THE TOXICOLOGY OF *BUTEA SUPERBA*, ROXB.

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Abstract. A study to determine the primary toxicological effects of Red Gwow Kreur (*Butea superba*, Roxb.) dry powder by micronucleus and dominant lethal tests was undertaken. Aqueous solution of Red Gwow Kreur dry powder in doses of 2, 20, 200 and 1,000 mg/kg/day were fed to male rats for 9 weeks. The results showed that 1,000 mg/kg/day of Red Gwow Kreur solution was significantly more effective in inducing the formation of micronuclei in polychromatic erythrocytes than the control ($p<0.01$), whereas dominant lethal test indicated that none of the doses had a toxic effect on male reproduction. There were no abnormal changes in the number of implantation sites and the number of dead fetuses produced by females that had mated with Red Gwow Kreur treated males in comparison with the controls. In addition, the Red Gwow Kreur solutions had no effect on the body weights of the treated rats.

INTRODUCTION

Gwow Kreur has been used for centuries as a traditional Thai herbal medicine; Gwow Kreur is believed to enhance longevity and is said to have rejuvenating and aphrodisiac properties. The use of the white strain (*Pueraria mirifica* Airy Shaw and Suvatabandhu) for breast enlargement has become a major focal point for modern research into Gwow Kreur. The red strain (*Butea superba* Roxb.), with its purported aphrodisiac potency, is little known to the scientific world. Recently, *B. superba* has enjoyed renewed interest, after a herbal specialist claimed that it may cure impotence. It is believed that *B. superba* promotes penile blood flow and therefore aids erection. Manosroi *et al* (1999) investigated the acute toxicity of *B. superba* in rats and reported that the LD50 was 20 g/kg. Extensive research of its sub-chronic toxicity by Manosroi *et al* (2000) revealed that *B. superba* had no effect on liver and kidney function; moreover, the highest dose used (625mg/kg) tended to increase the number of epididymal sperm and significantly increased the rats' testicular weight. These results seem to support the claims that *B. superba* promotes male sexual activity.

Despite the growth of research into the aphrodisiac effectiveness of *B. superba,* little work has been conducted in order to evaluate its genotoxicity. With the increased interest in the development of *B. superba* as a treatment for impotence, its toxicological evaluation is of ever greater importance. The aim of this study was to investigate the genotoxic effect of *B. superba* on rats using the micronucleus test and the dominant lethal test .

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MATERIALS AND METHODS

Plant materials

Fresh *B. superba* tubers were washed, peeled, and cut into 2-3 mm thick slices. The slices were dried at 50˚-60˚C and then ground to a fine powder. A solution was prepared by adding distilled water to the required doses.

Animal preparation

Male and female Wistar rats (*Rattus norvegicus*), of approximately 8 weeks of age and weighing between 150-200 g were purchased from the National Laboratory Animal Center, Salaya, Nakhon Pathom. They were allowed to acclimatize in the departmental animal facility for at least one week before the experiment. They had access to water and a standard pellet diet (CP No 082). The study room was maintained at 25 ± 2˚C; the photoperiod was 12-hours light followed by 12 hours of darkness.

Experimental design

Four groups of 10 male rats each were treated orally for 9 weeks with a solution of *B. superba* dried powder in doses of 2, 20, 200 and 1,000 mg/kg. Controls were treated with distilled water. A positive control group of rats was treated with cyclophosphamide (Asta Medica, Germany), a teratogen, in a daily dose of 30mg/kg for 7 days. At the end of treatment period, dominant lethal test and micronucleus testing were performed.

Dominant lethal test

Treated males were placed with virgin females of proven estrus cycle for overnight mating. The presence

of a copulation plug or sperm in the vaginal smears the following morning was regarded as day 1 of pregnancy. All pregnant females were isolated and sacrificed on day 14 of pregnancy. During autopsy, the numbers of implantations, viable fetuses, and resorptions along the uterine horns were recorded. After successful mating, treated males were sacrificed in order to perform the micronucleus test.

