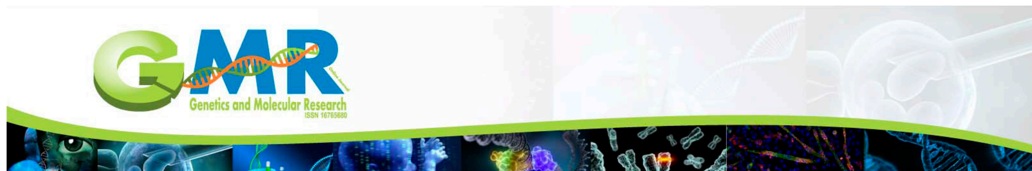

Campomanesia adamantium extract induces DNA damage, apoptosis, and affects cyclophosphamide metabolism

M.D. Martello[1,2]*, N. David[1,2]*, R. Matuo[2], P.C. Carvalho[1,3], S.D. Navarro[4], A.C.D. Monreal[2], A.L. Cunha-Laura[2], C.A.L. Cardoso[5], C.A.L. Kassuya[6] and R.J. Oliveira[1,2,3]

[1]Centro de Células Tronco, Terapia Celular e Genética Toxicológica, Hospital Universitário "Maria Aparecida Pedrossian", EBSERH, Campo Grande, MS, Brasil
[2]Programa de Mestrado em Farmácia, Centro de Ciências Biológicas e da Saúde, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brasil
[3]Programa de Pós-Graduação em Saúde e Desenvolvimento da Região Centro-Oeste, Faculdade de Medicina Dr. Hélio Mandetta, Campo Grande, MS, Brasil
[4]Programa de Pós-Graduação em Biotecnologia e Biodiversidade, Rede Pró-Centro-Oeste, Instituto de Química, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brasil
[5]Curso de Graduação em Química, Universidade Estadual de Mato Grosso do Sul, Dourados, MS, Brasil
[6]Curso de Ciências da Saúde, Universidade Federal da Grande Dourados, Dourados, MS, Brasil

*These authors contributed equally to this study.
Corresponding author: R.J. Oliveira
E-mail: rodrigo.oliveira@ufms.br

Genet. Mol. Res. 15 (2): gmr.15027678
Received September 18, 2015
Accepted November 18, 2015
Published April 26, 2016
DOI http://dx.doi.org/10.4238/gmr.15027678

ABSTRACT. *Campomanesia adamantium* (Cambess.) O. Berg. is originally from Brazil. Its leaves and fruits have medicinal properties such as anti-inflammatory, antidiarrheal and antiseptic properties. However,

 ©FUNPEC-RP www.funpecrp.com.br

the mutagenic potential of this species has been reported in few studies. This study describes the mutagenic/antimutagenic, splenic phagocytic, and apoptotic activities of *C. adamantium* hydroethanolic extract with or without cyclophosphamide in Swiss mice. The animals orally received the hydroethanolic extract at doses of 30, 100, or 300 mg/kg with or without 100 mg/kg cyclophosphamide. Mutagenesis was evaluated by performing the micronucleus assay after treatment for 24, 48, and 72 h, while splenic phagocytic and apoptotic effects were investigated after 72 h. Short-term exposure of 30 and 100 mg/kg extract induced mild clastogenic/aneugenic effects and increased splenic phagocytosis and apoptosis in the liver, spleen, and kidneys. When the extract was administered in combination with cyclophosphamide, micronucleus frequency and apoptosis reduced. Extract components might affect cyclophosphamide metabolism, which possibly leads to increased clearance of this chemotherapeutic agent. *C. adamantium* showed mutagenic activity and it may decrease the effectiveness of drugs with metabolic pathways similar to those associated with cyclophosphamide. Thus, caution should be exercised while consuming these extracts, especially when received in combination with other drugs.

Key words: *Campomanesia adamantium*; Antimutagenesis; Apoptosis; Micronucleus; Mutagenesis

INTRODUCTION

Natural products have been used as sources of active components of drugs (Cragg and Newman, 2013). Compounds isolated from plants function as models that provide the chemical backbones used in modern techniques, such as combinatorial chemistry techniques, for the discovery of novel drug candidates (Harvey, 2007; Cragg and Newman, 2013).

The high diversity of existing chemical agents can be ascribed to their discovery in natural products. With over 43,000 known plant species, Brazil has high biodiversity, and approximately 2000 of these species are unique to the country (Brazil, 2006, 2010).

