Mutagenicity of the *Musa paradisiaca* (Musaceae) fruit peel extract in mouse peripheral blood cells *in vivo*

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ABSTRACT. Plants are a source of many biologically active products and nowadays they are of great interest to the pharmaceutical industry. In the present study, the mutagenic potential of the *Musa paradisiaca* fruit peel extract was assessed by the single-cell gel electrophoresis (SCGE) and micronucleus assays. Animals were treated orally with three different concentrations of the extract (1000, 1500, and 2000 mg/kg body weight). Peripheral blood cells of Swiss mice were collected 24 h after treatment for the SCGE assay and 48 and 72 h for the micronucleus test. The results showed that the two higher doses of the extract of *M. paradisiaca* induced statistically significant increases in the average numbers of DNA damage in peripheral blood leukocytes for the two higher doses and a significant increase in the mean of micronucleated polychromatic erythrocytes in the three doses tested. The polychromatic/normochromatic erythrocyte ratio scored in the treated groups was not statistically different from the negative control. The data obtained indicate that fruit peel extract from *M. paradisiaca* showed mutagenic effect in the peripheral blood cells of Swiss albino mice.

Key words: *Musa paradisiaca*; Single-cell gel electrophoresis; Micronucleus test; Peripheral blood cells; Comet assay

INTRODUCTION

Since the beginning of civilization, people have used plants as medicine, because they are an important source of many biologically active products. Recently, there has been evidence of a growing interest in plants as a significant source of new pharmaceuticals. Many of today's drugs have been derived from plants, for example, digoxin from *Digitalis* spp, quinine and quinidine from *Chinchona* spp, vincristine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna*, morphine and codeine from *Papaver somniferum* (Rates, 2001). The medical and economical importance of plants has motivated several ethnopharmacological studies that have resulted in the discovery of many interesting properties of plants. Generally, these properties are based on the studies of how native people use plants therapeutically (Barrett, 1994; Coe and Anderson, 1999). Different communities and cultures often use the same plant in different ways. Therefore, it is important to investigate the use and the risks that these products may pose to health.

Musa paradisiaca L. (Musaceae), popularly known as "banana", is a perennial tree-like herb widely distributed in moist tropics. Due to the enriched food value and versatile medicinal value, banana is one of the most important fruits and vegetable crops of several countries. Fruits, leaves, peels, root, and stalks from banana plants have been used orally or topically as a medicine for treating diarrhoea and dysentery, in the healing of intestinal lesions in colitis (Stover and Simmonds, 1987), antilithic (Prasad et al., 1993), inflammation, pains and snakebite (Coe and Anderson, 1999), antiulcerogenic activity (Lewis et al., 1999; Goel et al., 2001), hypoglycemic effect (Ojewole and Adewunmi, 2003), and hypolipidemic and antioxidant actions (Krishnan and Vijayalakshmi, 2005). A constituent hydroxyanigorufone obtained from *M. paradisiaca* showed to be a potential cancer chemopreventive agent (Jang et al., 2002), and Houghton and Skari (1992) and Borges et al. (2005) have also reported the antivenom action of the stem juice from banana plant. Processes for the production of paper pulp, biogas, alcoholic beverages, etc., from banana plant have also been described (Tewari et al., 1986). In this report, we determined the mutagenic response of the *M. paradisiaca* fruit peel extract by measuring DNA damage by the single-cell gel electrophoresis (SCGE) assay and the mean number of micronucleated cells by the micronucleus test on peripheral blood cells from mice *in vivo*.

MATERIAL AND METHODS

Plant material

Fresh peels (500 g) of *Musa paradisiaca* (banana) were air dried at 40°C, powdered and extracted by maceration with water-ethanolic solution (4.0:l, 70.0%) for 2 days. The macerate was filtered, and the extraction procedure repeated. The concentration of the combined extracts under reduced pressure furnished 68.27 g (yield 13.65%) of crude water-ethanolic extract.

Chemicals

N-nitroso-N-ethylurea (ENU, CAS No. 759-73-9) was used as the DNA-damaging

agent detected by SCGE and micronucleus assays. ENU was dissolved in phosphate buffer, pH 6. The other main chemicals used were obtained from the following suppliers: normal melting point agarose (cat. No. 15510-019; Invitrogen); low melting point agarose (cat. No. 15517-014; Invitrogen); sodium salt *N*-lauroyl sarcosine (L-5125; Sigma) and ethylenedi-aminetetraacetic acid (EDTA; Merck).

