepared by Soxhlet extraction and evaporated by rotary evaporation. The crude extract was kept in a dry place and used after preparing at the required dose in distilled water. The leaves of *T. laurifolia* were sliced, blended with distilled water, and then filtered to obtain the fresh extract. The dried extract was prepared from dried slices in boiled water. Both kinds of extract were prepared at the

Correspondence: K Saenphet, Biology Department, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.
E-mail: K_Saenphet@yahoo.com

concentration of 0.2 mg/ml.

Micronucleus induction by *P. mirifica*

Rats were randomized into five groups (10 each). They were treated orally with 400, 600, and 800 g/kg bw, respectively, of aqueous extract from *P. mirifica* for 4 weeks. The controls were treated with distilled water. An additional group each had a single peritoneal injection of cyclophosphamide (Asta Medica, Germany), a mutagenic agent (80 mg/kg). This group served as a positive control. At the end of the treatment period all rats were sacrificed, and the femur bone was removed for micronucleus tests.

Suppression of micronucleus formation by *T. laurifolia*

From the results of micronucleus induction, the minimum dose of *P. mirifica* extract that could significantly induce micronucleus formation was used as a positive control. Rats were randomized into six groups (10 each) and treated orally for 4 weeks with the following regimens by group: 1) distilled water at 1 ml/day (control); 2) *P. mirifica* extract; 3-4) mixture of *P. mirifica* extract and fresh extract of *T. laurifolia* in proportions of 7:3 and 1:1, respectively; 5-6) mixture of *P. mirifica* extract and dried extract of *T. laurifolia* in proportions of 7:3 and 1:1, respectively. Also, micronucleus tests were performed at the end of the experiment.

Micronucleus test

The micronucleus assay from bone marrow was performed according to the protocol described by Dacie and Lewis (1984). The number of micronucleated cells was counted in 1,000 polychromatic erythrocytes (PCEs) per animal. The slides were analyzed in a blind test using a light microscope with a 100 x immersion objective and presented in percentages. The percentage of reduction in the frequency of micronuclei (MN) was calculated based on the following (Delmanto *et al*, 2001):

$$\text{Reduction (\%)} = \frac{\text{frequency of MN in A} - \text{frequency of MN in B}}{\text{frequency of MN in A} - \text{frequency of MN in C}} \times 100$$

A = group treated with aqueous extract of *P. mirifica* (positive control)

B = group treated with mixture of *P. mirifica* and *T. laurifolia*

C = group treated with distilled water (negative control)

Statistics

Means and standard deviations were calculated. The significance of difference was analyzed using the Student's *t*-test.

RESULTS

An aqueous extract of *P. mirifica* at doses of 600 and 800 mg/kg could have induced chromosomal damage in rats' PCEs. The frequencies of MN found in both groups were significantly higher than those of the controls (Table 1 and Fig 1). The increase of MN formation induced by the extracts was found to be dose-dependent. The ability of the extracts to induce MN formation, however, was significantly lower than that of cyclophosphamide, a genuine mutagenic agent.

Table 2 shows the frequencies of MN in the PCEs of rats treated with the mixture of extracts from *P. mirifica* and *T. laurifolia* in different proportions as compared to both negative and positive controls. It was found that the rats that received the mixture of extracts from both plants showed a significant reduction in the frequencies of MN as compared to the rats that received only *P. mirifica*. The dry extract of *T. laurifolia* mixed with the extract of *P. mirifica* in a proportion of 7:3 had the strongest effect of MN reduction. The results also showed that the frequencies of MN in PCEs of the rats treated with a mixture of extracts in all kinds of preparations were not different from those of controls.

DISCUSSION

It was reported in the earlier studies that high doses of *P. mirifica* caused a variety of toxicities in animals including genotoxicity by inducing MN formation in the PCEs of mice (Pongdam *et al*, 1987; Manoruang, 1996; Aritajat *et al*, 2001). Because *T. laurifolia* has been recommended by Thai traditional physicians for its antitoxic properties, and it has been reported to reduce the toxic effects of various known substances (Tejasen and Thongtapp, 1980), we therefore started to investigate the antimutagenic potential of this plant against *P. mirifica*. Although the frequencies of MN in PCEs found in *P. mirifica* treated rats were not as high as those found in rats treated with cyclophosphamide (Table 1 and Fig 1), *P. mirifica* alone induced a clear and dose-related increase in MN frequencies as compared to the controls. The significantly high frequencies of micronuclei found in *P. mirifica* and cyclophosphamide treated rats clearly indicated their genotoxic property. It is highly probable that the *P. mirifica* extract contains genotoxic substance(s). The high frequency of micronucleated PCEs was also reported in rats that received *Butea superba* Roxb, a plant in the same family of *P. mirifica* (Pongpanparadon *et al*, 2002). Such coincident results with the plants in a related taxonomy warrant special attention regarding the mutagenicities of other plants in the same family

Table 1

Frequencies of micronuclei in male rats treated with aqueous extracts of *P. mirifica* for 30 days as compare to negative (distilled water) and positive (cyclophosphamide) controls. Means and standard deviations are given.

