Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Modulatory effect of *Palicourea coriacea* (Rubiaceae) against damage induced by doxorubicin in somatic cells of *Drosophila melanogaster*

D.C.S. Passos[1], H.D. Ferreira[1], I.L.F.B. Vieira[1], W.B. Nunes[2], L.P. Felício[1], E.M. Silva[1], C.R. Vale[3], S.R. Duarte[3], E.S. Silva[1] and S. Carvalho[1]

[1]Instituto de Ciências Biológicas, Departamento de Biologia Geral, Universidade Federal de Goiás, Goiânia, GO, Brasil
[2]Instituto de Ciências Biológicas, Departamento de Morfologia, Universidade Federal de Goiás, Goiânia, GO, Brasil
[3]Unidade Universitária de Iporá, Iporá, GO, Brasil

Corresponding author: S. Carvalho
E-mail: salvadorcarvalho2005@yahoo.com.br

Genet. Mol. Res. 9 (2): 1153-1162 (2010)
Received February 24, 2010
Accepted March 25, 2010
Published June 15, 2010
DOI 10.4238/vol9-2gmr801

ABSTRACT. *Palicourea coriacea*, popularly known as "douradinha", is a medicinal plant from the Brazilian Cerrado region used in folk medicine to treat kidney and urethral stones and kidney inflammation. We evaluated the cytotoxic, genotoxic, and possible antigenotoxic activities of an aqueous extract of *P. coriacea* on somatic cells of *Drosophila melanogaster*, using the somatic mutation and recombination test. We used third-stage larvae of *D. melanogaster* from a standard cross and a high bioactivation cross and tested 10 different doses of *P. coriacea* aqueous extract (5, 15, 25, 35, 50, 65, 80, 95, 110, and 125 mg/mL). Doxorubicin (0.125 mg/mL) was used as a positive control and distilled water as a negative control. None of the doses was lethal to the larvae.

There was no genotoxic effect at 5, 10, or 15 mg extract/mL. However, a significant decrease in the frequency of spots induced by doxorubicin was observed when administered with *P. coriacea* aqueous extract at these same doses. We conclude that *P. coriacea* aqueous extract is not cytotoxic or genotoxic at these doses, but it does protect against the genotoxic action of doxorubicin.

Key words: *Palicourea coriacea*; Cytotoxicity; Genotoxicity; Antigenotoxicity; SMART/wing

INTRODUCTION

Studies on the genotoxicity and antigenotoxicity of natural plant extracts can help evaluate the safety and effectiveness of herbal products (Romero-Jiménez et al., 2005). Although herbal preparations have been traditionally used in cancer treatment, they could contain bioactivated components that can promote cancer development (Siddique et al., 2008).

In Brazil, many people use traditional natural preparations derived from plant materials for the treatment of various diseases (Lima et al., 2006); however, in many cases there is still limited scientific evidence of their therapeutic efficacy (Pereira et al., 2008). Therefore, it is very necessary to assess the mutagenic potential or modulating activity of plant extracts when combined with other substances (Siddique et al., 2008).

Palicourea coriacea (Cham.) K. Schum., popularly known as "douradinha" in Brazil, is a small tree with yellow inflorescences and flowers (da Silva et al., 2008) that is widely used in Brazilian Cerrado folk medicine as a leaf infusion, due to its potent diuretic effect (do Nascimento et al., 2008), to treat kidney and urethral calculus and kidney inflammation (Nunes et al., 2003). The main constituents of its leaves are alkaloids, triterpenes, phenolic acids, coumarins, tannins, a cyclic peptide, and allantoin (da Silva et al., 2008), as well as calycanthine and ursolic acid, which show antibacterial activity (do Nascimento et al., 2006). Furthermore, ursolic acid possesses antimutagenic, antitumor, aneugenic, and apoptotic properties (Dorai and Aggarwal, 2004; Liu, 2005; Ovesná et al., 2006; Aparecida Resende et al., 2006), and allantoin has excellent healing properties since it favors cell proliferation and accelerates skin regeneration (Ferreira et al., 2000). By contrast, calycanthine is considered to be a very powerful convulsant poison, causing effects similar to those of some neuropoisons such as strychnine (Adjibade et al., 1991).

