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Article

Evaluation of (anti)genotoxic activities of *Phyllanthus niruri* L. in rat bone marrow using the micronucleus test

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Phyllanthus niruri L. (Euphorbiaceae), known as "quebra-pedra" (Portuguese for "stonebreaker"), is an herb used for kidney disorders. In light of its frequent use by the population, the present study aimed to investigate the genotoxic, antigenotoxic and cytotoxic activities of a standardized *P. niruri* extract in bone marrow rats. Three groups of 12 animals were treated daily by gavage over a period of 30 days, with 50, 150 or 250 mg/kg of *P. niruri* extract aqueous solution. The control group (n = 12) received tap water. At the end of treatment (day 31), groups were divided into two minor subgroups (n=6/group) and received cyclophosphamide (50 mg/kg, i.p.) or saline 0.9% (i.p.). After 24 hours, we evaluated the frequency of micronucleated polychromatic erythrocytes for each animal (MNPCE) at 1000 PCE. Cytotoxicity was evaluated with the PCE/NCE ratio (NEC = normochromatic erythrocytes). General toxicity was assessed during treatment using the parameters of body weight gain, ration and water consumption. The dry extract did not provoke changes in body weight, weight gain, ration and water intake or changes in the frequency of MNPCE or cytotoxicity in bone marrow. We propose that the *P. niruri* extract used here showed no genotoxic, antigenotoxic and cytotoxic activities under the experimental conditions.

Uniterms: *Phyllanthus niruri* L./genotoxicity. *Phyllanthus niruri* L./toxicological analysis/experimental study. Medicinal plantas/evaluation.

Phyllanthus niruri L. (Euphorbiaceae), conhecida como "quebra-pedra", é uma planta medicinal utilizada frequentemente pela população no tratamento de problemas renais. Foram avaliadas as atividades genotóxicas, antigenotóxicas e citotóxicas de um extrato padronizado dessa espécie em ratos. Três grupos de doze animais foram tratados durante trinta dias, por gavagem, com 50, 150 ou 250 mg/kg/dia de solução aquosa do extrato de *P. niruri* e um grupo controle (n=12) recebeu água destilada pela mesma via. No final do tratamento os grupos foram divididos em dois subgrupos (6 animais/grupo) e receberam uma dose única de ciclofosfamida (50 mg/kg, i.p.) ou de solução salina 0,9% (i.p.). Após 24 horas, a frequência de eritrócitos policromáticos micronucleados (EPCMN) foi avaliada em 1000 EPC. A citotoxicidade foi avaliada pela relação entre eritrócitos policromáticos e normocromáticos (EPC/ENC) e a toxicidade geral foi avaliada através dos parâmetros de ganho de peso corporal, consumo de ração e ingestão hídrica. O extrato seco não provocou alterações significativas no peso corporal, ganho de peso e consumo de ração em relação ao grupo controle, nem alterações na frequência de EPCMN ou citotoxicidade em medula óssea. Dessa maneira, pode-se concluir que *P. niruri* não apresentou atividades genotóxica, antigenotóxica e/ou citotóxica nas condições experimentais executadas.

Unitermos: *Phyllanthus niruri* L./genotoxicidade. *Phyllanthus niruri* L./análise toxicological/estudo experimental. Plantas medicinais/avaliação.

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INTRODUCTION

Medicinal plants and their preparations have been used to treat several illnesses since the dawn of human history and are one of the foundations for healthcare worldwide (AESGP, 1998; Turolla, 2004; Mendes; Herdeiro; Pimentel, 2010). The observation and experience of primitive man elucidated the therapeutic properties of certain plants and promoted their use from one generation to another (Turolla, 2004).

It is well known that natural products traditionally consumed by a portion of the population may have mutagenic potential. As such, further evaluation and studies are needed in order to confirm this possibility (Freitas, 2007).

The species *Phyllanthus niruri* Linné, commonly known as "erva-pombinha", "quebra-pedras-de-arvorezinha", "quebra-pedra", "quebra-pedra legítimo" or "quebra-pedra verdadeiro" (names in Portuguese for the herb stonebreaker) (Simões *et al*., 1986; Garlet, 2000), belongs to the family Euphorbiaceae, which contains approximately three hundred and fifteen genera and eight thousand species (Filho *et al*., 1996). The herb has a thin straight stalk with many branches, is between 10 and 30 cm tall (Franco, 1997; Santos, Torres, Leonart, 1987) and can be found in Africa, Asia and the Americas, commonly on coastal plains (Castro-Chaves *et al*., 2002).