Campomanesia adamantium (Cambess.) O. Berg. belongs to the Myrtaceae family. Native to Brazilian cerrado, *C. adamantium* is found in 7 states in the central region of the country (Sobral et al., 2014). This species is characterized by a shrub form with height in the range of 60-80 cm, small cream-white flowers, and rounded yellowish-green fruit. The fruit is commonly known as guavira, gabiroba, or guabiroba, and appears from September to December (Avidos and Ferreira, 2000). Species belonging to the *Campomanesia* genus have been shown to have medicinal properties, such as anti-inflammatory, antidiarrheal, antiseptic (Rodrigues and Carvalho, 2001; Brandelli et al., 2011; de Souza et al., 2014), and antioxidant activities (Ramos et al., 2007; Coutinho et al., 2008; Rufino et al., 2010; Pascoal et al., 2011), which are mainly attributed to the presence of phenolic compounds, flavonoids, chalcones, carotenoids, and vitamins.

Fruit and infusions of *C. adamantium* are widely consumed by different populations in the form of fresh or fermented beverages, which are commonly consumed by the indigenous population (Duarte et al., 2009; Brandelli et al., 2011). Therefore, studies focused on the genetic

toxicity of *C. adamantium* are necessary to ensure its safety for consumption and validate its use for the prevention and treatment of diseases. This study evaluated *C. adamantium* for its acute toxicity, mutagenicity/antimutagenicity, and ability to induce apoptosis in Swiss mice.

MATERIAL AND METHODS

DNA damage-inducing agent

Cyclophosphamide (CP) (Fosfaseron®, Itaca Laboratórios Ltda., REG M.S. No. 1.2603.0056.002-1; Batch 063020, Brazil) was prepared in 0.9% NaCl sterile saline solution at pH 7.4 and administered as a single dose at 100 mg/kg body weight (bw) by intraperitoneal (*ip*) injection.

Collection and identification of the plant material

Plant material was collected in December 2011 at Horto de Plantas Medicinais - HPM, Universidade Federal da Grande Dourados - UFGD, in Dourados, Mato Grosso do Sul (MS), Brazil. The sample was identified by Dr. Maria do Carmo Vieira and a voucher specimen was deposited at the UFGD herbarium (record No. 2196).

C. adamantium hydroethanolic extract

C. adamantium extract was provided by Prof. Dr. Claudia Andréa Lima Cardoso, Universidade Estadual de Mato Grosso do Sul - UEMS. *C. adamantium* leaves were dried in a forced-air circulating oven at 45° ± 5°C and ground in a Wiley knife mill. The dried leaves were macerated in ethanol:water (70:30, v:v) at room temperature for 7 days. After this period, the extract was filtered and subjected to 2 additional extractions using the same procedure. After 21 days, the filtrate was collected, concentrated using a vacuum rotary evaporator (FISATOM), and then dried in a laminar flow hood until a specific yield of 28.1% was obtained. The extract was stored at 4°C and protected from light.

The extract was administered as a single dose at 30, 100, and 300 mg/kg (bw) by gavage. Doses were selected based on doses reported in studies by Fernandes and Vargas (2003) and de Souza et al. (2014).

Experimental design

A total of 140 sexually mature male Swiss mice (*Mus musculus*) obtained from Central Animal Facility of Biological and Health Sciences Center, Universidade Federal de Mato Grosso do Sul (UFMS), were divided into 14 experimental groups (N = 10 animals per group). The animals were housed in polypropylene cages lined with wood shavings and kept in ventilated racks (ALESCO®), and fed with commercial food (Nuvital®) and filtered water *ad libitum*. Lighting was maintained as a 12-h light:dark period; the temperature was set at 22° ± 2°C and humidity at 55 ± 10%. The experiment was conducted according to the guidelines of the Universal Declaration of Animal Rights and with the approval of Animal Research Ethics Committee of UFMS (Protocol No. 399/2012). Treatments and collection of biological material for assays were performed as shown in Figure 1.

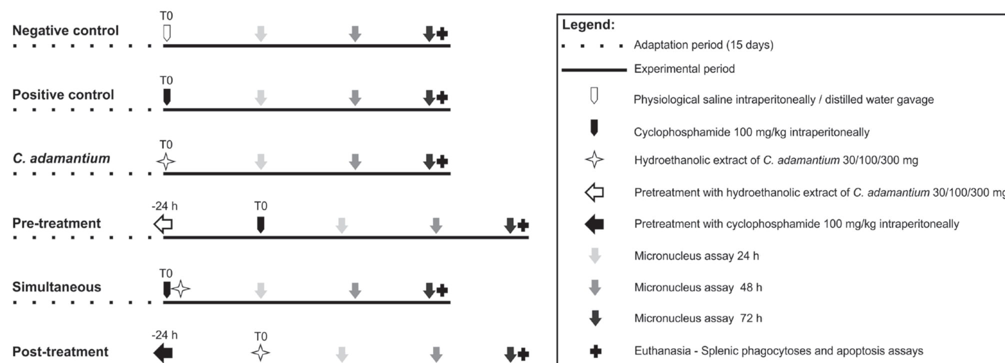

Figure 1. Experimental design, treatment protocols, and biological assays.