Although widely used as an antitumor agent to treat cancer, doxorubicin (DXR), a broad-spectrum anthracycline antibiotic, is genotoxic and carcinogenic (Minotti et al., 2004), with proven clastogenic effects on somatic and germ cells (Baumgartner et al., 2004), has mutagenic and recombinogenic properties (Lehmann et al., 2003), and is cytotoxic (Robert and Gianni, 1993), and cardiotoxic due to the production of oxygen radicals (Naidu et al., 2002).

The somatic mutation and recombination test (SMART) in wings of *Drosophila melanogaster* is a well-known eukaryotic assay based on the loss of heterozygosity for two genetic markers affecting the phenotype of wing hairs (Graf et al., 1984). It is a versatile and reliable system to test complex mixtures in genotoxicity and anti-genotoxicity assays, due to the capabilities of treated larvae to bio-activate metabolites either as single compounds or as complex

mixtures (Graf et al., 1998), through which a wide variety of compounds and complex mixtures have been assayed (Romero-Jiménez et al., 2005).

Deeper knowledge about particular plant extracts is likely to contribute to the fundamentals of herbal medicine (Roncada et al., 2004). Thus, the purpose of this study was to assess *P. coriacea* genotoxic activity and its potential antigenotoxic effect in combination with DXR using the SMART assay in *D. melanogaster*.

MATERIAL AND METHODS

Plant material

Samples of the aerial part of *P. coriacea* were collected in Goiânia, in the State of Goiás, Brazil, and identified by Prof. Heleno Dias Ferreira, Department of Botany at the Universidade Federal de Goiás (UFG), and a voucher specimen (UFG 27.169) was deposited in the Central Herbarium of UFG.

Dried leaves were ground in a mortar with a little water added, and the aqueous extract of *P. coriacea* (AEP) was lyophilized and stored in screw-cap vials in a common refrigerator at approximately -2°C.

We employed AEP concentrations of 5, 15, 25, 35, 50, 65, 80, 95, 110, and 125 mg/mL to generate the cell survival curves, and of 5, 10, and 15 mg/mL to carry out the experiments, by dissolving the lyophilized extract in distilled water at the time of use.

Positive and negative controls

DXR (Doxolen lyophilized, Eurofarma Laboratórios Ltda., São Paulo, Brazil) dissolved in distilled water in the dark was used as the positive control and distilled water as the negative control.

Survival curves

In order to determine the cytotoxic potential of AEP, we employed it at 10 different concentrations (5, 15, 25, 35, 50, 65, 80, 95, 110, and 125 mg/mL) to generate the cell survival curves. The standard solution was prepared at the time of treating *D. melanogaster* third-stage larvae using distilled water to dissolve AEP and concurrent negative controls treated with distilled water.

Somatic mutation and recombination test

Drosophila strains, crosses, and collection of larvae

Three strains of *D. melanogaster* (ORR), carrying either the genetic markers *multiple wing hairs* (*mwh*, 3-0.3) or *flare-3* (*flr*^{3}, 3-38.8), were used.

To produce the standard (ST) cross, stocks of *flr*^{3}*/In (3LR)TM3*, *ri p*^{p} *sep I(3)89Aa bx*^{34e}, and *Bd*^{S} virgin females were crossed with stocks of *mwh/mwh* males (Graf et al., 1989). The high bioactivation (HB) cross, which gives high levels of cytochrome P450, was obtained by crossing ORR/ORR; *flr*^{3}*/In(3LR)TM3, ri p*^{p} *sep I(3)89Aa bx*^{34e}, and *Bd*^{S} virgin females with *mwh/mwh* males (Graf and van Schaik, 1992).

