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Modulatory effects of *Duguetia furfuracea* (A. St. Hil) Benth. and Hook. f. in *Drosophila melanogaster* somatic and germinative cells

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ABSTRACT. Mutagenic and antimutagenic activities of the medicinal plant *Duguetia furfuracea* were assessed using SMART/wing and ring-X-loss tests. For the ring-X-loss test, 2- to 3-day-old *Drosophila melanogaster* ring-X-lineage males and virgin ywsn^3 females received *D. furfuracea* infusion at doses of 0.085, 0.042, or 0.014 g/mL for 24 h. We found that *D. furfuracea* did not produce any mutagenic effects in *D. melanogaster* germinative cells. The somatic cells of *D. melanogaster* were analyzed using the SMART/wing test involving three lineages - mwh, flr^3, and ORR - and the same doses of *D. furfuracea* infusion employed in the ring-X-loss test, as well as 20 mM urethane. The results of both standard (ST) and high bioactivation (HB) crosses showed absence of mutagenic activity of *D. furfuracea*. In contrast, in both ST and HB crosses, we

observed a modulatory effect of *D. furfuracea* against the genotoxic activity of urethane.

Key words: Antimutagenicity; *Duguetia furfuracea*; Genotoxicity; Mutagenicity

INTRODUCTION

The plant *Duguetia furfuracea* (A. St. Hil) Benth. and Hook. f. (Annonaceae) is frequently found in the Cerrado, a region covering mainly central Brazil, with populations composed of various individuals (Lorenzi, 2000). Tea made from the root has been used as a sedative, antirheumatic and for the treatment of renal and back pain. It has also been used against kidney disease. Screening of plant species in this family is of great importance since substances with cytotoxic, antiplasmodial, antiparasitic, pesticidal, antimicrobial, antifungal, antimalarial, antioxidant, and antitumor activities have been identified (Frana and Sufredini, 2000; Garcia et al., 2000; de Sousa et al., 2003).

Oxoaporphine alkaloids belong to the Annonaceae family and are known for their biological and pharmacological activities: cytotoxic, antiplatelet, antibacterial, and antifungal. Among these activities, the most representative in the *Duguetia* genus is cytotoxicity, which has been demonstrated in experiments using different strains of tumor cells. This is the case for the commonly isolated substance liriodenine (Silva et al., 2007). Liriodenine is a potent topoisomerase II inhibitor (Woo et al., 1999).

Studies on the chemical composition of *D. furfuracea* essential oil showed a high percentage of alkaloids, including monoterpenes and sesquiterpenes (Silva et al., 2007; Valter et al., 2008). Flavonoid glycosides are also found in this plant (Carollo et al., 2006).

Da Silva et al. (2009) showed the antitumoral, trypanocidal and antileishmanial activities of the extract and alkaloids isolated from *D. furfuracea*. Ethanolic extracts of *D. furfuracea* were tested for larvicidal activity against third-stage *Aedes aegypti* larvae and showed activity (>65% mortality) at 56.6, 162.31, 232.4, 285.76, and 384.37 µg/mL (Rodrigues et al., 2006).

In a phytochemical study of the plant *Hornschuchia obliqua* (Annonaceae), Fechine et al. (2002) reported the presence of the alkaloids roemerine, guadiscine, liriodenine, cleistopholine, and azanthraquinone, already described in the literature and found for the first time in this genus.

Silva et al. (2007) carried out biomonitored phytochemical studies of the extract obtained from the underground parts of *D. furfuracea* in a toxicity bioassay using brine shrimp (*Artemia salina*) and all extracts were active in the brine shrimp lethality bioassay.

Garcia et al. (2000) conducted a triage of extracts obtained from plants that belong to the families Combretaceae, Apocynaceae and Annonaceae in order to assess their possible anti-tumor activity and biomonitored isolates of active compounds against *A. salina.* Their results demonstrated that, among all the species tested, *Annona coriacea* (Annonaceae family) presented the highest toxicity. Tetrahydrofuranic acetogenine, possibly a gigantecin, isolated from *A. coriacea* leaves, was the most active compound, inhibiting human breast carcinoma cells.

