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Inhibitory Effects of Three Philippine Medicinal Plants on Somatic and Germ Cell Genotoxicity[1]

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Three medicinal plants, ampalaya, kamias and makabuhay possessing fertility regulating properties were screened for mutagenetic and antimutagenetic activity.

The micronucleus test showed that the plants did not have chromosome-breaking effects on mammalian somatic cells. Rather, they were anticlastogenic against dimethylnitrosamine (DMN) and tetracycline HCl. The dominant lethal assay proved that the samples were non-mutagenic to germ cells. The test plants also exhibited antigenotoxicity against tetracycline HCl.

Keywords: medicinal plants, somatic/germ cell genotoxicity, ampalaya, kamias, makabuhay.

Momordica charantia Linn. (ampalaya or bitter melon) family *Cucurbitaceae, Averrhoa bilimbi Linn.* (kamias) family *Oxalidaceae* and *Tinospora rumphii Boerl* (makabuhay) family *Menispermaceae* are indigenous medicinal plants used locally for fertility regulation. These plants are also known to possess numerous medicinal values (1,2,3,4). Juice extracted from ampalaya leaves are used to treat diarrhea, headaches and children's coughs. The fruit extract is a valuable cure for chronic colitis, bacillary dysentery and acts as astringent.

The leaves of *A. bilimbi* are used by Malays as paste applied to hot itches while the Javanese use this for mumps, rheumatism and pimples. The leaves either fresh or fermented are taken internally for syphilis. The fruit can be used for beri-beri, billiousness and cough.

The medicinal values of *T. rumphii* are well established too. An aqueous extract of the plant is used to treat stomach trouble, diarrhea and indigestion. A decoction of the stem is used internally as tonic and as an antimalarial agent and externally as parasiticide.

Since the complete chemical composition of each of these plants has not yet been established, crude preparations might contain substances which can have deleterious effects on the human system. A considerable number of plant have already been tested for mutagenic properties and while a majority yielded negative results (2,3,4,5,6,7), there were a few like siling labuyo and malunggay seeds which were found to be clastogenic to somatic cells.

In this study the mutagenic and anti-mutagenic potentials of the three plants on mammalian somatic and germ cells were determined using the micronucleus test and the dominant lethal assay.

MATERIALS AND METHODS

Test Animals

Japanese Namru mice used for both tests were purchased from Bio-Research in Cubao while Swiss Webster mice were obtained from the University of the Philippines College of Veterinary Medicine in Diliman, Quezon City.

Sample Preparation

Fruit concentrates of *M. charantia* and *A. bilimbi* were prepared by the Testing and Standardization Division of the Industrial Technology Development Institute (ITDI). Dried stems of *T. rumphii* were provided by U. P. Los Baños.

***M. charantia* fruit concentrate.** Fresh ampalaya fruits were washed, halved lengthwise and grated in a blender. The mixture was squeezed through cheesecloth to extract the juice. To remove fine particles, the juice was centrifuged. The supernatant was used as sample. A gram of fruit yielded 0.51 mL concentrate.

***A. bilimbi* fruit concentrate.** Fresh kamias fruits were washed, the calyx removed and the fruits cut crosswise. The fruit pieces were grated in a blender. To extract the juice, the mixture was squeezed through cheesecloth. The extracted juice was used as sample. Yield per gram fruit was 0.67 mL.

[1] Portion of M.S. Thesis of the Senior Author

T. rumphii decoction. Dried makabuhay stems were weighed, cut into small pieces and placed in a covered glass container. Enough distilled water was added to cover the material. This was boiled for fifteen to thirty minutes and filtered with the use of a coarse filter paper. The liquid obtained was measured and three concentration were prapared: 1mL/0.50 g, 1 mL/1g and 1 mL/2g of plant material.