Eggs from both crosses were collected over an 8-h period in culture bottles containing a solid base of 3% agar covered with a layer of live baker's yeast (*Saccharomyces cerevisiae*) supplemented with sucrose. For the treatments, 72-h-old larvae were removed from the culture bottles and washed in tap water with the help of a fine mesh stainless steel strainer.

The larvae from both crosses were transferred to 2.5-8.0-cm high glass tubes containing 0.9 g synthetic culture medium (form 4-24, Carolina Biological Supply Co., Burlington, USA) dissolved in 3.0 mL 5, 10, and 15 mg/mL AEP, with or without 0.125 mg/mL DXR, which is a concentration known to be genotoxic to somatic cells of *D. melanogaster*. The three concentrations of AEP were chosen based on the survival curves. A positive control was treated with 0.125 mg/mL DXR alone and a negative control only with distilled water.

Since some compounds are photosensitive, all tubes were wrapped in aluminum foil.

Preparation and microscopic analysis of the wings

After hatching, individual adults were transferred to a beaker containing 70% ethanol. Using a standard stereoscopic microscope and entomological tweezers, the wings were removed and mounted in Faure's solution (30 g gum arabic, 20 mL glycerol, 50 g chloral hydrate, 50 mL water). Both dorsal and ventral surfaces of the wings were analyzed under a compound microscope at 400X magnification. During the analysis, the positions of spots were recorded according to the wing sections (Graf et al., 1984).

Statistical analysis

The frequency of each type of spot for each treatment group was compared pairwise with the appropriate control (i.e., negative control *vs* each AEP treatment group; DXR alone *vs* each AEP DXR group), using the nonparametric Mann-Whitney U-test and the Wilcoxon rank sum test (Frei and Würgler, 1995). We calculated the percent inhibition of AEP using the mutant clone frequency/10^5 cells corrected using the control group, as follows: [(DXR alone - DXR plus EAP / DXR alone) x 100] (Abraham, 1994).

RESULTS AND DISCUSSION

According to the survival curves generated in the present study, the AEP concentrations used had no significant effects on the number of survivors, and consequently, the doses chosen were not cytotoxic to *D. melanogaster* larvae.

Table 1 shows the frequency of mutant spots observed in the trans-heterozygous descendants from ST and HB crosses treated with AEP alone. The three doses of AEP employed did not show significant effects on the frequency of any category of spots or on total spots, leading to the conclusion that AEP at these doses is not genotoxic.

The use of DXR alone produced a positive response in both trans-heterozygous and balancer-heterozygous descendants of ST and HB crosses, indicating that this compound was genotoxic in this assay (Tables 2 and 3). Additionally, the co-treatments using AEP and DXR significantly reduced the mutation rate that was induced by DXR alone.

Table 1. Frequency of mutant spots observed in marked (MH) trans-heterozygous descendants of *Drosophila melanogaster* from standard (ST) and high bioactivation (HB) crosses treated with three different concentrations (5, 10, and 15 mg/mL) of aqueous extract of *Palicourea coriacea* (AEP).

Treatment	Individuals (No.)	Frequency of mutant spots per individual (No. spots)[1]					Clone size class mean	Frequency of spot induction by cell division (per 10^5 cells)	
		SSS[2] (1-2 cells)[3] (m = 2)	SBS (>2 cells)[3] (m = 5)	TS (m = 5)	TMS (m = 5)	Total spots		Observed	Corrected
ST cross									
Negative control	40	0.58 (23)	0.18 (7)	0.05 (2)	0.80 (32)	32	2.13	1.64	1.79
5	40	0.28 (11)	0.00 (0)	0.08 (3)	0.35 (14)	14	2.07	0.72	0.75 (-)
10	40	0.33 (13)	0.03 (1)	0.00 (0)	0.35 (14)	14	1.43	0.72	0.48 (-)
15	40	0.20 (8)	0.03 (1)	0.00 (0)	0.23 (9)	9	1.44	0.46	0.31 (-)
HB cross									
Negative control	40	1.15 (46)	0.23 (9)	0.03 (1)	1.40 (56)	55	1.84	2.82	2.52
5	40	0.38 (15)	0.05 (2)	0.00 (0)	0.43 (17)	17	1.53	0.87	0.63 (-)
10	40	0.18 (7)	0.00 (0)	0.00 (0)	0.18 (7)	7	1.43	0.36	0.24 (-)
15	40	0.28 (11)	0.13 (5)	0.00 (0)	0.40 (16)	16	2.06	0.82	0.86 (-)