In addition to alkaloids, other compounds including tannins, flavonoids, phenols, triterpenoids, fatty acids, esters of phthalic acid, lignins such as phyllanthin and hypophyllanthin, and vitamin C have been isolated and characterized in several species of the genus *Phyllanthus* (Ahmad, Husain, Osman, 1981; Miguel *et al*., 1996; Quian-Cutrone, 1996; Ueno *et al*., 1988; Ishimaru *et al*., 1992; Rajeshkumar *et al*., 2002; Mahdi *et al*., 2011; Samali *et al*., 2012). Studies with *P. niruri* have found flavonoids such as quercetin and kaempferol (El-Mekkawy *et al*., 1995), lignin glycosides called phyllanthostatin A, 1 and 2 (Pettit, Schaufelberger, 1988; Pettit *et al*., 1990), ellagitannins such as geraniin and furosin (Miguel *et al*., 1996), niruroidine (Quian-Cutrone *et al*., 1996), lignans as nirtetralin A and B (Wei *et al*., 2012) among others.

In folk medicine, *P. niruri* is mainly used for urinary tract problems, such as kidney and bladder pain, renal lithiasis, and as a diuretic (Garlet, 2000). Extracts of *P. niruri* were found to normalize high levels of urinary calcium, reducing the formation of calculus in patients (Nishiura *et al*., 2004). This suggests it interferes in the first stages of stone formation and may be an alternative in the treatment and/or prevention of urolithiasis and inhibit the formation of calcium oxalate crystals in the urinary tract, preventing renal calculus (Barros *et al*., 2003; Freitas, Schoe, Boim, 2002). Studies show that the plant promotes relaxation of the ureters which, combined with analgesic action (Santos *et al*., 1995), facilitates the descent of stones. This generally occurs without pain or bleeding and increases glomerular filtration and the excretion of uric acid (Lorenzi; Matos, 2002), justifying its use for renal lithiasis. The aqueous extract inhibits reverse transcriptase in the human immunodeficiency virus type 1 (HIV-1-RT) (Ogata *et al*., 1992; El-Mekkawy *et al*., 1995; Notka, Meier, Wagner, 2003). However, other studies have been conducted in an attempt to prove several other biological activities, such as analgesic action (Miguel *et al*., 1996; Santos *et al*., 1995; Santos *et al*., 2000), anti-tumor activity in different cell lines (Powis, Moore, 1985; Pettit, Schaufelberger, 1988; Pettit *et al.*, 1990; Rajeshkumar, Kuttan, 2000), antioxidant effects in preventing gastric ulcers (Bandyopadhay, Pakrashi, 2000), anti-inflammatory (Ignácio *et al*., 2001; Kiemer *et al*., 2003) and anti-malaria activity (Muñoz *et al*., 2000) and reduced plasma lipid levels in rats (Khanna, Rizvi, Chander, 2002). It also exhibits abortive and purgative action in large doses (Martins *et al*., 1994). Manjrekar *et al*. (2008) demonstrated a reduction in carbon tetrachloride-induced hepatotoxic effects in rats treated with an aqueous extract of *P. niruri*.

The use of herbal drugs has long been well known. In order to popularize this knowledge and clarify when and how these drugs should be used to achieve beneficial effects, the National Agency for Sanitary Vigilance (ANVISA) provides notification of herbal drugs through Resolution-RDC Nº 10 of March 9, 2010 (Brasil, 2010). Standardization for popular use of *P. niruri* as an herbal drug among adults is 3 g (equivalent to a soup spoon) infused with 150mL of water (1 teacup), 2 or 3 times a day. It is recommended for renal lithiasis to help remove small kidney stones but contraindicated for large stones, as well as during pregnancy. Main adverse reactions include diarrhea and hypotension and it should not be used for more than three weeks (Brasil, 2010).