Biometric data evaluation and clinical signs of toxicity

The animals were weighed at the beginning and end of the experiment. Gain or loss of animal weight was calculated as the difference between the final and initial weights. Clinical observation for signs or characteristics of toxicity was performed in accordance to the guidelines of the Organization for Economic Co-Operation and Development (OECD, 2008).

Micronucleus assay using peripheral blood samples

Micronucleus assay was performed following a protocol described by Hayashi et al. (1990) and adapted by Oliveira et al. (2009). Peripheral blood samples (20 μL) were deposited on a slide pre-coated with 20 μL acridine orange (1 mg/mL) and covered with a coverslip. The slides were stored at -20°C for 15 days. For each animal, 2000 cells were analyzed using a fluorescence microscope at 400X magnification (Bioval®, Model L 2000A), a 420-490-nm excitation wavelength, and a 520-nm barrier filter.

Spleen phagocytosis evaluation

One-third of the spleen was macerated with 400 µL 0.9% NaCl sterile solution to obtain a cell suspension. One hundred microliters of the cell suspension was deposited on a slide containing 20 μL acridine orange (1 mg/mL) and covered with a coverslip. The slides were stored at -20°C until analysis. For each animal, 200 cells were analyzed using a fluorescence microscope at 400X magnification, a 420-490-nm excitation wavelength, and a 520-nm barrier filter. The analysis was performed as previously described by Hayashi et al. (1990) and adapted by de Lima et al. (2013).

Cell death by apoptosis

Cell death by apoptosis was investigated in different organs such as the liver, kidney, and spleen. Cell suspension was obtained from one-third of the spleen, one lobe of the liver, and the right kidney by macerating them separately with 200 µL 0.9% sterile saline solution using a crucible and pestle. One hundred microliters of the cell suspension was used to prepare a smear

on a histological slide. The slides were fixed in Carnoy's solution for 5 min and then subjected to a gradient of decreasing concentrations of ethanol (95-25%), washed with McIlvaine's buffer for 5 min, stained with 0.01% acridine orange for 5 min, and then washed again with buffer. A total of 100 cells per animal were assessed for apoptosis based on DNA fragmentation patterns described by Navarro et al. (2014).

Statistical analysis

Results are reported as means ± standard error of mean (SE). Data were analyzed by ANOVA or the Tukey parametric test using the Graph Pad Instat software (version 3.02; Graph-Pad Software Inc., San Diego, CA, USA). $P < 0.05$ was considered to indicate significance.

RESULTS

C. adamantium extract does not induce acute toxicity

There were no significant differences in weight gain between the mice treated with *C. adamantium* extract with or without CP (Table 1). In addition, there were no clinical signs of acute toxicity, such as changes in skin and fur, eyes and mucous membranes, respiratory function, somatomotor activity, and behavior (OECD, 2008).

Table 1. Initial weight and weight gain of animals during the experimental period (means ± SE).

Experimental groups	Initial weight (g)	Weight gain (g)
Control	35.40 ± 0.60[a]	1.60 ± 0.37[a]
CP 100 mg/kg	35.10 ± 0.82[a]	0.80 ± 0.75[a]
Camp 30 mg/kg	36.90 ± 0.97[a]	1.40 ± 1.13[a]
Camp 100 mg/kg	37.20 ± 1.57[a]	1.50 ± 1.54[a]
Camp 300 mg/kg	37.70 ± 0.71[a]	1.40 ± 1.12[a]
Associated groups		
Pre-treatment 30 mg/kg	34.00 ± 1.00[a]	1.50 ± 0.83[a]
Pre-treatment 100 mg/kg	33.80 ± 0.92[a]	1.80 ± 0.87[a]
Pre-treatment 300 mg/kg	33.90 ± 0.85[a]	1.60 ± 1.06[a]
Simultaneous 30 mg/kg	37.60 ± 0.80[a]	0.80 ± 0.32[a]
Simultaneous 100 mg/kg	36.50 ± 0.65[a]	1.00 ± 0.33[a]
Simultaneous 300 mg/kg	37.60 ± 0.77[a]	0.60 ± 0.42[a]
Post-treatment 30 mg/kg	36.10 ± 0.75[a]	0.90 ± 0.35[a]
Post-treatment 100 mg/kg	35.30 ± 0.89[a]	0.50 ± 0.43[a]
Post-treatment 300 mg/kg	35.20 ± 0.81[a]	0.30 ± 0.26[a]

CP = cyclophosphamide; Camp = *Campomanesia adamantium*. Same superscript letters indicate absence of significant differences (ANOVA/Tukey; $P > 0.05$).

Hydroethanolic extract is mutagenic and reduces DNA damage induced by CP

Mutagenic and antimutagenic activities were evaluated by performing the micronucleus assay in peripheral blood after 24, 48, and 72 h of treatment. After 24 h, there was a slight increase in the average number of micronuclei at doses of 30 and 100 mg/kg (7.70 ± 1.38 and 6.10 ± 1.76, respectively) compared to the number in the control (1.10 ± 0.45); however, the differences were not statistically significant. The highest dose (300 mg/kg) resulted in the lowest average micronucleus number among the groups that received the extract (2.00 ± 0.53). After 48 and 72 h, micronucleus averages decreased and no significant differences were observed among the *C. adamantium* and control groups.