Santos et al. (1996) isolated and determined the structure of a new acetogenine

obtained from the ethanolic extract of *A. crassiflora* seeds, named crassiflorine. The same compound received three different names, crassiflorine, bullatanocine, and cherimoline-2, in Brazil, United States, and France, where it was isolated from different species of the *Annona* genus, *A. crassiflora*, *A. bullata*, and *A. cherimolia*, respectively (Cortes et al., 1993). This substance presented significant activity against human tumor cell lines *in vitro* when compared to adriamicine, a well-known antineoplastic agent (Santos et al., 1996). Crassiflorine anti-tumor activity has aroused great interest in *A. crassiflora* Mart. as a possible source of one natural antineoplastic agent (Santos and Boaventura, 1994).

Most acetogenines found in plants of the Annonaceae family have presented anti-tumor activity *in vitro* and *in vivo* against different neoplastic cell lines (de Sousa et al., 2003). A possible anti-tumor activity attributed to the acetogenines found in the plants of the Annonaceae family was experimentally demonstrated by the interruption of ATP production through the inhibition of the enzymes oxi-NADH ubiquinone-reductase (complex I) of the mitochondrial electron transport system (Pollo-Zafra et al., 1996) and ubiquinone-NADH oxidase enzyme, present in the plasma membrane of tumor cells (Morré et al., 1995).

Drugs employed in chemotherapy and radiotherapy, broadly employed in the treatment of cancer, can have undesirable side effects, such as inducing DNA damage in normal cells. It would be useful to investigate compounds that inhibit or reduce these effects. Therefore, it is necessary to find other drugs, and natural products are a good source. From 1983 to 1994 more than 60% of the approved anticancer drugs were found in natural products (Newman et al., 2003). The new drugs are also necessary in the treatment of trypanosomiasis and leishmaniasis, since current chemotherapy is still inadequate and causes many undesirable side effects (Da Silva et al., 2009).

D. furfuracea is widely used in folk medicine, and studies indicate that it has great potential as a trypanocidal and leishmanicidal agent, and possible latent activity against *A. aegypti* mosquito larvae, a vector of important diseases such as dengue and yellow fever. The widespread use of this plant makes it necessary to investigate its possible genotoxic, mutagenic and antimutagenic activity. The present study shows the possible effects of the medicinal plant *D. furfuracea* using the ring-X-loss test, which permits the detection of its possible genotoxic activity against *Drosophila melanogaster* since it shows the response of germinative cells to mutagenic compounds (Cunha et al., 1992), and the SMART/wing test, which assesses mutagenicity in somatic cells (Graf et al., 1984).

MATERIAL AND METHODS

The leaves of *D. furfuracea* used to prepare the infusions were collected in the Cerrado region of the municipality of Paraúna, in the State of Goiás. In the laboratory, all leaves were washed with distilled water and dried at 45°C for 30 min. The dried leaves were used to prepare the infusion at the moment of the treatment of the adults (ring-X-loss test) and the third-instar larvae (SMART/wing) of *D. melanogaster*.

Ring-X-loss test

The ring-X-loss test has a short duration and permits the assessment of the muta-

genic activity of compounds by inducing genic mutations and chromosome breaks in *D. melanogaster* germinative cells. Using the ring-X-loss test in a single phase of spermatogenesis may lead to false-negative results. However, the brood method, which consists of successively mating treated males with virgin females at intervals of 3, 2 and 2 days, results in treated gametes at different phases of spermatogenesis, permitting the separation of sexual cells that become progressively younger at the time of treatment, thus ensuring the efficacy of the results obtained (Zijlstra and Vogel, 1988).