Among genotoxicity tests recommended by international regulatory agencies and government institutions, the *in vivo* Micronucleus Test (MN) in rat bone marrow is widely accepted and recommended to assess and record new chemical and pharmaceutical products that enter the world market (Krishna, Hayashi, 2000; Ribeiro, 2003; Speit, Zeller, Neuss, 2011). This test is frequently used to detect clastogenic agents (which break chomosomes) and aneugenic agents (which induce aneuploidy or abnormal chromosome segregation due to mitotic spindle dysfunction) (Macgregor *et al*., 1987; Hayashi *et al*.; 1994). It was initially developed in mouse bone marrow erythrocytes (Schmid, 1976), but can also be performed on rats (George, Wooton, Gatehouse, 1990).

In the present study, three different concentrations of dry *P. niruri* were tested to assess in vivo genotoxic, antigenotoxic and cytotoxic activities in the bone marrow cells of Wistar rats, using MN tests as a parameter. General toxicity was also evaluated after 30-day treatment with aqueous suspension of dry *P. niruri* extract.

MATERIAL AND METHODS

Materials

Dry *P. niruri* extract (lot: 024827, supplier: FLORES E ERVAS) was obtained from a compounding pharmacy in the city of Natal and suspended in water at a concentration of 20 mg/mL. Total tannin and gallic acid content in the lot used was 6.5% and 0.15% respectively, in accordance with supplier specifications. Cyclophosphamide was acquired from Sigma Chemical Co., USA; methanol (Merck); Giemsa (Merck); May-Grunwald (Merck); saline solution (NaCl 0.9%).

Animals

Adult male rats (48) with an initial weight of 280g were obtained from the vivarium of the Health Sciences Center at the Federal University of Rio Grande do Norte (UFRN). Individuals were from the same strain of the species *Rattus novergicus*, family Wistar, and were randomly separated into four groups (n = 12/group). Over a period of 30 days, three experimental groups received doses of 50, 150 and 250 mg/kg/day of dry *P. niruri* extract, by gavage, suspended in distilled water. The control group received only water, using the same method and time period. Body weight, ration consumption and water intake were monitored during treatment and weight gain was also calculated. Animals were housed in polypropylene cages with metal lids measuring 40 x 50 x 20 cm, for no less than 5 days before being submitted to the different experimental conditions. Individuals were kept in a room at approximately constant temperature (23 to 26 °C) in a 12 hour light/dark cycle, where lights were turned on at 6.00 h. Water and feed (Labina® - Purina) were offered *ad libitum* throughout the experimental process. The research project was approved by the Ethics Committee on Animal Use at the Federal University of Rio Grande do Norte (CEUA – UFRN) under protocol number 024/2010. The methodology used was in accordance with guidelines of the Brazilian Animal Experimentation College (COBEA) and standards described in Law nº 11.794/2008.

Doses adopted were calculated considering the recommended dose for use in human adults to treat renal lithiasis; around 130 mg/kg/day (Brasil, 2010). Thus, a smaller, a similar and a larger dose were used to evaluate general toxicity.

Genotoxicity assessment by the MN test in mouse bone marrow

At the end of the 30-day treatment, animals from experimental and control groups were subdivided into smaller groups of six animals each. One of the new smaller groups for each treatment received 0.9% saline solution i.p. (at a volume of 1.0 mL) and the remaining groups received 50mg/kg i.p of cyclophosphamide.

After 24 hours of the above treatments, animals were euthanized with sodium thiopental (50mg/kg i.p.) and the femur was aseptically exteriorized and removed. The Schmid method (1976) was used to obtain bone marrow cells for the MN frequency study, modified by Zambrano, Targa and Rabello-Gay (1982). The far end of the proximal femur was cut to expose the spinal canal. Next, a syringe needle previously filled with 1.0 mL of 0.9% sterile saline solution was inserted firmly into the opening, injecting the serum and extracting the cells from the femoral cavity of each animal. Cells were place in 15 mL conical centrifuge tubes.

Slide preparation for MN research

Centrifuge tubes containing the bone marrow cells were centrifuged for 5 minutes at 1000 rpm, discarding the supernatant. From the resulting suspension, a small drop was transferred to a glass slide for distension. Two smears were performed per animal and slides were kept at room temperature for drying. May-Grunwald-Giemsa stain was used, modified by Rosenfeld. Staining allowed for differentiation between polychromatic (PCE) and normochromatic erythrocytes (NCE). Genotoxic and antigenotoxic activity was assessed on an optical microscope with a 100x objective lens (zigzag orientation), using the frequency of micronucleated polychromatic erythrocytes (MNPCE) in 1000 polychromatic erythrocytes, for each animal. We also determined the polychromatic erythrocyte count in 200 total erythrocytes (PCE + NCE) by the PCE/NCE ratio in order to confirm the presence of cytotoxicity.