[1]Statistical analysis according to Frei and Würgler (1988): (-) negative. Significance level: $\alpha = \beta = 0.05$; m = multiplication factor to determine the significant negative results. [2]SSS = simple small spots; SBS = simple big spots; TS = twin spots; TMS = total *mwh* spots. [3]Including simple rare *flr*[3] spots.

Table 2. Frequency of mutant spots observed in marked (MH) balancer trans-heterozygous descendants of *Drosophila melanogaster* from the high bioactivation (HB) cross treated with three different concentrations (5, 10, and 15 mg/mL) of aqueous extract of *Palicourea coriacea* (AEP) combined with doxorubicin (DXR).

Treatment	Individuals (No.)	Frequency of mutant spots per individual (No. of spots)[1]					Clone size class mean	Frequency of spot induction by cell division (per 10^5 cells)		Inhibition (%)
		SSS[2] (1-2 cells)[3] (m = 2)	SBS (>2 cells)[3] (m = 5)	TS (m = 5)	TMS (m = 5)	Total spots		Observed	Corrected	
mwh/flr[3]										
DXR	40	5.38 (215)	10.80 (432)	16.90 (676)	33.08 (1323)	252	3.14	12.91	28.43	
5 + DXR	40	1.55 (62)	0.33 (13)	0.35 (14)	2.23 (89)	41	1.76	2.10	1.77*	93.8
10 + DXR	40	1.23 (49)	0.30 (12)	0.13 (5)	1.65 (66)	52	1.58	2.66	1.99*	93
15 + DXR	40	1.08 (43)	0.13 (5)	0.05 (2)	1.25 (50)	24	1.42	1.23	0.82*	97
mwh/TM3										
DXR	40	0.93 (37)	0.33 (13)		1.25 (50)	45	2.16	2.31	2.57	
5 + DXR	40	0.13 (5)	0.00 (0)		0.13 (5)	5	1.20	0.26	0.15*	94.2
10 + DXR	40	0.18 (7)	0.03 (1)		0.20 (8)	8	1.50	0.41	0.29*	88.7
15 + DXR	40	0.08 (3)	0.03 (1)		0.10 (4)	3	1.33	0.15	0.10*	96.1

[1]Statistical analysis according to Frei and Würgler (1988); Mann-Whitney U-test: * = statistically significant reduction (compared with the positive control - DXR 0.125 mg/mL). Significance level: $\alpha = \beta = 0.05$; m = multiplication factor to determine the significant negative results. [2]SSS = simple small spots; SBS = simple big spots; TS = twin spots; TMS = total *mwh* spots. [3]Including simple rare *flr*[3] spots.

Table 3. Frequency of mutant spots observed in marked (MH) balancer trans-heterozygous descendants of *Drosophila melanogaster* from the standard (ST) cross treated with three different concentrations (5, 10, and 15 mg/mL) of aqueous extract of *Palicourea coriacea* (AEP) in association with doxorubicin (DXR).