Equipment and Chemicals

Mice were weighed on a Mettler top-loading balance P1000 (Van Waters and Rogers, Switzerland). Centrifugation of fetal calf serum containing bone marrow cells was carried out in an ordinary table centrifuge Model X1708 (International Clinical Centrifuge, USA), with a speed of 2300 rpm. Cells were counted under a binocular microscope, Olympus Model CHB-213, Japan.

May-Grunwald stain and dimethylnitrosamine [DMN] analytical grade were obtained from Sigma Chemical Company, United States; fetal calf serum from Gibco laboratories, United States and Giemsa stain from Fluka Germany. Tetracycline hydrochloride (Pfizer) in capsule form was purchased from a local drugstore.

Determination of Chromosome-Breaking Effects

The clastogenic and anti-clastogenic properties of the samples were measured by the Micronucleus test developed by Schmid (12).

Three preparations of each of the test substances were administered orally at a constant dose of 0.5 mL/20gBW; each preparation was given to 5 mice of either sex. The known mutagens, dimethylnitrosamine [DMN: 15 mg/kg body weight (BW)] and tetracycline HCl (110 mg/kgBW) which served as positive controls were administered by intraperitoneal injection. The treatments were made 30 and 6 h before the mice were sacrificed. Immediately after sacrificing the mice, bone marrow was extracted and smears were prepared (3 slides/mice). The slides were stained and scored under a high power microscope by counting mnPCEs/1,000 PCEs.

The dominant lethal assay devised by Generoso (16) was used to determine the genotoxic and antigenotoxic effects of the test substances on germ cells. Male animals were given the plant preparations orally for genotoxic determination while the animals were injected intraperitoneally with tetracycline HCl and immediately administered the test substances orally for antigenotoxicity determination. Parallel experiments using distilled water and tetracycline HCl as negative and positive controls respectively were done. Dosage used for each sample was 0.50 mL/ 20 gBW and for tetracycline HCl 110 mg/KgBW.

Six days after treatment, the males were caged with two females. Each for two weeks per trial for a total of ten trials. Females were checked for vaginal plugs in the mornings during the mating period. The first day of the appearance of a vaginal plug was counted as day one of pregnancy. Pregnant females were sacrificed at 18 days of gestation. On the day of sacrifice, the following parameters were measured:

mean fetal weight

$$\text{fertility index} = \frac{\text{no. of pregnant females}}{\text{no. of females mated}} \times 100$$

$$\text{gestation index} = \frac{\text{no. of live implants}}{\text{no. of total implants}} \times 100$$

$$\%\ \text{dead implants} = \frac{\text{no. of dead implants}}{\text{no. of total implants}} \times 100$$

$$\text{implantation index} = \frac{\text{total implants}}{\text{no. of pregnant females}}$$

RESULTS AND DISCUSSIONS

Antimutagenecity Potential

The micronucleus test is an in vivo assay that determines chromosome damage inflicted on somatic cells with the production of micronucleated polychromatic erythrocytes. Table 1 shows that all the three samples, *M. charantia*, *A. bilimbi* and *T. rumphii at* all dose levels are non-mutagenic there being no significant increase in the number of micronuclei formed over the negative control group after treatment of the test animals with the plant samples. The plant preparations though were found to exhibit antimutagenic properties against the known mutagens DMN and tetracycline HCl. The chromosome-breaking effects of the two clastogens are significantly reduced as shown by the decrease in the number of micronucleated polychromatic erythrocytes in animals given both the sample and the mutagens. It is surmised that the presence of the B vitamins and mineral ions like iron in the plant systems is responsible for this anti-mutagenic effect.

The extent of chromosome damage induced by mutagenic chemicals on the germ cells of male mice is measured by the dominant lethal test. A dominant lethal mutation indicates major genetic damage which leads to the death of an individual heterozygote (13,14,15,16,17).

These are manifested either as unimplanted eggs or as deciduomata. A deciduomata or "mole" is an outgrowth of the uterus at the site of the implanting blastocysts which itself fails to develop much after implantation.