Animals and assay procedures

Experiments were carried out in 12-week-old Swiss mice (*Mus musculus*), weighing 25-30 g. The animals were acquired from the animal house of the Jose do Rosario Vellano University (UNIFENAS), kept in polyethylene boxes (N = 6), in a climate-controlled environment (25 ± 4°C, 55 ± 5% humidity) with a 12-h light/dark cycle (7:00 am to 7:00 pm). Food (Labina-Purina) and water were available *ad libitum*. Mice were divided into 5 experimental groups of six animals each (three females and three males). *Musa paradisiaca* extract was administered in a single dose of 0.5 mL, by gavage, at concentrations of 1000, 1500, and 2000 mg/kg body weight, chosen on the basis of our acute toxicity studies in mice, which was higher than 2000 mg/kg. The negative control group received distilled water. The positive control group received 50 mg ENU/kg. The SCGE test was carried out according to the method described by Speit and Hartmann (1999), which was based on the original study of Singh et al. (1988) and included modifications introduced by Klaude et al. (1996) as well as additional modifications. Twenty-four hours after the treatment, peripheral blood leukocytes were examined. A 10-μL aliquot of the blood cells from each animal was mixed with 120 μL 0.5% low melting point agarose at 37°C, and rapidly spread onto microscope slides pre-coated with 1.5% normal melting point agarose. Coverslips were added and the slides were allowed to gel at 4°C for 20 min. The coverslips were removed gently and the slides were then immersed in cold, freshly prepared lysing solution consisting of 89 mL of a stock solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH set to 10.0 with ~8 g solid NaOH, 890 mL distilled water and 1% sodium lauryl sarcosine), plus 1 mL Triton X-100 (Merck) and 10 mL DMSO. After protecting from light, the slides were left to stand at 4°C for 1 h and then placed in the gel box, positioned at the anode end, and left in a high pH (>13) electrophoresis buffer (300 mM NaOH per 1 mM EDTA, prepared from a stock solution of 10 N NaOH and 200 mM EDTA, pH 10.0) at 4°C for 20 min before electrophoresis to allow the DNA to unwind. The electrophoresis run was performed in an ice bath (4°C) for 20 min at 25 V and 300 mA. The slides were then submerged in a neutralized buffer (0.4 M Tris-HCl, pH 7.5) for 15 min, dried at room temperature and fixed in 100% ethyl alcohol for 10 min. The slides were dried and stored at least overnight before staining. For the process of staining, slides were briefly rinsed in distilled water, covered with 30 μL 1X ethidium bromide-staining solution prepared from a 10X stock (200 μg/mL) and covered with a coverslip. The material was evaluated immediately at a 400X magnification, using a fluorescence microscope (Nikon) with a 515-560-nm excitation filter and a 590-nm barrier filter. For the micronucleus assay, peripheral blood from the same animals used in the SCGE procedure was collected from an orbital vein, 48 and 72 h after the treatment and then slides of blood smears were made. All slides were coded, fixed with methanol and stained with Giemsa solution. For the micronucleus assay, 4000 polychromatic erythrocytes were scored from each Swiss mouse

(2000 cells from the 48-h blood sample and 2000 cells from the 72-h blood sample). One thousand cells were analyzed per animal to determine the polychromatic/normochromatic erythrocyte ratio. All animals were submitted to euthanasia after the 72-h blood sample collection. The Animal Bioethical Committee of the UNIFENAS, Brazil, approved the present study on September 28, 2005 (protocol No. 13A/2005), in accordance with the Federal Government legislation on animal care.

Scoring procedures and data evaluation

The extent and distribution of DNA damage indicated by the SCGE assay were evaluated by examining at least 100 randomly selected and non-overlapping cells on the slides of each animal. These cells were scored visually according to tail size into four classes, as follow: i) class 0: undamaged, with no tail; ii) class 1: with a tail shorter than the diameter of the head (nucleus); iii) class 2: with a tail length 1 to 2 times the diameter of the head, and iv) class 3: with a tail longer than 2 times the diameter of the head. Comets with no heads and images with nearly all DNA in the tail, or with a very wide tail, were excluded from evaluation because they probably represented dead cells (Hartmann and Speit, 1997). The total score for 100 comets was obtained by multiplying the number of cells in each class by the damage class, ranging from 0 (all undamaged) to 300 (all maximally damaged).