Statistical analysis

Data were assessed with the analysis of variance (ANOVA) method and the Tukey-Kramer test, when necessary. Data were considered statistically significant for all groups when $P<0.05$.

RESULTS

Statistical analysis revealed no changes in body weight (Table 1) and food consumption (Figure 1) for animals treated with *P. niruri* when compared with controls. However, body weight gain was lower in the group treated with a dose of 150 mg/kg/day from the first to the sixth day of treatment [$F(3/44)= 3.790$; $p<0.05$] and over the entire treatment period [$F(3/44)= 2.961$; $p<0.05$] (Figure 2).

Only isolated alterations in water intake were detected (Figure 3). Statistical analysis revealed reduced water consumption in groups treated with doses of 50 mg/kg/day

TABLE I - Body weight (g) of male rats treated or not (control) with different doses of dry *P. niruri* extract (50, 150 or 250 mg/kg/day; p.o.) for 31 days. Means and the respective standard errors are shown (n= 12/group)

Day of treatment	Control	*P. niruri* 50 mg/kg/day	*P. niruri* 150 mg/kg/day	*P. niruri* 250 mg/kg/day
1	269.42 ± 13.02	291.00 ± 10.36	278.75 ± 12.36	279.75 ± 9.34
3	277.58 ± 10.55	293.42 ± 10.14	291.92 ± 17.47	279.75 ± 10.03
5	281.08 ± 10.22	293.92 ± 10.15	277.50 ± 12.44	283.83 ± 10.13
7	283.92 ± 9.03	286.17 ± 7.32	279.00 ± 12.60	286.83 ± 9.28
9	288.08 ± 9.07	294.92 ± 9.85	280.92 ± 12.90	285.92 ± 9.67
11	290.17 ± 8.84	296.08 ± 9.92	281,33 ± 12.43	289.50 ± 8.97
13	290.50 ± 9.04	298.83 ± 10.08	280.83 ± 12.73	290.50 ± 9.26
15	293.92 ± 8.13	297.92 ± 10.29	281.17 ± 12.49	291.67 ± 9.28
17	292.58 ± 7.87	300.75 ± 10.72	283.83 ± 12.70	292.83 ± 9.21
19	296.08 ± 8.35	302.58 ± 10.69	284.83 ± 12.53	294.17 ± 9.87
21	298.67 ± 8.27	305.00 ± 10.39	288.50 ± 12.89	295.75 ± 9.78
23	299.58 ± 7.31	306.50 ± 10.40	288.58 ± 12.77	298.00 ± 9.38
25	293.83 ± 10.72	308.50 ± 10.69	288.92 ± 13.10	300.25 ± 9.45
27	303.92 ± 7.73	310.17 ± 10.60	291.42 ± 12.85	300.25 ± 9.39
29	304.25 ± 7.37	310.42 ± 10.64	292.00 ± 12.81	301.75 ± 9.85
31	305.00 ± 7.13	313.92 ± 10.69	294.58 ± 12.97	303.50 ± 9.68