In order to carry out the ring-X-loss test, 2- to 3-day-old *D. melanogaster* ring-X-lineage males and virgin ywsn^3 females were used, whose genotypes are, respectively, C1 (2) yB/y+ YBS and ywsn^3/ywsn^3 (Lindsley and Grell, 1968; Lindsley and Zimm, 1992). For the experimental procedure, aliquots of 2.0 mL of different doses of *D. furfuracea* infusion (0.085, 0.042, and 0.014 g/mL) were distributed in vials and 10 ring-X-lineage males, which had previously undergone a 4-h fast, were placed per vial and kept for 24 h. After being exposed to the solutions for 24 h, the males were mated to produce F1 offspring by removing them from the trip tubes and placing them into tubes containing banana-agar and virgin ywsn^3 females where they were allowed to mate to produce Brood 1 offspring (metabolically inactive sperm cells). After 3 days the males were transferred to fresh tubes containing banana-agar and fresh ywsn^3 virgin females where they mated and produced Brood 2 offspring (metabolically active mature cells). After a further 2 days the males were again transferred to fresh tubes containing banana-agar and fresh virgin ywsn^3 females where they mated to produce Brood 3 offspring (immature sperm cells), the males being discarded after 2 days. In all cases the females were discarded after the eggs were collected and a thick layer of live fermenting yeast supplemented with sucrose was added to the tubes containing the larvae every 3 days. Urethane (20 mM) was used as positive control and sterile distilled water as negative control. The experiment was performed in triplicate at 25° ± 1°C and relative humidity of 60% (Lewis and Bacher, 1968). The individuals of this progeny were classified into phenotypic classes for statistical evaluation (Frei and Würgler, 1988).

The adult forms started to emerge on the 10th day. The F1 progeny were analyzed for 4 days by entomological microscopy technique. Assessment was made possible because the sexual chromosomes of both progenitors were marked with specific visible mutations, which enabled each chromosome (or segment) to be identified based on 1 of the 11 different possible F1 phenotype classes. The loss of one of the phenotypic markers could occur through several mechanisms, i.e., deletion of a section containing a given marker (1 or 2 breaks) or the loss or gain of an entire chromosome by non-disjunction.

The flies were divided into 11 classes, class 1-2 = ring-X-loss and 3 = complete ring-X-loss; 4 and 8 = mosaicism; 6 and 7 = partial loss of the Y-chromosome; 5, 9, 10, and 11 = non-disjunction. The calculation of the frequency of complete loss of the ring-X-chromosome was obtained using the formula: % complete loss of the ring-X-chromosome = class 3 + class 4 divided by class 1 + class 3 + class 4 + class 5 + class 11. The ring-X-loss test was performed according to the model proposed by Frei and Würgler (1988), and the results were classified as inconclusive, positive, weak positive, or negative.

SMART/wing test

Graf et al. (1984) developed a test system named somatic mutation and recombination test (SMART) based on two wing cell markers, *multiple wing hair* (*mwh*) and *flare* (*flr^3*),

for the detection of mutagenic and recombinagenic activity of chemicals in *D. melanogaster*. Larvae trans-heterozygous for the mutations *mwh* and *flr³* are exposed to the test compounds and induced mutations are detected as single spots on the wing blade of surviving adults, while induced recombination leads to *mwh* and *flr³* twin spots and *mwh* single spots. Two different crosses are currently used in the SMART: the standard (ST) cross (flr^3/TM^3 Bd^s females mated with *mwh/mwh* males) (Graf et al., 1989) and the high bioactivation (HB) cross (ORR; flr^3/TM^3 Bd^s females mated with *mwh/mwh* males) (Graf and van Schaik, 1992). The latter cross is characterized by high sensitivity to promutagens and procarcinogens, since the ORR; flr^3/TM^3 Bd^s strains carry chromosomes 1 and 2 from a DDT-resistant Oregon *R*(*R*) line (Dapkus and Merrell, 1977), which is characterized by an increased level of cytochrome p450 enzyme complex (Fragiorge et al., 2008).