For antimutagenesis experiments, *C. adamantium* extract was combined with DNA damage-inducing agent CP and three different protocols were used: pre-treatment, simultaneous treatment, and post-treatment. Results showed that there was a reduction of micronucleus frequency in all three experiments and at different exposure time points compared to the frequency associated with CP treatment. For all of these protocols, the greatest reduction was obtained at 72 h (Table 2).

Table 2. Micronucleus frequency observed at different time points in animals treated with *Campomanesia adamantium* (Camp) hydroethanolic extract with or without cyclophosphamide (CP).

Experimental groups	Micronuclei (means ± SE)		
	24 h	48 h	72 h
Control	1.10 ± 0.45[a]	0.70 ± 0.33[a]	1.00 ± 0.39[a]
CP 100 mg/kg	29.22 ± 2.04[c]	16.89 ± 2.75[d]	26.10 ± 1.95[b]
Camp 30 mg/kg	7.70 ± 1.38[a,b]	2.10 ± 0.65[a]	2.10 ± 0.45[a]
Camp 100 mg/kg	6.10 ± 1.76[a,b]	2.40 ± 0.65[a,b]	2.25 ± 0.77[a]
Camp 300 mg/kg	2.00 ± 0.53[a]	3.30 ± 0.65[a,b]	0.75 ± 0.41[a]
Associated groups			
Pre-treatment 30 mg/kg	6.66 ± 1.84[a,b]	4.55 ± 0.88[a,b,c]	0.90 ± 0.40[a]
Pre-treatment 100 mg/kg	10.78 ± 3.43[a,b]	2.20 ± 0.62[a]	1.80 ± 0.48[a]
Pre-treatment 300 mg/kg	8.80 ± 2.18[a,b]	1.50 ± 0.65[a]	2.33 ± 0.66[a]
Simultaneous 30 mg/kg	13.40 ± 3.78[b]	7.30 ± 1.15[b,c]	0.75 ± 0.49[a]
Simultaneous 100 mg/kg	10.33 ± 1.58[a,b]	8.75 ± 1.62[c]	1.40 ± 0.56[a]
Simultaneous 300 mg/kg	12.10 ± 1.76[b]	1.50 ± 0.53[a]	2.00 ± 0.95[a]
Post-treatment 30 mg/kg	5.77 ± 1.32[a,b]	1.10 ± 0.45[a]	1.00 ± 0.53[a]
Post-treatment 100 mg/kg	7.33 ± 1.23[a,b]	1.80 ± 0.64[a]	1.30 ± 0.71[a]
Post-treatment 300 mg/kg	6.55 ± 2.06[a,b]	2.37 ± 1.13[a,b]	1.44 ± 0.68[a]

Different superscript letters indicate statistically significant differences (ANOVA/Tukey; $P < 0.05$).

C. adamantium increases splenic phagocytic activity

Compared to the control group, the *C. adamantium*-treated groups showed increased phagocytosis in the spleen (39.37 ± 2.90, 38.50 ± 2.32, and 58.00 ± 1.87 for the 30, 100, and 300 mg/kg doses, respectively, *vs* 20.37 ± 0.80 in the control group); the intergroup difference was statistically significant.

CP increased phagocytic activity in the spleen (119.25 ± 3.22). Combined treatment with the extract significantly increased the average number of phagocytosed cells in relation to the number in the positive control group (Figure 2).

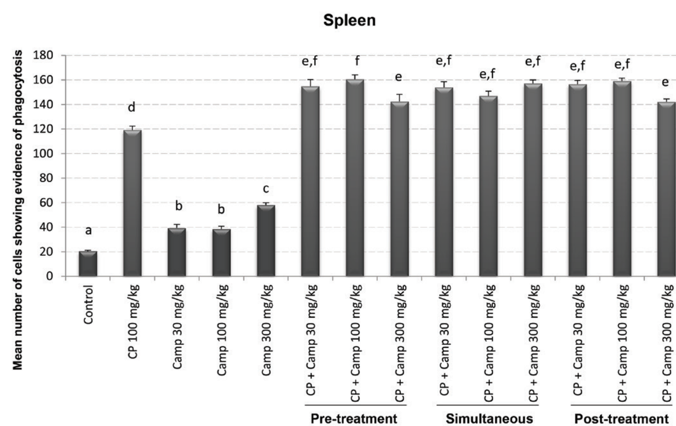

Figure 2. Spleen phagocytosis after treatment with *Campomanesia adamantium* (Camp) hydroethanolic extract with or without cyclophosphamide (CP). Different letters indicate statistically significant differences (ANOVA/Tukey; $P < 0.05$).