P>0.05; ANOVA

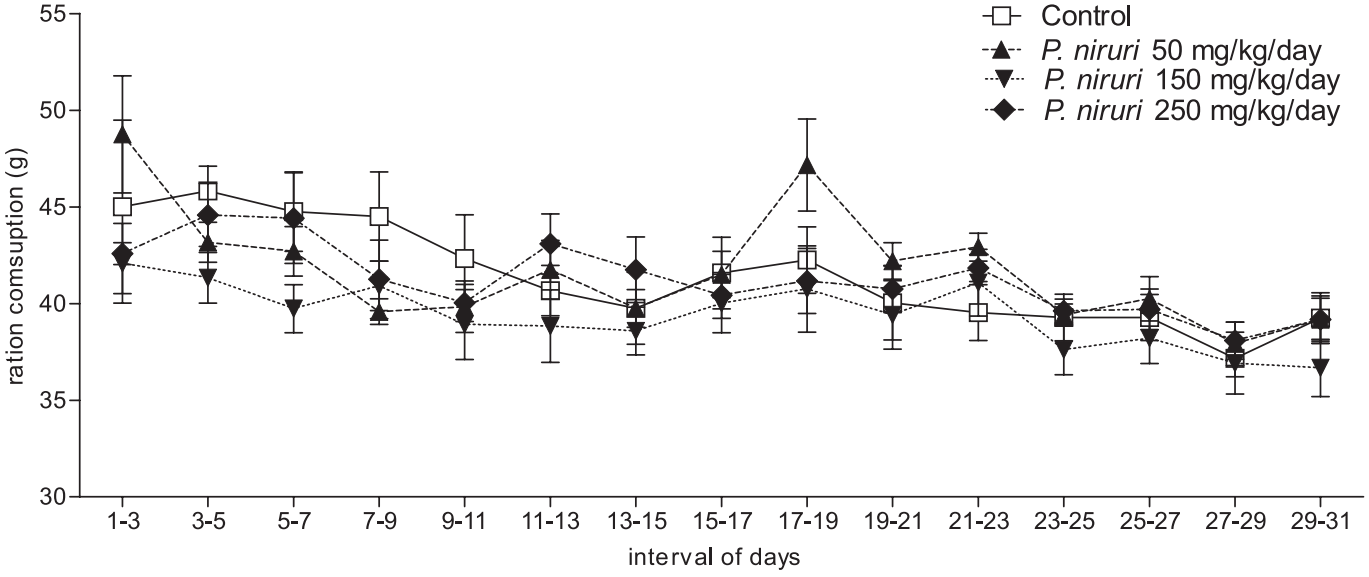

FIGURE 1 - Feed intake (g) of male rats treated or not (control) with different doses of dry *P. niruri* extract (50, 150 or 250 mg/kg/day; p.o.) for 31 days. The above values represent the mean and respective standard error for each group (p>0.05; ANOVA).