In this study, for the SMART wing test in the ST and HB crosses we used three lines: a) multiple wing hair (mwh); b) flr^3 In(3LR)TM^3, ri pp sep I(3)89Aa bx34e and Bd^S; c) ORR;flr^3/In(3LR)TM^3, ri pp sep I(3)89Aa bx34e and Bd^S. The doses of *D. furfuracea* infusion, positive and negative controls, and culture media used to maintain *D. melanogaster* lines and in the assays were the same ones used in the ring-X-loss test. The oviposition medium was used to obtain the larvae and the instant culture medium (Formula 4-24 Carolina Biological Supply Company, Burlington, NC, USA) was employed for the development of the treated third-instar larvae (Graf et al., 1984). The experimental procedure for mutagenesis testing involved two types of crosses: 1) ST - mwh males mated with virgin flr^3 females; 2) HB - mwh males mated with virgin ORR females. The flies were left to cross for 2 days. After that the males and females were transferred to vials containing the oviposition media and kept there for 8 h. After 72 ± 4 h, third-instar larvae were collected by flotation in running water and distributed in vials containing 0.9 g synthetic media for *D. melanogaster*, 3 mL of the different doses of *D. furfuracea* infusion (0.085, 0.042, and 0.014 g/mL), 20 mM urethane as positive control and sterile distilled water as negative control. The adult individuals were conserved in 70% ethanol. These assays were performed in triplicate at 25° ± 1°C and approximately 60% relative humidity (Graf et al., 1984). The dorsal and ventral surfaces of *D. melanogaster* wings were analyzed with an optical microscope at a magnification of 400X. The spots observed were classified according to their size, position in the wing, and type (single, twin) for evaluation and statistical analysis (Frei and Würgler, 1988, 1995).

RESULTS AND DISCUSSION

Table 1 presents the results of the ring-X-loss test using germinative cells of *D. melanogaster*. The progeny was classified into 11 phenotypic classes: class 1 (Xp/Xm); class 2 (Xm/y+YB^s); class 3 (Xm/0); class 4 (Xm/0-Xm/Xp); classes 6, 7, and 8 (Xm/y+Y; Xm/YB^s; Xm/y+YB^s-or Xm/YB^s); classes 5, 9, 10, and 11 (Xm/Xp/y+YB^s; Xm/Xmy+YB^s; Xm/Xmy Xp/o) of the Broods 1, 2, and 3.

Nunes and Carvalho (2003) evaluated the mutagenic potential of *Cochlospermum regium* in *D. melanogaster* germinative cells using the ring-X-loss test and reported a 1.13% loss of the ring-X-chromosome at the dose of 0.013 g/mL in Brood 3. A similar result (1.15%) was found in the present study for Brood 2 using 0.085 g/mL *D. furfuracea* infusion and both studies presented negative results for mutagenesis.

Table 1. Results of ring-X-loss test on *Drosophila melanogaster* males fed with different concentrations of *Duguetia furfuracea* extract or positive (20 mM urethane) and negative (sterile distilled water) controls.

Brood number and concentration of substance tested	Number of progeny with genetic changes							
	Number of normal progeny		P.C.X*	P.P.Y*	M	N.D.	Percentage of progeny with complete ring-X-loss	Mean ± SD
	Female	Male						
Brood 1 (3 days mating) *D. furfuracea*								
0.085 g/mL	1570	1564	1.38	0	13	53	1.38 (-)	1.38 ± 0.06
0.042 g/mL	1636	1594	1.17	0	8	32	1.17 (-)	1.17 ± 0.11
0.014 g/mL	1645	1600	1.19	0	8	26	1.17 (-)	1.17 ± 0.15
Urethane (20 mM)	1535	1597	6.42	22	50	80	6.42 (+)	6.42 ± 0.79
Water	1619	1563	1.12	2	9	37	1.12 (-)	1.12 ± 0.14
Brood 2 (3 days mating) *D. furfuracea*								
0.085 g/mL	1680	1769	1.15	0	9	18	1.15 (-)	1.15 ± 0.03
0.042 g/mL	1628	1704	1.22	0	9	46	1.22 (-)	1.22 ± 0.12
0.014 g/mL	1613	1537	1.14	0	8	19	1.14 (-)	1.14 ± 0.16
Urethane (20 mM)	1661	1715	7.36	4	60	200	7.40 (+)	7.40 ± 1.10
Water	1721	1726	1.34	0	8	72	1.33 (-)	1.33 ± 0.39
Brood 3 (2 days mating) *D. furfuracea*								
0.085 g/mL	1622	1560	1.18	0	10	36	1.18 (-)	1.18 ± 0.11
0.042 g/mL	1698	1597	1.19	0	9	28	1.18 (-)	1.18 ± 0.11
0.014 g/mL	1604	1576	1.09	0	9	17	1.09 (-)	1.09 ± 0.07
Urethane (20 mM)	1728	1583	6.52	12	47	111	6.43 (+)	6.43 ± 0.50
Water	1590	1611	1.21	0	9	22	1.21 (-)	1.21 ± 0.11

P.C.X = complete ring-X-loss; P.P.Y = partial loss of the Y-chromosome; M = mosaicism; N.D. = non-disjunction; * = no statistically significant increases were detected between the negative control and the series treated with the infusion of *D. furfuraceae* (χ^2 for 2 x 2 contingency table).