A low level of dominant lethal effects is usually expressed in terms of increased number of moles. A high dose of mutagen usually results in high dominant lethal effects and the mutations are expressed in embryos which fail to implant. To be considered mutagenic, a chemical must exhibit post-implantation lethality. Conversely, there should be no evidence of both pre- and post- implantation lethality if it is to be pronounced non-mutagenic in a particular species (14,16,18,19).

Table 1. Antimutagenicity potential of *M. charantia*, *A. bilimbi* and *T. rumphii* agains somatic cell genotosicity

Sample	*M. charantia*			*A. bilimbi*			*T. rumphii*		
	Mutagen dose (mg/KgBW)	Sample dose (mL/20gBW)a	No. of mnPCEs per 1000 PCEs ± S.D. b	Mutagen dose (mg/KgBW)	Sample dose (mL/20gBW)a	No. of mnPCEs per 1000 PCEs ± S.D. b	Mutagen dose (mg/KgBW)	Sample dose (mL/20gBW)a	No. of mnPCEs per 1000 PCEs ± S.D. b
c (Negative control) Distilled water	---	---	1.00 ± 0.23	---	---	2.33 ± 0.66	---	---	1.73 ± 0.60
Test Substance alone	---	0.50	1.33 ± 0.24	---	100	1.47 ± 0.38	---	1.00	2.60 ± 0.37
DMN	15	---	6.87 ± 0.56	15	---	7.60 ± 0.60	15	---	8.00 ± 0.91
Test Substance + DMN	15	0.50	* 2.00 ± 0.53	15	100	* 1.87 ± 0.61	15	1.00	* 1.93 ± 0.55
Tetracycline-HC1	110	---	6.40 ± 0.44	110	---	6.87 ± 0.18	110	---	8.20 ± 0.61
Test Substance + T. HC1.	110	0.50	* 2.00 ± 0.41	110	100	* 1.67 ± 0.34	110	1.00	* 2.00 ± 0.33
d (Negative control) Distilled water	---	---	3.67 ± 0.34	---	---	3.53 ± 0.65	---	---	3.87 ± 0.77
Test Substance alone	---	0.25	4.20 ± 0.81	---	50	2.73 ± 0.55	---	0.50	2.60 ± 0.44
Test Substance alone	---	1.00	3.93 ± 0.95	---	75	3.93 ± 0.69	---	2.00	3.93 ± 0.86
DMN	15	---	10.40 ± 0.86	15	---	9.73 ± 1.01	15	---	10.67 ± 0.91
Test Substance + DMN	15	0.25	* 6.40 ± 0.80	15	50	* 4.53 ± 1.62	15	0.50	* 4.93 ± 0. 64
Test Substance + DMN	15	1.00	* 4.93 ± 0.55	15	75	* 4.00 ± 0.85	15	2.00	* 2.60 ± 0.49
Tetracycline-HC1	110	---	11.87 ± 0.87	110	---	10.93 ± 0.55	110	---	10.73 ± 1.28
Test Substance + T. HC1	110	0.25	* 8.33 ± 1.05	110	50	* 3.93 ± 0.83	110	0.50	* 4.00 ± 0.78
Test Substance + T. HC1	110	1.00	* 5.93 ± 1.93	110	75	* 5.33 ± 1.62	110	2.00	* 2.80 ± 0.49

a - 0.50 mL/gBW
b - average of 15 slides
* - significantly different from respective controls (alpha = 0.01)
c - Japanese Mamru mice used
d - Swiss Webster mice used

TABLE 2. Antigenotoxicity potential of *M. charantia*, *A. bilimbi* and *T. rumphii* against germ cell genotoxicity

