

ACTION OF AN EXTRACT OF *Stryphnodendron obovatum* BENTH SEED ON RAT BONE MARROW AND ON HUMAN LYMPHOCYTES

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ABSTRACT

Stryphnodendron obovatum Benth (Barbatimão) is an important source of tannin used in industry. This plant has different biological activities, including inhibition of embryo development in rats and toxicity to cattle when ingested, and is also used to treat some human diseases. Because of its wide distribution and use, we studied the effect of an extract of Barbatimão seeds on Wistar rat bone marrow cells (*in vivo*) and on human peripheral blood lymphocytes (*in vitro*) in terms of mutagenesis and induction of colchicine-metaphases. The treatments did not cause a statistically significant increase in the number of chromosome aberrations or SCEs in either test system used. In the tests on rats there were a small induction of colchicine-metaphases, and in the lymphocyte cultures, this induction was dependent on time and form of treatment. A cytotoxic effect occurred in the lymphocyte cultures, which was dependent on increasing concentrations and time of exposure to the extract. Some treated metaphases presented banded chromosomes.

INTRODUCTION

Brazil has a great variety of medicinal plants. *Stryphnodendron obovatum* Benth (Leguminosae, Mimosoideae), popularly called "Barbatimão" is a typically South American plant with semi-fleshy beans. During periods of drought in the Northeastern part of the country, starving cattle eat these beans and then die of intoxication, with sizable

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losses to the cattle raising industry of this region. Intoxicated animals present signs of photosensitivity and hepatic damage, possibly caused by saponins present in the beans (Rizzini and Mors, 1976). Similar data concerning intoxication have been reported for cattle treated with *S. barbatimão* Mart. beans. The malnutrition, dehydration and edema observed in these animals were attributed to tannin, which is the major toxic component of the plant (Pereira *et al.*, 1989a,b,c). The bark of the plant contains tannin, which confers an astringent action, explaining the medicinal use for healing purposes (Panizza *et al.*, 1988).

The extract of *S. obovatum* Benth seeds inhibits the growth of rat embryos, as well as the development of bones and nervous system, and causes hypotrophy of tongue muscles. In adult rats, the extract also delays the development of palatine, Weber, submandibular and parotid glands (Contrera *et al.*, 1983). The extract also affects the life cycle of *Drosophila melanogaster* by inhibiting fly development, by causing a progressive decrease in the number of imagoes and, at certain concentrations, by interrupting development during the pupal phase (Rodrigues, 1982).

Because of its astringent properties, Barbatimão is important in folk medicine. It is used topically or ingested as a seed or as a bark infusion against hemorrhage, leucorrhea, diarrhea, hernia, and ulcers (Gomes, 1972). Thus, in view of the wide distribution of the genus *Stryphnodendron* and of its use in folk medicine, it is important to evaluate the possible mutagenic, cell-division blocking and colchicine-metaphase inducing effects of its seed extract as these will indicate its mutagenic and/or carcinogenic potential for man.

MATERIALS AND METHODS

S. obovatum Benth seeds

Barbatimão fruits were collected in the cerrado from the interior of São Paulo State. Seeds were removed from ripe fruits, selected, dried, and ground. The solutions for the treatments were prepared by diluting the seed powder in distilled water. After soaking for 12 h, the solutions were lightly boiled for 3-5 min and filtered.

Wistar rat bone marrow cells

The treatment of the Wistar rats (*Rattus norvegicus*) was performed *in vivo* by injecting each rat intraperitoneally (i.p.) with a single dose of Barbatimão seed extract (13.2 mg/100 g body weight (b.w.) or 105.6 mg/100 g b.w.), with and without administration of 0.16% colchicine (Merck) 1.5 h before sacrifice. The animals were

sacrificed at different times after treatment. Two control groups were used, one with and one without administration of colchicine.

Metaphase cell preparations were obtained from rat bone marrow by the technique of Ford and Hamerton (1956), modified. A total of fifty metaphase preparations were analyzed for each rat submitted to colchicine treatment, and thirty were analyzed for each rat not treated with colchicine. For the cells not submitted to the action of colchicine, analysis depended on the presence of chromosomes of adequate structure and morphology, and these preparations were called colchicine-metaphases.

Human peripheral blood lymphocytes

Group 1 - Peripheral blood lymphocytes were obtained from healthy individuals (2 males and 3 females) aged 20 to 30 years. Cultures were treated at the beginning of incubation with Barbatimão seed extract at a concentration of 0.7 µg/1 ml culture medium for 72 h, with and without addition of 10 µg/1 ml 5-bromo-2-deoxyuridine (Sigma). Negative controls were done for the analysis of chromosome aberrations and SCEs, and 0.16 µg/1 ml colchicine (Merck) was added to all cultures 2 h before harvesting.