©FUNPEC-RP www.funpecrp.com.br

Apoptosis increased in the kidney, spleen, and liver

Cell death by apoptosis was investigated by morphological analysis in different types of organs, such as the spleen, liver, and kidneys. The extract induced apoptosis in all the organs studied.

In groups treated with the combined treatments, there was a significant reduction in apoptotic cell frequency in all organs compared to that in the CP groups. More pronounced reduction was observed after treatments (Figure 3).

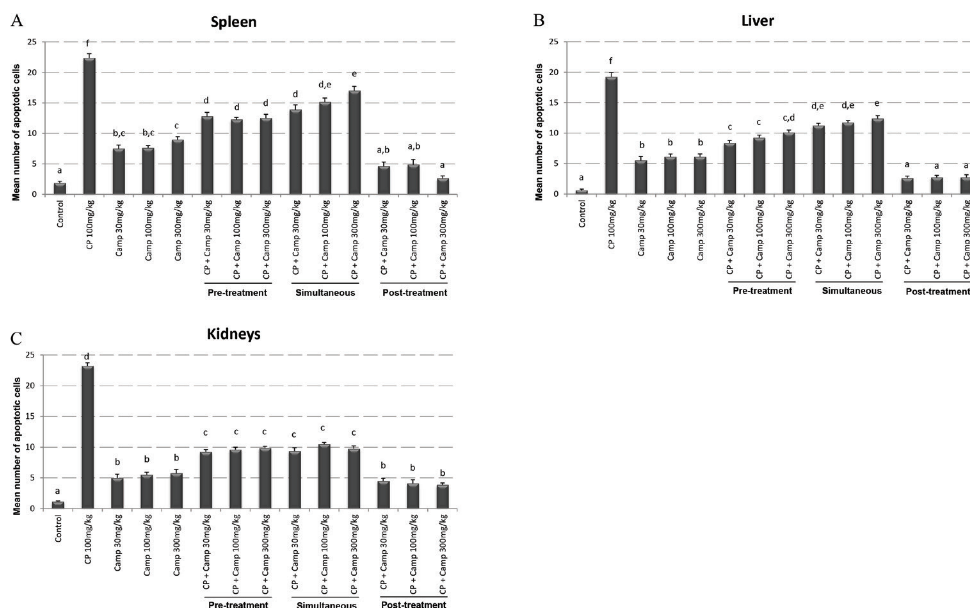

Figure 3. Apoptosis evaluation after treatment with *Campomanesia adamantium* hydroethanolic extract with or without cyclophosphamide in the (**A**) spleen, (**B**) liver, and (**C**) kidneys. Different letters indicate statistically significant differences (ANOVA/Tukey; $P < 0.05$).

DISCUSSION

The widespread use of plants to treat diseases is an important indicator that plants can serve as sources of new chemical compounds with therapeutic potential. *C. adamantium* is a common species in Brazilian cerrado, and its anti-inflammatory, antidiarrheal, antiseptic, and antioxidant activities, reported by the native population, have been confirmed in previous studies (Ramos et al., 2007; Coutinho et al., 2008; Pascoal et al., 2011; Ferreira et al., 2013; de Souza et al., 2014). However, few studies on its mutagenic potential have been reported.

In this study, no clinical signs of toxicity were observed after *C. adamantium* extract administration in the experimental animals. These findings are consistent with those of de Souza et al. (2014), who observed low toxicity of *C. adamantium* fruit extracts, for which the lethal dose (LD_{50}) was higher than 2000 mg/kg. Furthermore, this extract was also classified as having low toxicity in Guidelines of the Organization for Economic Co-Operation and Development (OECD, 2008). Ramos et al. (2007) evaluated the toxicity of hexane extract of *C. adamantium* leaves in *Artemia* sp and concluded that 2 mg/mL extract was not toxic, since no microcrustacean death was observed.

 ©FUNPEC-RP www.funpecrp.com.br

Mutagenicity evaluation showed an increase in the average number of micronuclei at 30 and 100 mg/kg after 24 h. The highest concentration (300 mg/kg) resulted in the lowest average number among groups that received the extract. Micronuclei are derived from acentric chromosomal fragments, chromatids, or whole chromosomes that do not migrate along the spindle during anaphase and stay in the cytoplasm. These components are surrounded by a nuclear membrane and acquire the same morphological characteristics as the nucleus, with the exception of size (Fenech et al., 2011). One cause of micronucleus formation is the effect of chemical agents on DNA, which was first reported by Klein and Klein (1952) in Ehrlich tumor cells treated with colchicine. These compounds induce single- and double-strand DNA breaks, which generate DNA fragments that form micronuclei if they were not repaired (Fenech et al., 2011).