Treatment	Individuals (No.)	Frequency of mutant spots per individual (No. of spots)[1]					Clone size class mean	Frequency of spot induction by cell division (per 10^5 cells)		Inhibition (%)
		SSS[2] (1-2 cells)[3] (m = 2)	SBS (>2 cells)[3] (m = 5)	TS (m = 5)	TMS (m = 5)	Total spots		Observed	Corrected	
mwh/flr[3]										
DXR	40	3.95 (158)	6.60 (264)	8.45 (338)	19.00 (760)	372	3.43	19.06	51.26	
5 + DXR	40	0.88 (35)	0.23 (9)	0.03 (1)	1.13 (45)	22	1.59	1.13	0.85*	98.3
10 + DXR	40	0.70 (28)	0.25 (10)	0.10 (4)	1.05 (42)	28	1.64	1.43	1.12*	97.8
15 + DXR	40	0.73 (29)	0.15 (6)	0.03 (1)	0.90 (36)	36	1.92	1.84	1.74*	96.6
mwh/TM3										
DXR	40	0.70 (28)	0.23 (9)		0.93 (37)	37	2.30	1.90	2.33	
5 + DXR	40	0.18 (7)	0.00 (0)		0.18 (7)	7	1.57	0.36	0.27*	88.4
10 + DXR	40	0.10 (4)	0.03 (1)		0.13 (5)	5	1.80	0.26	0.22*	90.5
15 + DXR	40	0.18 (7)	0.03 (1)		0.20 (8)	8	1.63	0.41	0.32*	86.3

[1]Statistical analysis according to Frei and Würgler (1988); Mann-Whitney U-test: * = statistically significant reduction (compared with the positive control - DXR 0.125 mg/mL). Significance level: $\alpha = \beta = 0.05$; m = multiplication factor to determine the significant negative results. [2]SSS = simple small spots; SBS = simple big spots; TS = twin spots; TMS: total *mwh* spots. [3]Including simple rare *flr*[3] spots.

Tables 2 and 3 show the results of the AEP-DXR co-treatments using ST and HB cross flies. In both crosses, AEP reduced the genotoxicity induced by DXR in each mutant spot category. The inhibition in the marker-trans-heterozygous descendants from the HB cross (*mwh/flr*^{3}) ranged from 93.8% (for 5 mg/mL AEP) to 97% (for 15 mg/mL AEP). Comparing these results with the positive control, we see that the addition of 5 and 15 mg/mL AEP in the co-treatment reduced the total number of spots induced by AEP by 98.3 and 96.6%, respectively.

Using wing heterozygosity for multiple inversions on the *TM3* balancer chromosome, it was possible to separate mutational events from recombinational events since the latter are eliminated in flies with this genotype. Comparing the clone induction frequency in both genotypes obtained with DXR alone, we observed that 9.97% of the mutant clones produced in ST flies were a result of mutation and 90.03% of recombination, and that 17.89% of the spots induced in HB flies occurred due to mutation and 82.11% to recombination. The strong recombinogenic activity of DXR in somatic cells of *D. melanogaster* has been previously reported by Lehmann et al. (2003), Costa and Nepomuceno (2006) and Fragiorge et al. (2007). In ST cross descendants, recombination was responsible for reducing the frequency of spots produced by DXR in combination with 5, 10, and 15 mg/mL AEP by 68.14, 81.82, and 77.72%, respectively. Also, in HB cross descendants, the frequency of spots produced by DXR in combination with 5, 10, and 15 mg/mL AEP was reduced by 87.62, 84.62, and 87.81%, respectively. These findings indicate that this phytotherapeutic compound primarily reduced the recombinogenic effects induced by DXR.

Some plants may possess substances that can modulate the genotoxicity of other compounds (Siddique et al., 2008). The mechanisms through which AEP inhibited the genotoxicity of DXR were not directly analyzed. However, due to the way DXR generates free radicals and produces DNA damage (Keizer et al., 1990), it is possible to suggest mechanisms by which AEP may protect cells against DXR genotoxicity, and the data obtained in the present study suggest that the compounds present in AEP are not mutagenic.

When present during exposure, AEP may have a desmutagenic effect. Desmutagens deactivate genotoxic agents either chemically or enzymatically (Kada et al., 1982). In this case, AEP may deactivate DXR or the free radicals that are produced during its transformation. This deactivation could be related to the antioxidant activity of phenolic compounds, such as tannin and ursolic acid in AEP, as observed by several authors who have attributed this fact to the antigenotoxic action of these phenolics (Tanaka et al., 1998; Dhawan et al., 2002; Maurich et al., 2004; Pellegrina et al., 2005).