Statistical analysis

Micronucleus and SCGE assay data were submitted to one-way analysis of variance (ANOVA) and the Tukey-Kramer multiple comparison test (Sokal and Rohlf, 1995), using the GraphPad Instat® software, version 3.01. Results were considered to be statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

The alkaline SCGE (Comet) assay is a rapid and sensitive procedure for quantifying DNA damage in mammalian cells (Singh et al., 1988; Sasaki et al., 1997). In this assay, relaxed and broken DNA fragments stream further from the nucleus than intact DNA, so the extent of DNA damage can be measured by the length of the stream. Furthermore, DNA lesions can be measured by the absence of mitotic activity.

Table 1 shows the effects of a 24-h treatment with the *M. paradisiaca* extract on DNA migration in peripheral blood leukocytes from Swiss albino mice on the comet assay. As expected, ENU used as positive control lead to some fragmentation and migration of peripheral blood leukocytes in the SCGE assay. Significant effects on DNA migration were found at the two higher *M. paradisiaca* extract concentrations tested. The 1000 mg/kg dose of the extract produced some DNA damage in the cells but this increase was not statistically significant. When cells were exposed to the test extract, most cells examined on slides were undamaged (class 0), few cells showed minor damage (class 1) and very few showed a large amount of damage (classes 2 and 3). Furthermore, there was a significant difference in DNA migration among the three extract concentrations tested (Table 1).

Table 1. DNA migration in the comet assay for the assessment of genotoxicity of *Musa paradisiaca* extract in peripheral blood leukocytes (collected 24 h after the treatment) from female (F) and male (M) Swiss mice *in vivo*.

Treatment	Animals	Total[1]	Comet class				Scores
			0	1	2	3	
Control	F_1	3	97	3	0	0	3
	F_2	3	97	3	0	0	3
	F_3	1	99	1	0	0	1
	M_1	5	95	5	0	0	5
	M_2	2	98	2	0	0	2
	M_3	4	96	4	0	0	4
	Mean ± SD	3.0 ± 1.41	97.0 ± 1.41	3.0 ± 1.41	0 ± 0	0 ± 0	3.0 ± 1.41
M. paradisiaca extract	F_1	6	94	6	0	0	6
(1000 mg/kg)	F_2	8	92	8	0	0	8
	F_3	5	95	5	0	0	5
	M_1	9	91	9	0	0	9
	M_2	9	91	9	0	0	9
	M_3	4	96	4	0	0	4
	Mean ± SD	6.83 ± 2.13	93.1 ± 2.13	6.83 ± 2.13	0 ± 0	0 ± 0	6.83 ± 2.13
M. paradisiaca extract	F_1	19	81	10	6	3	31
(1500 mg/kg)	F_2	20	80	10	5	5	35
	F_3	12	88	7	3	2	19
	M_1	14	86	7	7	0	21
	M_2	16	84	7	5	4	29
	M_3	32	68	21	10	1	44
	Mean ± SD	18.8 ± 7.11***	81.1 ± 7.11***	10.3 ± 5.42*	6.0 ± 2.36*	2.5 ± 1.87	29.8 ± 9.2**
M. paradisiaca extract	F_1	35	65	22	8	5	53
(2000 mg/kg)	F_2	20	80	17	1	2	25
	F_3	35	65	11	13	11	70
	M_1	26	74	14	5	7	65
	M_2	32	68	19	10	3	48
	M_3	25	75	13	5	7	64
	Mean ± SD	28.8 ± 6.11***	71.1 ± 6.11***	16.0 ± 4.09***	7.0 ± 4.24*	5.8 ± 3.25	54.1 ± 16.4***
N-nitroso-N-ethylurea	F_1	93	7	14	25	54	226
(50 mg/kg)	F_2	79	21	18	23	38	178
	F_3	80	20	8	37	35	187
	M_1	82	18	11	31	40	193
	M_2	81	19	19	33	29	172
	M_3	83	17	11	31	41	196
	Mean ± SD	83.0 ± 5.09***	17.0 ± 5.09***	13.5 ± 4.32***	30.0 ± 5.1***	39.5 ± 8.3***	192 ± 18.9***

[1]Total number of cells with damage (classes 1 + 2 + 3).
*Significantly different from the negative control (P < 0.05).
**Significantly different from the negative control (P < 0.01).
***Significantly different from the negative control (P < 0.001).

To have a parameter of comparison with the SCGE assay, in the present study we also applied a micronucleus test. The most attractive features of this assay are the rapidity and ease with which *in vivo* genetic activity can be demonstrated and the knowledge that these events, being chromosomal in nature, are likely to be significant ones in terms of human risk (Salamone et al., 1980; Mavournin et al., 1990).