The increases in the average number of micronuclei at doses of 30 and 100 mg/kg after 24 h may be related to the extract potential to cause DNA damage. In this case, DNA damage could not be repaired and resulted in micronuclei. At 24 h after the administration of the 300 mg/kg, the extent of damage was greater than that produced at the lower doses. This observation may be explained by comparing results from micronuclei and splenic phagocytosis, which showed increased phagocytic activity in spleens from *C. adamantium*-treated mice. It is known that the spleen removes defective cells from the bloodstream, including micronucleated cells (de Lima et al., 2013). Therefore, this finding supports the hypothesis that the extract is capable of inducing cellular DNA damage.

Extracts also increased the number of apoptotic cells, which are consistent with previously discussed data. In particular, extract at 300 mg/kg resulted in highest apoptosis average, which suggests that higher concentrations are directly related to increased non-reparable cell damage that leads to cell death. This speculation is supported by the effect observed at lower doses; the initial damage caused by the extract is less extensive and can still be detected as increased number of micronuclei over 24 h. After 48 and 72 h, micronucleus frequency decreased, and damage may be detected as increased number of apoptotic cells.

Pascoal et al. (2014) reported the cytotoxic potential of ethanolic extract from leaves and fruit of *C. adamantium*, and a chalcone isolated from leaves, referred to as cardamonin. In a prostate cancer cell line (PC-3), leaf extract demonstrated optimal antiproliferative response, which was associated with higher level of cardamonin. In addition, chalcone induced apoptosis along with DNA fragmentation after 6 h of exposure. These findings are consistent with our data that showed increased damage at shorter post-exposure times.

Previous study using aqueous leaf extract in *Salmonella*/microsome test demonstrated the mutagenic potential of *Campomanesia xanthocarpa* in a dose-dependent manner, in TA97 strain (frameshift mutation) without metabolic activation (Fernandes and Vargas, 2003).

Our data showed a considerable decrease in micronucleus formation in all groups treated with extract and CP, which suggests that extract has antimutagenic activity. Considering the other above-mentioned data, which suggests that extract is capable of producing potentially non-repairable DNA damage and increases cell death by apoptosis, these findings suggest that: i) extract may chemically inactivate CP (desmutagenic activity) and/or ii) extract directly affects the metabolism of CP, increasing its excretion.

CP is an alkylating agent that induces intrastrand and interstrand DNA crosslinks. However, CP is a prodrug that is metabolized by cytochrome P450 family of hepatic microsomal enzymes, specifically mixed-function oxidases, to form the active molecule (Cohen and Jao, 1970; Pinto et al., 2009). P450 metabolic system is the most well-known drug metabolism system (Cohen and

Jao, 1970). Plant extracts affect drug metabolism. When taken concomitantly, they can directly affect enzyme activity, inhibiting or increasing drug metabolism, which may result in increased clearance rates and side effects (Hu et al., 2005).

The main components of *C. adamantium* extract are chalcones, flavonones, and phenolic compounds (Ramos et al., 2007; Coutinho et al., 2008; Ferreira et al., 2013; Pascoal et al., 2011, 2014). Compounds belonging to the chemical classes such as flavonones and chalcones are known to affect the metabolizing activity of microsomal fraction of liver (Shimada et al., 2010; Liu et al., 2013). Additionally, they can also affect the phase 2 reactions and modulating activity and/or expression of glutathione-S-transferases (GSTs) (Cabrera et al., 2010; Boušová and Skálová, 2012). These enzymes are involved in detoxification of several chemotherapeutic agents and their metabolites, including CP (Pinto et al., 2009). Taking these findings into consideration, decreased micronucleus frequency and apoptosis in combined treatment groups may be related to the ability of *C. adamantium* extract components to increase GST activity, thereby causing a significant increase in the excretion of CP and its metabolites. Pre-treatment and simultaneous treatments differed significantly from positive control. In contrast, post-treatment groups had lower means similar to negative control and groups that only received extract.

Apoptotic cell death process basically involves two steps, cell condensation and fragmentation (formation of apoptotic bodies) and elimination of apoptotic bodies by phagocytes (Kerr et al., 1972). It is difficult to estimate the amount of time required to complete this process. Analysis of several experimental models suggests that it is reasonably fast, approximately 24 h (Kerr, 1971; Crawford et al., 1972; Wyllie et al., 1973). Therefore, lower means observed for the post-treatment group may not only be related to extract effect on CP metabolism, but may also be related to the removal of apoptotic cells by the body because in this study time interval between CP treatment and execution of the apoptosis assay was longer than that in other studies.

Similarly, phagocytic activity increased significantly. This effect was most likely owing to additive effect of both extract and CP, because both are capable of inducing DNA damage and consequently producing defective cells that are removed from circulation by the spleen.