Group 2 - Blood from a female was used for this experiment. Two controls were performed, i.e., with and without the addition of colchicine 2 h before harvesting. Two treatments were performed with the Barbatimão seed extract: after 24 h of incubation, the cultures were treated with 0.95 mg/1 ml culture medium for 2, 3, 4, 5 or 6 h. Cultures were then washed to interrupt exposure to the drug by changing the medium. The cells were fixed after 72 h of culture, and no colchicine was added. After 24 h, the same concentration was used for exposure up to 48 h, i.e., up to the time for harvesting, with colchicine. In the second treatment the cultures were treated with 4.22 mg/1 ml medium for 1 and 2 h before harvesting, with and without joint addition of colchicine and Barbatimão.

Metaphase preparations were obtained by the technique of Moorhead *et al.* (1960), modified. Fifty metaphases from each culture were analyzed for chromosome aberrations. SCEs were analyzed by the technique of fluorescence plus Giemsa (FPG) of Perry and Wolff (1974) and of Korenberg and Freedlender (1974), modified. Twenty metaphases per culture were analyzed.

RESULTS

Wistar rats

Table I presents the percentage of metaphases with aberrations, the number of chromosome aberrations, which were of the chromatid, isochromatid and centromeric

Table I - Frequency of chromosome aberrations and of colchicine-metaphases in Wistar rat bone marrow cells from controls and from animals treated with a single i.p. dose of *Stryphnodendron obovatum* Benth seed extract, with and without colchicine administration.

Treat. mg/100 g	Colchicine	Time of sacrifice	Number of animals	% of metaphases altered*	Aberrations*				% Total colchicine- metaphases*
					Gap		Breaks		
					C	IC	C	CE	
-	-	24:00	8	0.0	0	0	0	0	16.3
-	+	24:00	8	1.8	5	0	2	0	100.0
13.2	-	24:00	4	0.0	0	0	0	0	35.0
13.2	+	24:00	4	1.0	1	0	1	0	100.0
105.6	-	1:30	8	0.0	0	0	0	0	5.0
	-	2:00	4	0.0	0	0	0	0	3.3
	-	2:30	4	0.0	0	0	0	0	11.7
	-	3:00	4	0.0	0	0	0	0	4.2
	-	6:00	4	0.0	0	0	0	0	40.0
	-	12:00	4	0.8	0	0	1	0	37.5
	-	24:00	8	2.5	0	0	7	2	30.4
105.6	+	1:30	8	3.3	6	1	13	0	100.0
	+	6:00	4	0.5	0	2	0	0	100.0
	+	12:00	4	0.5	1	0	0	0	100.0
	+	24:00	8	1.3	4	0	3	0	100.0

C, Chromatid aberration; IC, isochromatid aberration; CE, centromeric aberration.

* $p > 0.05$, i.e., no statistically significant difference between controls and/or between treatments.

break and gap types, and the percentage of colchicine-metaphases which were obtained spontaneously or induced with colchicine and/or Barbatimão at concentrations of 13.2 mg and 105.6 mg/100 g b.w., for different periods. The number of chromosome aberrations did not differ significantly ($p > 0.05$, Kruskal-Wallis test) (Hollander and Wolfe, 1973). At the concentrations and times of exposure used, Barbatimão did not increase the frequency of chromosome changes or the number of altered metaphases in

relation to the control. The number of colchicine-metaphases induced by Barbatimão without the addition of colchicine also varied in this experiment, but the difference was not statistically significant when compared to the untreated control.

Human lymphocytes

The concentrations of Barbatimão seed extract used (7-714 $\mu\text{g}/1\text{ ml}$) were cytotoxic and inhibited culture growth. Table II presents the number of metaphases with aberrations and the number of chromosome aberrations, which were of the chromatid, isochromatid and centromeric break and gap types. Table III presents the total and mean (\pm SE) number of SCEs per cell for control cultures and for cultures of lymphocytes from five individuals treated with 0.7 $\mu\text{g}/1\text{ ml}$ Barbatimão for 72 h. As shown by the binomial sign test (Siegel, 1956; Conover, 1971), there was no statistically significant difference at the 5% level in induction of chromosome changes and SCEs by Barbatimão seed extract compared to the control.