CONCLUSION

AEP was not cytotoxic or genotoxic at the doses tested and under the assay conditions used in the present study. Furthermore, it protected cells against the genotoxic effects of the free radical producing chemotherapeutic agent DXR in the *D. melanogaster* wing spot assay.

REFERENCES

Abraham SK (1994). Antigenotoxicity of coffee in the *Drosophila* assay for somatic mutation and recombination. *Mutagenesis* 9: 383-386.

Adjibade Y, Hue B, Pelhate M and Anton R (1991). Action of calycanthine on nervous transmission in cockroach central nervous system. *Planta Med.* 57: 99-101.

Aparecida Resende F, de Andrade Barcala CA, da Silva Faria MC, Kato FH, et al. (2006). Antimutagenicity of ursolic acid and oleanolic acid against doxorubicin-induced clastogenesis in BALB/c mice. *Life Sci.* 79: 1268-1273.

Baumgartner A, Schmid TE, Cemeli E and Anderson D (2004). Parallel evaluation of doxorubicin-induced genetic damage in human lymphocytes and sperm using the comet assay and spectral karyotyping. *Mutagenesis* 19: 313-318.

Costa WF and Nepomuceno JC (2006). Protective effects of a mixture of antioxidant vitamins and minerals on the genotoxicity of doxorubicin in somatic cells of *Drosophila melanogaster*. *Environ. Mol. Mutagen.* 47: 18-24.

da Silva VC, de Carvalho MG and Alves AN (2008). Chemical constituents from leaves of *Palicourea coriacea* (Rubiaceae). *J. Nat. Med.* 62: 356-357.

Dhawan A, Anderson D, de Pascual-Teresa S, Santos-Buelga C, et al. (2002). Evaluation of the antigenotoxic potential of monomeric and dimeric flavanols, and black tea polyphenols against heterocyclic amine-induced DNA damage in human lymphocytes using the Comet assay. *Mutat. Res.* 515: 39-56.

do Nascimento CA, Gomes MS, Lião LM, de Oliveira CMA, et al. (2006). Alkaloids from *Palicourea coriacea* (Cham.) K. Schum. *Z. Naturforsch.* 61b: 1443-1446.

do Nascimento CA, Liao LM, Kato L, da Silva CC, et al. (2008). A tetrahydro β-carboline trisaccharide from *Palicourea coriacea* (Cham.) K. Schum. *Carbohydr. Res.* 343: 1104-1107.

Dorai T and Aggarwal BB (2004). Role of chemopreventive agents in cancer therapy. *Cancer Lett.* 215: 129-140.

Ferreira DT, Alvares PSM, Houghton PJ and Braz-Filho R (2000). Chemical constituents from roots of *Pyrostegia venusta* and considerations about its medicinal importance. *Quím. Nova* 23: 42-46.

Fragiorge EJ, Spanó MA and Antunes LMG (2007). Modulatory effects of the antioxidant ascorbic acid on the direct genotoxicity of doxorubicin in somatic cells of *Drosophila melanogaster*. *Genet. Mol. Biol.* 30: 449-455.

Frei H and Würgler FE (1988). Statistical methods to decide whether mutagenicity test data from *Drosophila* assays indicate a positive, negative, or inconclusive result. *Mutat. Res.* 203: 297-308.

Frei H and Würgler FE (1995). Optimal experimental design and sample size for the statistical evaluation of data from somatic mutation and recombination tests (SMART) in *Drosophila*. *Mutat. Res.* 334: 247-258.

Graf U and van Schaik N (1992). Improved high bioactivation cross for the wing somatic mutation and recombination test in *Drosophila melanogaster*. *Mutat. Res.* 271: 59-67.

Graf U, Würgler FE, Katz AJ, Frei H, et al. (1984). Somatic mutation and recombination test in *Drosophila melanogaster*. *Environ. Mutagen.* 6: 153-188.

Graf U, Frei H, Kägi A, Katz AJ, et al. (1989). Thirty compounds tested in the *Drosophila* wing spot test. *Mutat. Res.* 222: 359-373.