Table 2 shows the micronucleus test results obtained in female and male Swiss mice treated with *M. paradisiaca* extract: the number of micronucleated polychromatic erythro-

cytes of each animal and means, for untreated controls and treated animals. The results obtained showed a statistically significant increase in the average number of micronucleated, polychromatic erythrocytes in the 3 doses tested of the plant extract. This table also shows the ratio between the average number of polychromatic erythrocytes with respect to normochromatic erythrocytes in 1000 randomly cells analyzed from each animal. The increased number of micronucleated cells was not associated with cytotoxicity because the polychromatic/normochromatic erythrocyte ratio was not significantly decreased at any doses tested.

Table 2. Number of micronucleated polychromatic erythrocytes (MNPCE) observed in the peripheral blood cells of female (F) and male (M) Swiss mice treated with a *Musa paradisiaca* extract, and respective controls.

Treatment	Time of blood collection	Number of MNPCE per animal						MNPCE (mean ± SD)	PCE/NCE (mean ± SD)
		F_1	F_2	F_3	M_1	M_2	M_3		
Negative control	48 h	3	2	5	3	2	3	3.0 ± 1.09	2.62 ± 0.59
(water)	72 h	3	4	1	3	3	6	3.33 ± 1.63	2.07 ± 0.24
Musa paradisiaca	48 h	6	9	7	4	8	8	7.0 ± 1.78***	2.10 ± 0.50
(1000 mg/kg)	72 h	6	8	7	6	4	6	6.16 ± 1.32*	1.97 ± 0.46
Musa paradisiaca	48 h	11	7	8	7	7	9	8.16 ± 1.60***	3.17 ± 0.90
(1500 mg/kg)	72 h	6	7	7	7	9	6	7.0 ± 1.09**	2.27 ± 1.03
Musa paradisiaca	48 h	7	10	8	8	6	7	7.66 ± 1.36***	1.62 ± 0.17
(2000 mg/kg)	72 h	9	9	7	8	7	11	8.5 ± 1.51***	2.40 ± 0.62
Positive control	48 h	12	15	10	12	12	11	12.0 ± 1.67***	1.98 ± 0.78
N-nitroso-N-ethylurea	72 h	11	9	12	10	11	12	10.83 ± 1.16***	1.93 ± 0.41
(50 mg/kg)									

For each time (48 and 72 h), 2000 cells were analyzed. SD = standard deviation; PCE = polychromatic erythrocytes; NCE = normochromatic erythrocytes.
*Significantly different from the negative control ($P < 0.05$).
**Significantly different from the negative control ($P < 0.01$).
***Significantly different from the negative control ($P < 0.001$).

The phytochemistry screen of *M. paradisiaca* extracts has shown the presence of several polysaccharides. The stalk of the rhizome (scape) of *M. paradisiaca* contains at least 4 different polysaccharide fractions. Its analysis indicated that the scape is essentially a solid state gel containing ~1.5% polyphenols and ~10% polysaccharides (D-Glc, L-Ara, D-Xyl, D-Glucan) with the rest being water (~88.5%) in association with minerals, potassium, silica, lignin, and polyphenolic pigments (Anjaneyalu et al., 1997). Methanol extracts of the fruits contain diarylheptanoid, 1,2-dihydro-1,2,3-trihydroxy-9-(4-methoxyphenyl)phenalene, hydroxyanigorufone, 2-(4-hydroxyphenyl)naphthalic anhydride and 1,7-bis(4-hydroxyphenyl)-hepta-e(E),6(E)-dien-3-one (Jang et al., 2002). Mucilaginous exudates from the pseudo-stem contain uronic acid, neutral sugars, proteins, rhamnose, arabinose, xylose, mannose, galactose, glucose as well as other specific sugars (Mondal et al., 2001). The trace element concentrations of the fruit peels and trunks were described by Selema and Farago (1996); however, there are no data about *M. paradisiaca* fruit peel extract in the literature.

Many studies have shown positive results for the mutagenic action of plant extracts (Sánchez-Lamar et al., 2002; Zanoni et al., 2005). Studies such as ours indicate the importance

of scientifically evaluating the security of the products used in popular medicine, especially those derived from plants. The mutagenic effect of the *M. paradisiaca* fruit peel extract on peripheral blood cells of Swiss mice was studied for the first time in the present article. The results indicated that this crude extract presented a mutagenic effect on blood cells. As fruit peel extract from *M. paradisiaca* is commonly included in folk medicine used by numerous individuals, it seems advisable to moderate its use, and to develop other mutagenic tests to finally establish the health risk for humans.

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