Table IV presents the results of an additional test carried out on lymphocyte cultures, to evaluate the potential of the Barbatimão seed extract for inducing typical colchicine- metaphases at concentrations of 0.95 and 4.22 $\text{mg}/1\text{ ml}$ and at different times of treatment, followed or not by a change of medium. In the control, the metaphases presented long and joined chromatids and their morphology was not ideal for standard

Table II - Frequency of chromosome aberrations in the control (CON), human lymphocytes and in lymphocytes treated (TR) with *Stryphnodendron obovatum* Benth seed extract at a concentration of 0.7 $\mu\text{g}/1\text{ ml}$ culture medium.

Individual (sex)	Metaphase altered		Aberrations*			
			CON		TR	
	CON	TR	Gap	Break	Gap	Break
1 (F)	3	3	2	2	2	2
2 (F)	3	4	1	2	6	2
3 (F)	6	12	2	5	3	13
4 (M)	4	1	2	5	0	2
5 (M)	3	7	3	2	1	8
Mean	3.8	5.4	2.0	3.2	2.4	5.4

* $p > 0.05$, i.e., no statistically significant difference between controls and/or between treatments.

Table III - Total frequency and mean number of SCEs/cell in control human lymphocytes and in lymphocytes treated with *Stryphnodendron obovatum* Benth seed extract at a concentration of 0.7 µg/1 ml culture medium.

Individual (sex)	Total SCEs*		SCEs/cell + Standard error	
	Control	Treated	Control	Treated
1 (F)	196	173	9.80 ± 4.44	8.65 ± 3.05
2 (F)	133	176	6.65 ± 2.56	8.80 ± 2.53
3 (F)	62	99	3.10 ± 2.75	4.95 ± 3.86
4 (M)	104	115	5.20 ± 3.78	5.75 ± 3.65
5 (M)	113	172	5.65 ± 3.51	8.60 ± 5.86
Mean ± SE	121.6	147.0	6.08 ± 3.41	7.35 ± 3.79

* $p > 0.05$, i.e., no statistically significant difference between controls and/or between treatments.

chromosome analysis, as is the case for colchicine-induced metaphases. In the treatments of 2 and 3 h of exposure with Barbatimão alone (0.95 mg/1 ml), after 24 h of incubation the extract induced the formation of normal colchicine-metaphases in 72 h cultures. In the treatments lasting 4, 5 and 6 h, also with later recovery, there was a decrease in the number of dividing cells, the chromosomes showed a banded pattern and the chromatids remained joined after the last two exposures, thus preventing appropriate standard analysis. At the same concentration at which Barbatimão was added to the culture 24 h after incubation and colchicine was added 2 h before harvesting (72 h) there was a cytotoxic effect which fully inhibited cell division. In the third treatment, in which Barbatimão (4.22 mg/1 ml) was added 1 to 2 h before harvesting (72 h), the metaphases presented joined and banded chromatids. After the same type of treatment, with the addition of colchicine for 1 and 2 h, some metaphases presented banded chromosomes.

DISCUSSION

S. obovatum Benth seed extracts did not have clastogenic effects on the bone marrow of Wistar rats treated *in vivo* (Table I) or on human peripheral blood lymphocytes treated *in vitro* (Table II), even though the cells were exposed at all stages of the cell cycle and for the entire duration of the experiment. The lack of induction of chromosome aberrations in human lymphocytes may have been due to the low Barbatimão concentration used, since elevated doses caused a cytotoxic effect which inhibited culture

Table IV - Percentage of colchicine-metaphases obtained in control human lymphocyte cultures and in cultures treated with *Stryphnodendron obovatum* Benth seed extract, with and without the addition of colchicine.

Treat. (mg/1 ml)	Colchicine	Time of treatment (hour)	% of colchicine-metaphases	Observations
-	-	-	0	Long and joined chromatids
-	+	-	100	
0.95	-	2	100	
	-	3	100	
	-	4	50	Banded chromosomes
	-	5	0	Joined and banded chromatids
	-	6	0	Joined and banded chromatids
0.95	+	48	0	Cytotoxic effect
4.22	-	1	0	Joined and banded chromatids
	-	2	0	Joined and banded chromatids
4.22	+	1	100	Some metaphases with banded chromosomes
	+	2	100	Some metaphases with banded chromosomes

growth and therefore prevented analysis. The extract also induced no statistically significant increase in SCE number in the exposed lymphocytes (Table III).

The percentage of colchicine-metaphases induced by Barbatimão seed extract treatment did not reach 50%, and some of the values were close to those obtained for untreated controls. Thus this treatment is not indicated as a substitute for colchicine, which always induces 100% of these metaphases.