Test substance	Cone/dose	Fertility index (%)	Gestation index (%)	Implantation index	% Dead implants	Mean fetal weight (g)
A. For *M. charantia*						
Negative control (32) (distilled water)	---	75.00	95.98	8.29 ± 2.71	4.02	1.21 ± 0.25
M. charantia (16) (undiluted fruit concentrate)	0.50mL/20gBW	68.75	98.70	7.00 ± 1.76	1.30 *	1.36 ± 0.13
Positive control (16) (Tetracycline HCl)	110mg/kgBW	12.50	82.35	8.50 ± 0.50	17.64	0.95 ± 0.08
T HCl +	110mg/kgBW	61.11 *	97.00	9.09 ± 1.68	3.00 **	1.12 ± 0.28 **
M. charantia (18) fruit concentrate	0.50 mL/20gBW					
B. For *A. bilimbi*						
Negative control (16)	---	75.00	98.75	6.67 ± 2.01	1.25	1.31 ± 0.25
A. bilimbi (20)	0.50mL/20gBW	70.00	97.27	7.86 ± 2.70	2.73	1.10 ± 0.23
Positive control (20) (Tetracycline HCl)	110mg/kgBW	55.00	90.29	9.36 ± 1.61	9.71	1.10 ± 0.23
T HCl *	110mg/kgBW	75.00 **	98.99 **	8.25 ± 3.17	1.01 **	1.07 ± 0.33
A. bilimbi (16)	0.50mL/20gBW					
C. For *T. rumphii*						
Negative control (36) (distilled water)	---	66.67	98.76	8.67 ± 2.62	1.44	1.14 ± 0.30
T. rumphii (20) (1.00g/mL)	0.50mL/20gBW	65.00	97.35	8.69 ± 3.54	2.65	1.00 ± 0.26
Positive control (32) (Tetracycline HCl)	110mg/kgBW	37.50	92.00	8.33 ± 3.88	8.00	1.11 ± 0.26
T. rumphii (28) (1.00g/mL)	0.50mL/20gBW					

a - figure in parenthesis represent sample size

** - significantly different from positive control (alpha = 0.05)

Strain of mice used: SWISS WEBSTER

Antigenotoxicity Potential

Table 2 summarizes the antigenotoxicity potential of *M. charantia, A. bilimbi* and *T. rumphii* against germ cells.

Fertility Index. Relative to their negative controls, there were no significant differences observed in the fertility index shown by *M. charantia, A. bilimbi* and *T. rumphii*. However, with tetracycline, there was a very sharp drop in the fertility index. Administration of the three plant samples together with tetracycline resulted in a considerable increase in fertility index.

Implantation Index. There were no significant differences in implantation indices shown by all the test samples, their negative and positive controls. The same is true when samples were administered together with the positive controls.

Gestation Index. There was an observed reduction in the gestation index produced by tetracycline as compared to the negative control. However, there were no significant differences between the results of the test samples and their corresponding controls. A significant increase in gestation index was observed only in *M. charantia* and *A. bilimbi* when they were given simultaneously with tetracycline HCl.

% Dead Implants. *M. charantia* showed a significant decrease in percent dead implants in relation to the negative control but no obvious difference was observed in *A. bilimbi* and *T. rumphii*. *A. bilimbi* significantly decreased the % dead implants when given with tetracycline as compared to that produced by tetracycline alone.

Mean fetal weights. A significant difference in mean fetal weight was only observed when *M. charantia* and tetracycline HCl were administered together as compared to the positive control. Results with *A. bilimbi* and *T. rumphii* were not significant.

All these results indicate that *M. charantia* and *A. bilimbi* are antigenotoxic against tetracycline in the micronucleus test. **It is suggested that vitamins and mineral ions in the test systems are responsible for the exhibited antigenotoxic effects.**

The same is also proposed for the antigenotoxic effects on germ cells. Hydrogen-bonded interactions with Vitamins A and B and chelation with iron could have been responsible for the inhibition of tetracycline interaction with DNA.