The ring-X-loss test results using *D. furfuracea* infusion were also compared to those reported by de Sousa et al. (2003), who investigated the effect of *Stryphnodendron adstringens* in *D. melanogaster* germinative cells. Using 66% of the plant extract, the researchers observed a 1.37% loss of the ring-X-chromosome for Brood 3. In our experiments, we observed a 1.38% loss of the ring-X-chromosome for Brood 1 using the 0.085 g/mL *D. furfuracea* infusion. Also, there was a similarity between the results for the negative control in both studies: a 1.82% loss of the ring-X-chromosome for Brood 2 using *S. adstringens* and a 1.21% loss of the ring-X-chromosome for Brood 3 using *D. furfuracea*. Furthermore, for the positive control (20 mM urethane), the results for the loss of the ring-X-chromosome were similar: 4.58% for Brood 1, 7.71% for Brood 2, and 7.52% for Brood 3 using *S. adstringens*; 6.42% for Brood 1, 7.40% for Brood 2, and 6.43% for Brood 3 using *D. furfuracea*. Thus, the results found in the present study are in accordance with those reported in the literature.

Table 2 presents the results for mutagenesis of *D. furfuracea* in *D. melanogaster* somatic cells, using the SMART/wing test, ST and HB crosses. The ST cross descendents treated with different doses of *D. furfuracea* infusion (0.085, 0.042, and 0.014 g/mL) did not present a statistically significant increase ($P > 0.05$) in the frequency of small single spots, large single spots, twin spots, or total spots compared to the negative control (sterile distilled water).

The HB cross descendents treated with 0.085, 0.042, and 0.014 g/mL *D. furfuracea*

infusion did not present a statistically significant increase ($P > 0.05$) in the frequency of small single spots, large single spots, twin spots, or total spots compared to the negative control (sterile distilled water).

Table 2. Frequency of mutant spots observed in marker heterozygous trans-heterozygous descendants of *Drosophila melanogaster* from standard (ST) and high bioactivation (HB) crosses treated with three different concentrations (0.085, 0.042, and 0.014 g/mL) of aqueous extract of *Duguetia furfuracea* (AED).

Cross and AED concentrations	No. of Individuals	Frequency of mutant spots per individual (No. of spots)[a]							
		SSS (1-2 cells)[b] *m* = 2		LSS (>2 cells)[b] *m* = 5		TWS *m* = 5		TOS *m* = 2	
ST									
Negative control	60	0.06	(4)	0.08	(5)	0.06	(4)	0.21	(13)
Urethane (20 mM)									
D. furfuracea	60	0.31	(19) +	0.76	(46) +	0.76	(46) +	1.76	(106)f+
0.085 g/mL	60	0.03	(2) -	0.03	(2) -	0.03	(2) -	0.10	(6) -
0.042 g/mL	60	0.03	(2) -	0.05	(3) -	0.03	(2) -	0.11	(7) -
0.014 g/mL	60	0.03	(2) -	0.05	(3) -	0.03	(2) -	0.11	(7) -
HB									
Negative control	60	0.13	(8)	0.06	(4)	0.08	(5)	0.28	(17)
Urethane (20 mM)									
D. furfuracea	60	0.50	(30) +	0.80	(48) +	0.76	(46) +	1.33	(124)f+
0.085 g/mL	60	0.06	(4) -	0.03	(2) -	0.03	(2) -	0.13	(8) -
0.042 g/mL	60	0.05	(3) -	0.03	(2) -	0.05	(3) -	0.13	(8) -
0.014 g/mL	60	0.06	(4) -	0.03	(2) -	0.03	(2) -	0.13	(98) -

[a]Statistical diagnosis according to Frei and Würgler (1988): *m* = multiplication factor; (+) = positive; (-) = negative; f = weakly positive. Significance level: $\alpha = \beta = 0.05$. [b]Including rare single spot *flr*^{3}. SSS = small single spots; LSS = large single spots; TWS = twin spots; TOS = total spots. Negative control = sterile distilled water.