Micronucleus test

The tests were conducted according to the methods of Dacie and Lewis, (1984) and Adler (1984). The femurs were removed and the bone marrow was washed out with repeated larvage with 0.5 ml of fetal calf serum. The sample was shaken and then centrifuged at 1,000 rpm for 5 minutes. The supernatant was carefully removed and the precipitant was homogeneously mixed and used to prepare bone marrow smears. 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 by light microscopy for micronuclei. The micronucleated polychromatic erythrocytes per 1,000 cells were counted; the results were presented as percentages. The normochromatic erythrocytes (NCE) were also counted. Analysis of bone marrow function was based on ratio of PCE to PCE + NCE.

Statistical analysis

Means and standard deviations were calculated. Differences between groups of micronucleus test were compared by ANOVA and LSD; the Chi-square test was used for the dominant lethal test.

RESULTS

Micronucleus test

Only the highest dose of *B. superba* (1,000mg/kg) used in this study could induce chromosomal damage in rat polychromatic erythrocytes. The frequency of micronuclei found in this group was significantly higher than that of the controls ($p<0.01$). Cyclophosphamide, however, induced micronucleus frequency that was almost twenty-five times greater than that of the controls.

The significantly high ratio of PCE to PEC + NCE found in *B. superba* (1,000 mg/kg) and cyclophosphamide treated rats (Table 1) indicated the suppression of bone marrow function.

Dominant lethal test

Although not all the males treated with *B. superba* could impregnate females, the numbers of live and dead fetuses were found to be similar to those of the controls. The number of dead fetuses in the 1,000mg/kg group was slightly higher than those of the other groups (Table 2). Nevertheless, it was not high enough to be regarded as the unusual percentage of pre-birth death in rats. No sign of implantation was found in the females that had mated with males that had received cyclophosphamide.

DISCUSSION

It has been reported that the spontaneous frequency of micronuclei in laboratory rats is in the range of 0.12-0.41% (Wild, 1988). The average of micronucleus

Table 1
Induction of micronuclei by *B. superba* in rats.

Group	No. rats	MNPCE(%) (X±SD)	Ratio of PCE:PCE+NCE (X±SD)
Control	10	0.088 ± 0.014^{a}	0.0008 ± 0.00014^{a}
B. superba (2 mg/kg)	10	0.013 ± 0.027^{a}	0.0013 ± 0.00027^{a}
B. superba (20mg/kg)	10	0.157 ± 0.021^{ab}	0.0015 ± 0.00021^{ab}
B. superba (200 mg/kg)	10	0.167 ± 0.048^{ab}	0.0016 ± 0.00048^{ab}
B. superba (1,000 mg/kg)	10	0.271 ± 0.084^{b}	0.0027 ± 0.00084^{b}
Cyclophosphamide	10	1.937 ± 0.211^{c}	0.1937 ± 0.00212^{c}

^{a,b,c} indicate significant difference between groups ($p< 0.01$)
MNCPE= micronucleated polychromatic erythrocytes;
PCE= polychromatic erythrocytes; NCE = normochromatic erythrocytes;
Cyclophosphamide served as positive control.

Table 2
Dominant lethal testing of females that mated with male rats that were treated with *B. superba*.

DLT	Control	*B. superba* 2mg/kg	*B. superba* 20mg/kg	*B. superba* 200mg/kg	*B. superba* 1,000mg/kg	CYP
Number of pregnant rats	10 (10)	4 (10)	6 (10)	5 (10)	6 (10)	0 (10)
Number of implantations	11.39 ±1.17	12.25 ±0.96	12.17 ±2.23	11.20 ±8.80	12.50 ±1.38	0
Number of live fetuses	10.50 ±0.84	11.25±1.5	11.67±1.97	10.80±2.77	10.33±3.67	0
Number of dead fetuses	0.90 ± 0.99	1.00±1.41	0.50±0.84	1.00±0.71	2.17±2.48	[a]

Results are expressed as means ± SD
CYP=cyclophosphamide
[a] No implantation. All fetuses were dead.

formation in control group was lower than this range (0.08%); this finding may be explained by the standard conditions of our experiment. As shown in Table 1, *B. superba* at the highest dose (1,000 mg/kg) induced a statistically significant increase in the frequencies of micronuclei detected in polychromatic bone marrow erythrocytes ($p<0.01$). Nevertheless the number of micronuclei in this group were lower than the spontaneous frequency of micronucleus and not as high as that found in the cyclophosphamide-treated group. The 1,000 mg/kg dose seems likely to be in the normal level that may not be genotoxic. *B. superba*'s induction of micronuclei was, however, dose-dependent; it is possible that its genotoxic effect is cumulative, and therefore, its use in higher doses may lead to significant abnormalities.