A short-term exposure to *C. adamantium* hydroethanolic extract is capable of causing potentially non-repairable DNA damage, which leads to cell death. Extract components may have a modulatory effect on microsomal enzymes, especially GSTs, thereby directly affecting metabolism of CP. Our data suggest caution in concomitant consumption of preparations containing *C. adamantium* leaves with drugs, since it may decrease drug efficacy through metabolic modulations produced by extract components.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES, Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq, and Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia - FUNDECT do Estado de Mato Grosso do Sul. M.D. Martello and N. David received a Master's scholarship from CAPES, and R. Matuo received grants from PNPD/CAPES.

REFERENCES

Avidos MFD and Ferreira LT (2000). Frutos dos cerrados: preservação gera muitos frutos. *Biotecnolog. Cienc. Desenvolv.* 3: 36-41.

Boušová I and Skálová L (2012). Inhibition and induction of glutathione S-transferases by flavonoids: possible pharmacological and toxicological consequences. *Drug Metab. Rev.* 44: 267-286. http://dx.doi.org/10.3109/03602532.2012.713969

Brandelli CLC, Giordani RB, Macedo AJ, de Carli GA, et al. (2011). Indigenous traditional medicine: plants for the treatment of diarrhea. In: Nature Helps: How Plants and Other Organisms Contribute to Solve Health Problems (Mehlhorn H, ed.). 1st ed. Springer.

Brazil, Ministry of the Environment (2006). Assessment of the Status of Knowledge on Brazilian Biodiversity. In: Coordinator. First National Report for the Convention on Biological Diversity (Lewinsohn T, ed.). Brasília, Brazil, 21-30.

Brazil, Ministry of the Environment (2010). Status of Knowledge on Brazil's Biodiversity. In: Coordinator. Fourth National Report to the Convention on Biological Diversity (Dias BFS, ed.). Brasília, Brazil, 35-43.

Cabrera M, Lavaggi ML, Croce F, Celano L, et al. (2010). Identification of chalcones as *in vivo* liver monofunctional phase II enzymes inducers. *Bioorg. Med. Chem.* 18: 5391-5399. http://dx.doi.org/10.1016/j.bmc.2010.05.033

Cohen JL and Jao JY (1970). Enzymatic basis of cyclophosphamide activation by hepatic microsomes of the rat. *J. Pharmacol. Exp. Ther.* 174: 206-210.

Coutinho ID, Coelho RG, Kataoka VMF, Honda NK, et al. (2008). Determination of phenolic compounds and evaluation of antioxidant capacity of *Campomanesia adamantium* leaves. *Eclet. Quím.* 33: 53-60.

Cragg GM and Newman DJ (2013). Natural products: a continuing source of novel drug leads. *Biochim. Biophys. Acta* 1830: 3670-3695. http://dx.doi.org/10.1016/j.bbagen.2013.02.008

Crawford AM, Kerr JF and Currie AR (1972). The relationship of acute mesodermal cell death to the teratogenic effects of 7-OHM-12-MBA in the foetal rat. *Br. J. Cancer* 26: 498-503. http://dx.doi.org/10.1038/bjc.1972.67

de Lima PL, Sugui MM, Petrício AI, Vilela LC, et al. (2013). *Lentinula edodes* (shiitake) modulates chemically induced mutagenesis by enhancing pitting. *J. Med. Food* 16: 733-739. http://dx.doi.org/10.1089/jmf.2013.0015

de Souza JC, Piccinelli AC, Aquino DFS, de Souza VV, et al. (2014). Toxicological analysis and antihyperalgesic, antidepressant, and anti-inflammatory effects of *Campomanesia adamantium* fruit barks. *Nutr. Neurosci.* http://dx.doi.org/10.1179/1476830514Y.0000000145

Duarte WF, Dias DR, de Melo Pereira GV, Gervásio IM, et al. (2009). Indigenous and inoculated yeast fermentation of gabiroba (*Campomanesia pubescens*) pulp for fruit wine production. *J. Ind. Microbiol. Biotechnol.* 36: 557-569. http://dx.doi.org/10.1007/s10295-009-0526-y

Fenech M, Kirsch-Volders M, Natarajan AT, Surralles J, et al. (2011). Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. *Mutagenesis* 26: 125-132. http://dx.doi.org/10.1093/mutage/geq052

Fernandes JB and Vargas VM (2003). Mutagenic and antimutagenic potential of the medicinal plants *M. laevigata* and *C. xanthocarpa*. *Phytother. Res.* 17: 269-273. http://dx.doi.org/10.1002/ptr.1058

Ferreira LC, Grabe-Guimarães A, de Paula CA, Michel MC, et al. (2013). Anti-inflammatory and antinociceptive activities of *Campomanesia adamantium*. *J. Ethnopharmacol.* 145: 100-108. http://dx.doi.org/10.1016/j.jep.2012.10.037

Harvey AL (2007). Natural products as a screening resource. *Curr. Opin. Chem. Biol.* 11: 480-484. http://dx.doi.org/10.1016/j.cbpa.2007.08.012