The data obtained for human lymphocyte cultures (Table IV) showed that the effect was determined by the time and form of treatment. The times of 2 and 3 h of exposure to Barbatimão (0.95 mg/1 ml), 24 h after the culture was started, with later recovery and with no further contact with the drug for the remainder of the experiment

(45-46 h) were the most adequate for the induction of metaphases of ideal morphology and condensation for standard chromosome analysis, similar to those induced by colchicine. For this same treatment, when the time of exposure to the drug was increased to 4-6 h, the number of metaphases was decreased, possibly due to a cytotoxic effect of Barbatimão, despite the fact that the cells were allowed to recover with no further contact with the drug for 42-44 h. These metaphases presented banded chromosomes and, with increasing times of exposure, they also presented joined chromatids. This cytotoxic effect was highest when the cultures were not recovered and the time of treatment was increased to 48 h, with full inhibition of cell division. Effects similar to those observed with treatment of 0.95 mg/1 ml (5 and 6 h) and for the untreated control were obtained when the Barbatimão concentration was increased to 4.22 mg/1 ml for only 1 and 2 h. When colchicine was also added some metaphases with banded chromosomes were observed.

Alterations in chromosome morphology similar to those we observed in human lymphocytes treated with Barbatimão have been obtained in several studies (Arrighi and Hsu, 1965; Waring, 1965; Stubblefield, 1966; Crawford and Waring, 1967; Angerer and Moudrianahis, 1973; Hsu *et al.*, 1973; McGill *et al.*, 1974; Pathak *et al.*, 1975; Linnainmaa *et al.*, 1978). Goodpasture and Arrighi (1976), in a study of mammalian cells treated *in vitro* with food seasonings, noted that these substances were cytotoxic, causing the appearance of decondensed and banded chromosomes. Turmeric immediately interrupted mitosis, causing an accumulation of metaphases, which suggested that this seasoning affects spindle proteins; and the chromosomes showed a progression of alterations including unfolding, chromatid separation and fragmentation, depending on dose and duration of treatment. In some cells we treated with Barbatimão extract only (data not shown), chromatid separation occurred in metaphase chromosomes, probably due to longer lymphocyte exposure to the drug.

Results similar to the present ones, i.e., negative for SCE induction and positive for the induction of colchicine-metaphases in some treatments, were obtained by Morgan and Crossen (1980). They found no significant difference in SCE incidence between the control and human lymphocyte cultures treated with the compounds of plant origin vincristine and colcemid, which inhibit the mitotic spindle. SCE formation requires the cell to go through an S period of DNA synthesis after the occurrence of damage and, according to the authors, it is unlikely that vincristine can induce DNA damage that would result in an SCE during mitosis and, even though colcemid interferes with DNA synthesis, it does not increase the number of SCEs. The primary effect of both drugs seems to be the interruption of metaphase by interference with, or inhibition of, the formation of a mitotic spindle, with little interaction with DNA.

In the present study, the effect of induction of colchicine-metaphases was also evaluated because this is a cytologic parameter which indicates a change in mitotic spindle function, probably occurring through the inhibition of tubulin polymerization.

In conclusion, under the analysis conditions used and the concentrations and methods tested, the extract of *S. obovatum* Benth seeds did not induce a statistically significant increase in the frequency of chromosome aberrations in Wistar rat bone marrow cells and human lymphocytes, or in the frequency of SCEs in human lymphocytes. Additional studies are needed to determine whether this extract, which is widely used in folk medicine, can be used without risk to human health.

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RESUMO

O Brasil é um país rico em plantas medicinais e o *Stryphnodendron obovatum* Benth (Barbatimão), é uma importante fonte de tanino usado em indústrias. Esta planta tem diferentes atividades biológicas causando inibição do desenvolvimento embrionário em ratos, toxicidade ao gado que a ingere e é usada também, na cura de diversas doenças do homem. Devido a sua ampla distribuição e uso estudamos o efeito do extrato de sementes de Barbatimão em células de medula óssea de ratos Wistar (*in vivo*) e em linfócitos de sangue periférico humano (*in vitro*), avaliando o seu efeito ao nível mutagênico e na indução de metáfases-colchicínicas. Os tratamentos não causaram um aumento estatisticamente significativo no número de alterações cromossômicas e de SCEs nos dois sistemas-testes avaliados. Nos testes em ratos houve pequena indução de metáfases-colchicínicas e nas culturas de linfócitos esta indução foi dependente do tempo e da forma do tratamento. Nas culturas de linfócitos ocorreu um efeito citotóxico dependente do aumento da concentração e do tempo de exposição ao extrato. Algumas metáfases tratadas apresentaram cromossomos bandados.

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