Graf U, Abraham SK, Guzmán-Rincón J and Würgler FE (1998). Antigenotoxicity studies in *Drosophila melanogaster*. *Mutat. Res.* 402: 203-209.

Kada T, Inoue T and Namiki M (1982). Environmental Desmutagens and Antimutagen. In: Environmental Mutagenesis, Carcinogenesis, and Plant Biology (Klekowski EJ, ed.). Praeger Publishers, New York, 133-152.

Keizer HG, Pinedo HM, Schuurhuis GJ and Joenje H (1990). Doxorubicin (adriamycin): a critical review of free radical-dependent mechanisms of cytotoxicity. *Pharmacol. Ther.* 47: 219-231.

Lehmann M, Franco A, de Souza Prudente Vilar K, Lukza RM, et al. (2003). Doxorubicin and two of its analogues are preferential inducers of homologous recombination compared with mutational events in somatic cells of *Drosophila melanogaster*. *Mutat. Res.* 539: 167-175.

Lima MRF, Luna JS, Santos AF, Andradea MCC, et al. (2006). Anti-bacterial activity of some Brazilian medicinal plants. *J. Ethnopharmacol.* 105: 137-147.

Liu J (2005). Oleanolic acid and ursolic acid: research perspectives. *J. Ethnopharmacol.* 100: 92-94.

Maurich T, Pistelli L and Turchi G (2004). Anti-clastogenic activity of two structurally related pterocarpans purified from *Bituminaria bituminosa* in cultured human lymphocytes. *Mutat. Res.* 561: 75-81.

Minotti G, Menna P, Salvatorelli E, Cairo G, et al. (2004). Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol. Rev.* 56: 185-229.

Naidu MU, Kumar KV, Mohan IK, Sundaram C, et al. (2002). Protective effect of *Gingko biloba* extract against doxorubicin-induced cardiotoxicity in mice. *Indian J. Exp. Biol.* 40: 894-900.

Nunes GP, Silva MF, Resende UM and Siqueira JM (2003). Medicinal plants from herb sellers operating in downtown Campo Grande, Mato Grosso do Sul, Brazil. *Rev. Bras. Farmacogn.* 13: 83-92.

Ovesná Z, Kozics K and Slamenová D (2006). Protective effects of ursolic acid and oleanolic acid in leukemic cells. *Mutat. Res.* 600: 131-137.

Pellegrina CD, Padovani G, Mainente F, Zoccatelli G, et al. (2005). Anti-tumour potential of a gallic acid-containing phenolic fraction from *Oenothera biennis*. *Cancer Lett.* 226: 17-25.

Pereira DG, Antunes LGM, Graf U and Spanó MA (2008). Protection by *Panax ginseng* C.A. Meyer against the

genotoxicity of doxorubicin in somatic cells of *Drosophila melanogaster*. *Genet. Mol. Biol.* 31: 947-955.
Robert J and Gianni L (1993). Pharmacokinetics and metabolism of anthracyclines. *Cancer Surv.* 17: 219-252.
Romero-Jiménez M, Campos-Sánchez J, Analla M, Muñoz-Serrano A, et al. (2005). Genotoxicity and anti-genotoxicity of some traditional medicinal herbs. *Mutat. Res.* 585: 147-155.
Roncada T, Vicentini VE and Mantovani MS (2004). Possible modulating actions of plant extracts on the chromosome breaking activity of MMC and Ara-C in human lymphocytes *in vitro*. *Toxicol. In Vitro* 18: 617-622.
Siddique YH, Ara G, Beg T, Faisal M, et al. (2008). Antigenotoxic role of *Centella asiatica* L. extract against cyproterone acetate induced genotoxic damage in cultured human lymphocytes. *Toxicol. In Vitro* 22: 10-17.
Tanaka K, Hayatsu T, Negishi T and Hayatsu H (1998). Inhibition of N-nitrosation of secondary amines *in vitro* by tea extracts and catechins. *Mutat. Res.* 412: 91-98.