Table 3 presents the results for antimutagenesis of *D. furfuracea* in *D. melanogaster* somatic cells, using the SMART/wing test, ST and HB crosses.

The results of ST cross repetition 1 using 0.085 g/mL *D. furfuracea* infusion + 20 mM urethane and 0.042 g/mL *D. furfuracea* infusion + 20 mM urethane showed a statistically significant increase ($P > 0.05$) in the frequency of large single spots and twin spots compared to the positive control and a statistically significant decrease ($P < 0.05$) in the frequency of small single spots and total spots compared to the positive control.

The results of the ST cross using 0.085 g/mL *D. furfuracea* infusion + 20 mM urethane presented a statistically significant decrease ($P < 0.05$) in the frequency of small single spots and total spots compared to the positive control (20 mM urethane). The same situation was observed for 0.042 g/mL *D. furfuracea* infusion + 20 mM urethane and for 0.014 g/mL *D. furfuracea* infusion + 20 mM urethane.

The results of the HB cross using the same doses of *D. furfuracea* infusion + 20 mM urethane presented a statistically significant decrease ($P < 0.05$) in the frequency of small single spots, large single spots and total spots.

de Sousa et al. (2003) studied the possible genotoxic activity of the phytotherapic *S. adstringens* (Mart) in *D. melanogaster* somatic cells using the SMART/wing test. They concluded that there was a statistically significant increase in results, and can be correlated to the present study since it presented similar characteristics. The authors found the frequency of 0.41 (23) and 0.37 (20), respectively, for total spots in the negative control of the ST and HB crosses using *S. ad-*

Table 3. Frequency of mutant spots observed in marker heterozygous trans-heterozygous descendants of *Drosophila melanogaster* from standard (ST) and high bioactivation (HB) crosses treated with three different concentrations (0.085, 0.042, and 0.014 g/mL) of aqueous extract of *Duguetia furfuracea* (AED) in association with 20 mM urethane (URE).

Cross and AED + URE concentrations	No. of Individuals	Frequency of mutant spots per individual (No. of spots)[a]							
		SSS (1-2 cells)[b] *m* = 2		LSS (>2 cells)[b] *m* = 5		TWS *m* = 5		TOS *m* = 2	
ST									
Negative control	60	0.05	(3)	0.00	(0)	0.00	(0)	0.05	(3)
Urethane (20 mM)	60	3.70	(222)	0.22	(13)	0.01	(6)	4.01	(241)
D. furfuracea + urethane									
0.085 g/mL + 20 mM	60	0.28	(17)↓	0.06	(4)	0.01	(1)	0.36	(22)↓
0.042 g/mL + 20 mM	60	0.30	(18)↓	0.11	(7)	0.03	(2)	0.45	(27)↓
0.014 g/mL + 20 mM	60	0.15	(9)↓	0.10	(6)	0.01	(1)	0.26	(16)↓
HB									
Negative control	60	0.08	(5)	0.00	(0)	0.00	(0)	0.08	(5)
Urethane (20 mM)	60	5.15	(309)	0.73	(44)	0.15	(9)	6.03	(362)
D. furfuracea + urethane									
0.085 g/mL + 20 mM	60	0.45	(27)↓	0.08	(5)↓	0.05	(3)	0.58	(35)↓
0.042 g/mL + 20 mM	60	0.33	(20)↓	0.06	(4)↓	0.03	(2)	0.43	(26)↓
0.014 g/mL + 20 mM	60	0.30	(18)↓	0.10	(6)↓	0.00	(0)	0.40	(24)↓

[a]Statistical diagnosis according to Frei and Würgler (1995): ↓ = frequency reduction of mutant spots; *m* = multiplication factor (χ^2 test, two-tailed, to compare proportions). [b]Including rare single spot *flr*^{3}. Significance level: $\alpha = \beta = 0.05$. SSS = small single spots; LSS = large single spots; TWS = twin spots; TOS = total spots. Negative control = sterile distilled water.