Treatment	Number of analyzed cells	Micronucleated polychromatic erythrocytes (%)
Control	10,000	0.44 ± 0.28 ^a
<i>P.mirifica</i> (400 mg/kg)	10,000	0.55 ± 0.41 ^a
<i>P.mirifica</i> (600 mg/kg)	10,000	1.37 ± 0.66 ^b
<i>P.mirifica</i> (800 mg/kg)	10,000	2.67 ± 1.09 ^b
Cyclophosphamide (80 mg/kg)	10,000	5.27 ± 1.33 ^c

a, b, c indicate significant differences between groups (p < 0.05).

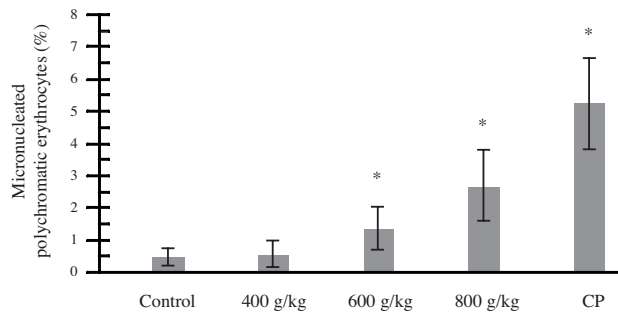


Fig 1- Frequencies of micronuclei in male rats treated with aqueous extract of *P. mirifica* for 30 days as compare to negative (distilled water) and positive (cyclophosphamide, CP) controls. Means and standard deviations are given.

* p < 0.05

Table 2

The effect of *T. laurifolia* (fresh and dry extracts) on the frequencies of micronuclei induced by *P. mirifica* extract in polychromatic erythrocytes of male rats.

Treatment	Number of analyzed cells	Micronucleated polychromatic erythrocytes		Reduction (%)
		Number	%	
Control	10,000	30	0.30 ^a	-
<i>P.mirifica</i> (600 mg/kg)	10,000	139	1.39 ^b	-
<i>P.mirifica</i> + <i>T. laurifolia</i>				
- Fresh extract 7:3	10,000	40	0.40 ^{ac}	90.82
- Fresh extract 1:1	10,000	55	0.55 ^{ac}	77.06
- Dry extract 7:3	10,000	49	0.31 ^{ac}	99.08
- Dry extract 1:1	10,000	31	0.49 ^{ac}	82.56

a, b, c indicate significant differences between group (p < 0.05).

(Papilionaceae). The average frequency of MN formation in the control group was 0.35%, which was in the normal range of spontaneous frequency of

micronuclei (0.12-0.41%) found in laboratory rats (Wild, 1988). This finding supports the standard conditions of our experiment.

To study the antimutagenicity of *T. laurifolia*, an aqueous extract of *P. mirifica* at the dose of 600 mg/kg was used as a positive control for inducing MN formation in this study. The data summarized in Fig 2 demonstrates that both fresh and dry extracts of *T. laurifolia* were capable of reducing the micronucleated PCE frequencies of rats induced by the *P. mirifica* extract. Since medicinal plants can induce a variety of responses in animals, according to the kind of preparation that is ingested, we have also tested the possible influence of the kind of preparation on the antimutagenic effect. It was found that both kinds of preparation, fresh and dry extracts, gave the appreciable results in reducing the frequency of MN formation. This evidence suggested that an aqueous extract of *T. laurifolia* provided protection against the genotoxicity of *P. mirifica*. Many Thai plant species have been proven to have antimutagenic activity, for instance, *Cymbopogon citratus* Stapf (Vinitketkumnueen *et al*, 1994) and *Hibiscus sabdariffa* Linn (Chewonarin *et al*, 1999). These authors suggested that the components of those plants might induce chemoprotective enzymes. Although mechanisms of inhibition of MN formation by *T. laurifolia* extract were not clearly understood, it was shown that antimutagenic substance in this plant was not destroyed by high temperature. Beside its general known antitoxic activity, our results bring about new striking information concerning the antimutagenic activity of *T. laurifolia*. Due to the popular use of *P. mirifica* in Thailand, the results of this primary research would provide valuable information for the intensive studies on mechanisms of antimutagenic effect of *T. laurifolia*, so as the development of the effective natural products from mixture of *P. mirifica* and *T. laurifolia*.

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