It would be worthwhile to ascertain the identity of the substances in *T. rumphii* responsible for the anticlastogenic properties proven in the micronucleus test and the mechanism of action which these substances employ to bring about the desired result. This would be of value in the elucidation on its mode of action on germ cells and perhaps explain the results obtained in this study.

LITERATURE CITED

1. Philippine National Formulary. 1980. National Science Development Board. First Edition, Second Printing. 60-63.
2. Quisumbing E. 1978. Medicinal Plants of the Philippines. Katha Publishing Co., Inc. 300-301.
3. Agravante. 1985. Philippine Plants: Their Medicinal, Culinary and Cosmetic Values. Rex Bookstore. 19.
4. Divinagracia GL. 1984. Medicinal plants. Farm Horizons. 4:22.
5. Sylianco CYL, FRB Blanco and CM Lim. 1987. Mutagenicity, clastogenicity and antimutagenicity of medicinal plant tablet produced by the NSTA Pilot Plant II. Sambong tablets. Philipp J Sci 116:13-18.
6. Yaplito MA and CYL Sylianco. 1985. Mutagenicity, clastogenicity and antimutagenicity potential of *Barringtonia asiatica* L. seed extracts. Nat Appl Sci Bull 37:149-154.
7. Sylianco CYL, FRB Blanco and CM Lim. 1986. Mutagenicity, clastogenicity and antimutagenicity of medicinal plant tablets produced by the NSTA Pilot Plant I. yerba buena tablets. Philipp J Scie 115:299-305.
8. Sylianco CYL, JA Concha, AP Jocano and CM Lim. 1986. Antimutagenic effects of expressions from twelve medicinal plants. Philipp J Sci 115:23-30.
9. Sylianco CYL, JA Concha, AP Jocano and CM Lim. 1986. Antimutagenic effects of eighteen Philippine plants. Philipp J Sci 115 (4):293-298.
10. Sylianco CYL, JA Concha, AP Jocano and CM Lim. 1984. Non-mutagenic and non-clastogenic Philippine medicinal plants. **Asian J Pharm Sci 6:49-69.**
11. Sylianco CYL, I Panizares and AP Jocano. 1985. Clastogenic effects on bone marrow erythrocytes of some medicinal plants. Philipp J Sci 114:39-52.
12. Schmid W. 1976. The micronucleus test for cytogenetic analysis. In Chemical Mutagens: Principles and Methods for their Detection. Ed A. Hollaender. New York: Plenum Press. 1:31-53.
13. Bateman AJ and SS Epstein. 1972. Dominant lethal mutations in mammals. In Chemical Mutagens: Principles and Methods for their Detection. Ed A. Hollaender, New York: Plenum Press. 2:541-567.
14. Sundaram K and PS Chauhan. 1974. Dominant lethal and mutagenicity testing. In Laboratory Manual for a Course on Mutagenicity Testing of Drugs and Chemicals. Bhabba Atomic Research Center, Bombay.
15. Searle AG. 1974. The Dominant lethal test in mammals. In Laboratory Manual for a Course on Mutagenicity Testing of Drugs and Chemicals. Bhabba Atomic Research Center, Bombay.
16. Generoso WM. 1980. Dominant lethal mutations and heritable translocation tests in mice. In Proceedings of the Southeast Asian Conference on Detection and Regulation of Environmental Mutagens, Carcinogens and Teratogens. University of the Philippines, Diliman, Quezon City. 146-150.
17. Generoso WM, RJ Preston, and JG Brewen. 1975. 6-Mercaptopurine, an inducer of cytogenetic and dominant lethal effects in premeiotic cells of male mice. Mutation Research. 28:437-447.
18. Bateman AJ. 1966. Testing Chemicals for Mutagenicity in Mammal. Nature. 210:205-205.
19. Bateman AJ. 1960. The induction of dominant lethal mutations in rats and mice with triethylenemelamine. Genetic Research 1:381-392.