Hayashi M, Morita T, Kodama Y, Sofuni T, et al. (1990). The micronucleus assay with mouse peripheral blood reticulocytes using acridine orange-coated slides. *Mutat. Res.* 245: 245-249. http://dx.doi.org/10.1016/0165-7992(90)90153-B

Hu Z, Yang X, Ho PC, Chan SY, et al. (2005). Herb-drug interactions: a literature review. *Drugs* 65: 1239-1282. http://dx.doi.org/10.2165/00003495-200565090-00005

Kerr JF (1971). Shrinkage necrosis: a distinct mode of cellular death. *J. Pathol.* 105: 13-20. http://dx.doi.org/10.1002/path.1711050103

Kerr JFR, Wyllie AH and Currie AR (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* 26: 239-257. http://dx.doi.org/10.1038/bjc.1972.33

Klein G and Klein E (1952). The viability and the average desoxypentosenucleic acid content of micronuclei-containing cells produced by colchicine treatment in the Ehrlich ascites tumor. *Cancer Res.* 12: 484-489.

Liu J, Taylor SF, Dupart PS, Arnold CL, et al. (2013). Pyranoflavones: a group of small-molecule probes for exploring the active site cavities of cytochrome P450 enzymes 1A1, 1A2, and 1B1. *J. Med. Chem.* 56: 4082-4092. http://dx.doi.org/10.1021/jm4003654

Navarro SD, Beatriz A, Meza A, Pesarini JR, et al. (2014). A new synthetic resorcinolic lipid 3-heptyl-3,4,6-trimethoxy-3H-isobenzofuran-1-one: evaluation of toxicology and ability to potentiate the mutagenic and apoptotic effects of

 ©FUNPEC-RP www.funpecrp.com.br

cyclophosphamide. *Eur. J. Med. Chem.* 75: 132-142. http://dx.doi.org/10.1016/j.ejmech.2014.01.057

OECD (2008). Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing.

Oliveira RJ, Salles MJ, da Silva AF, Kanno TY, et al. (2009). Effects of the polysaccharide β-glucan on clastogenicity and teratogenicity caused by acute exposure to cyclophosphamide in mice. *Regul. Toxicol. Pharmacol.* 53: 164-173. http://dx.doi.org/10.1016/j.yrtph.2008.12.007

Pascoal AC, Ehrenfried CA, Eberline MN, Stefanello MEA, et al. (2011). Free radical scavenging activity, determination of phenolic compounds and HPLC-DAD/ESI-MS profile of *Campomanesia adamantium* leaves. *Nat. Prod. Commun.* 6: 969-972.

Pascoal AC, Ehrenfried CA, Lopez BG, de Araujo TM, et al. (2014). Antiproliferative activity and induction of apoptosis in PC-3 cells by the chalcone cardamonin from *Campomanesia adamantium* (Myrtaceae) in a bioactivity-guided study. *Molecules* 19: 1843-1855. http://dx.doi.org/10.3390/molecules19021843

Pinto N, Ludeman SM and Dolan ME (2009). Drug focus: Pharmacogenetic studies related to cyclophosphamide-based therapy. *Pharmacogenomics* 10: 1897-1903. http://dx.doi.org/10.2217/pgs.09.134

Ramos DD, Cardoso CAL and Yamamoto NT (2007). Avaliação do potencial citotóxico e atividade antioxidante em *Campomanesia adamantium* (Cambess.) O. Berg (*Myrtaceae*). *Rev. Bras. Biocienc.* 5: 774-776.

Rodrigues VEG and Carvalho DA (2001). Plantas medicinais no domínio dos cerrados [Medicinal plants of the Cerrado]. Ed. UFLA, Lavras.

Rufino MSM, Alve ER and Brito SE (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chem.* 121: 996-1002. http://dx.doi.org/10.1016/j.foodchem.2010.01.037

Shimada T, Tanaka K, Takenaka S, Murayama N, et al. (2010). Structure-function relationships of inhibition of human cytochromes P450 1A1, 1A2, 1B1, 2C9, and 3A4 by 33 flavonoid derivatives. *Chem. Res. Toxicol.* 23: 1921-1935. http://dx.doi.org/10.1021/tx100286d

Sobral M, Proença C, Souza M, Mazine F, et al. (2014). *Myrtaceae*. Lista de Espécies da Flora do Brasil [List of Plants Species of Brazil]. Jardim Botânico do Rio de Janeiro [Rio de Janeiro Botanical Garden]. Available at [http://www.floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB10308]. Accessed December 6, 2014.

Wyllie AH, Kerr JFR, Macaskill IAM and Currie AR (1973). Adrenocortical cell deletion: the role of ACTH. *J. Pathol.* 111: 85-94. http://dx.doi.org/10.1002/path.1711110203

 ©FUNPEC-RP www.funpecrp.com.br