Medicinal plants can induce a variety of responses in animals, according to the kind of preparation that is ingested. *B. superba*'s induction of micronuclei was limited, even in long-term treatment (9 weeks); it is likely that the powdered form is less effective in comparison with other preparations, *eg* the extract.

B. superba at the doses of 2, 20 and 200mg/kg did not suppress bone marrow function. This fact was indicated by the ratio of PCE to PCE + NCE, which was not different from that of the controls. At a dose of 1,000 mg/kg a more marked suppression of bone marrow was noted. The ratio of PCE to PCE + NCE in this group was found to be significantly lower than that of the controls ($p<0.01$). It was also significantly lower than that of the cyclophosphamide group. This may conclude that all the doses of *B. superba* used could not suppress bone marrow function.

With the exception of those in the cyclophosphamide-treated group, males were able to impregnate females. The failure of implantation in females mated with males that received cyclophosphamide was attributed to the teratogenic effect of cyclophosphamide, which can cause chromosomal aberrations (Fishbein *et al*, 1970) and lead to the production of abnorma of sperm. This loss of capacity for fertilization was evident in the present study. The absence of fetal implantation in this group, was not surprising. After 9 weeks of receiving *B. superba*, rats in all the groups were able to impregnate females. Dead fetuses were also observed in all the groups, including the control. The rate of fetal death was less than 4%, which is the usual fetal mortality rate in rats (Zimnan, 1970). *B. superba* in the form of dried powder may have very low toxic constituent or may not have it. It was clear from our results, obtained during both micronucleus and dominant lethal tests that *B. superba* as a dried powder at the doses used in this experiment, is not mutagenic to male germ cells. Similar findings, which suggested that *B. superba* was a nontoxic herb, has been reported by Manosroi *et al* (1999). The administrations of *B. superba* at different concentrations (0.5-250 mg/ml) for 8 weeks had no effect on liver and kidney function. Furthermore, *B. superba* increased testicular weight and the number of epididymal sperm: a finding echoed by Manosroi *et al* (2000). However, the dose-dependent induction of

micronuclei and the rate of fetal death were clearly evident. The use of *B. superta* in higher doses may prove toxic to animals and humans.

REFERENCES

Adler ID. Cytogenetic test in mammal. In: Venitt S, Parry JM, eds. Mutagenicity testing. Oxford: IRL Press 1984:1-353.

Dacie JV, Lewis SM. Practical haematology. 6th ed. Churchill Livingstone International Student Editions, 1984.

Fishbein L, Flamm WG, Falk HL. Chemical mutagens, environmental effects on biological system. London: Academic Press, 1970: 159-61.

Manosroi J, Manosroi A, Aritajat S, *et al*. The research and development of natural products for Thai traditional medicine. The progress report submitted to the Institute for Thai Traditional Medicine, Department of Medicine, Ministry of Public Health, Thailand, 1999 (in Thai with English abstract).

Manosroi A, Aritajat S, Kaweewat K, *et al*. Subacute toxicity and effects of Red Kwao Krua (*Butea superba*, Roxb.) on reproductive system of male albino rats. Chiang Mai: The 2nd National Seminar on Pharmaceutical Biotechnology, 2000 (in Thai with English abstract).

Wild B. Summary report on the performance of the in vivo micronucleus assays. Evaluation of short term test for carcinogens. 1st ed. New York: Cambridge University Press, 1988: 145-1.148.

Zimnan D. DDT linked to genetic damage. *Newsletter* 1970;1:13.