stringens, whereas in our research using *D. furfuracea* these values were 0.21 (13) and 0.28 (17), respectively. Using 100% *S. adstringens*, they observed the frequency of 0.18 (11) for induced spots in the ST cross, while with 0.085 and 0.042 g/mL *D. furfuracea* infusions we found 0.11 (7). In the HB cross, using 66, 75, and 100% *S. adstringens* the authors observed the frequencies of 0.30 (18), 0.42 (25), and 0.42 (25), respectively, for total induced spots, and in our research, using 0.085, 0.042, and 0.014 g/mL *D. furfuracea* infusion, we found the frequency of 0.13 (08) in the HB cross. Thus, there was evidence of absence of genotoxic effects for both plants.

Silva (1998) studied the mutagenesis and antimutagenesis of *D. furfuracea* in mice using the micronucleus technique (MN). The animals were treated with 100, 200, and 300 mg/kg leaf extract and sacrificed after 24 and 48 h. The frequencies of MN/2000 cells were not significant when compared to the negative control, indicating an absence of mutagenic activity, corroborating the results of the present study, using *D. melanogaster* somatic and germinative cells. Nevertheless, they reported significant antimutagenesis results, presenting modulating effects on the damage caused by mitomycin C applied together with the phytotherapic. The authors observed the frequency of 21.2 MN/2000 cells, 24 h after the treatment with 300 mg/kg *D. furfuracea* leaf extract, while the positive control presented 35.6 MN/2000 cells, whereas after 48 h the positive control showed 9.6 MN/2000 cells falling to 5.8 MN/2000 cells. These results are in accordance with those observed in the present study, which also showed the modulatory effect of *D. furfuracea* infusion on the damage caused by urethane.

Mendanha et al. (2010) observed the antigenotoxic activity of the aqueous extract of *Byrsonima verbascifolia* using the SMART/wing test/wing. This medicinal plant has chemicals that are also present in *D. furfuracea*, such as flavonoids and terpenes, corroborating the results found in this study.

Passos et al. (2010) have observed a protective effect of the medicinal plant *Palicourea coriacea* against damage induced by doxorubicin, using the SMART/wing. The main constituents of its leaves are alkaloids, triterpene and tannins.

Vilar et al. (2009) found that all doses of *Ginkgo biloba* extracts co-administered with mitomycin or cyclophosphamide to mice (50, 100 and 200 mg/kg) were significantly ($P < 0.05$) effective, reducing the frequency of MN, suggesting that *G. biloba* possesses both direct and indirect antimutagenic potential. This plant has chemical compounds, such as flavonoids and terpenoids, found in *D. furfuracea*.

Toledo et al. (2006) studied the possible abortive and toxic effects of *D. furfuracea* aqueous extract in pregnant rats (*Rattus novergicus*). Those researchers observed that the uterus of most treated rats presented cysts, which proves blastocyst implantation but without fetal development. The total number of corpus luteum of the treatment animals presented a significant decrease compared to the control group, therefore, showing the embryotoxicity of *D. furfuracea*. Nonetheless, in the present research, we detected an absence of mutagenic activity of *D. furfuracea* infusion in *D. melanogaster* somatic and germinative cells.

The presence of certain compounds, such as flavonoids and terpenoids, in *D. furfuracea* may protect cells against DNA damage (Silva et al., 2006), and they are likely to be responsible for the modulatory effect of this phytotherapeutic agent and absence of genotoxicity and mutagenicity. Nonetheless, further experiments using other models should be carried out to confirm these results, to elucidate the mechanisms used to modulate genotoxic effects, and to determine the ideal conditions to use *D. furfuracea* extracts without risks to human health.

This medicinal plant is a desmutagenic agent that, when combined with urethane, can block the interaction of fly DNA-urethane.

CONCLUSION

Following the procedures herein described, we conclude that the phytotherapic *D. furfuracea* did not present genotoxic effects in *D. melanogaster* germinative cells considering the loss of the ring-X-chromosome, partial loss of the Y-chromosome, mosaicism, and non-disjunction, and that *D. furfuracea* did not induce mutations and recombinations in the somatic cells of *D. melanogaster*, in ST and HB cross descendents. It did, however, present a protective effect against damage induced by urethane in both ST and HB crosses.

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