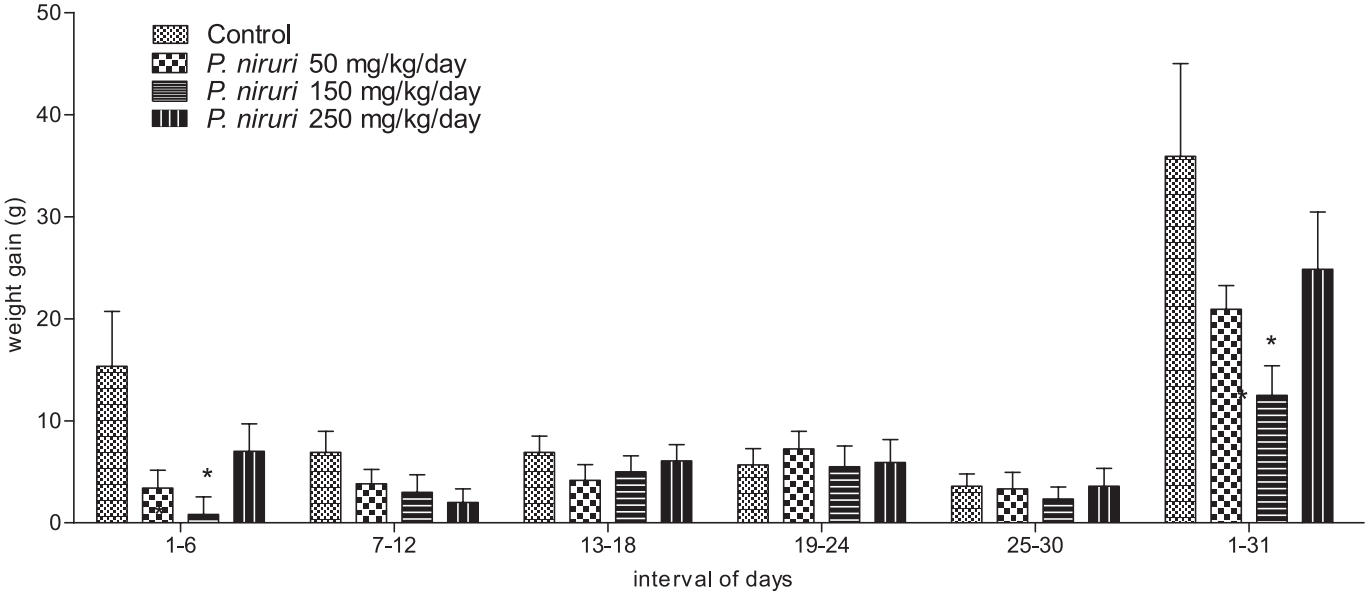

FIGURE 2 - Weight gain (g) among male rats treated or not (control) with different doses of dry *P. niruri* extract (50, 150 or 250 mg/kg/day; p.o.) for 31 days. The above values represent the mean and respective standard error for each group. (ANOVA, Tukey-Kramer Test, *p<0.05 in relation to the control group).

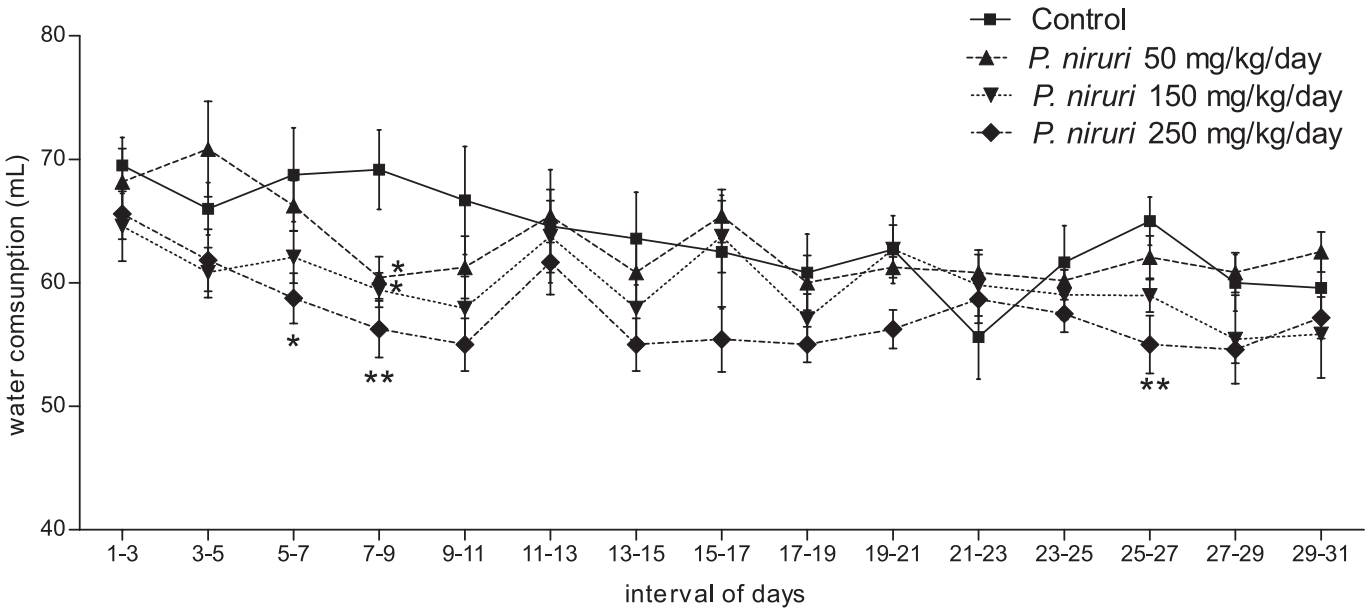

FIGURE 3 - Water intake (ml) for male rats treated or not (control) with different doses of dried *P. niruri* extract (50, 150 or 250 mg/kg/day; p.o.) for 31 days. The above values represent the mean and respective standard error for each group. ANOVA, Tukey-Kramer test, *p<0.05, **p<0.01 in relation to the control.

[F(3/44)= 5.976; p<0.05] and 150 mg/kg/day [F(3/44)= 5.976; p<0.05] during day 9 of treatment and by the group treated with the larger dose on day 7 [F(3/44)= 2.884; p<0.05], 9 [F(3/44)= 5.976; p<0.01] and 27 [F(3/44)= 5.285; p<0.01].

When evaluating the mutagenicity of dry *P. niruri* extract, the animals treated only with this extract exhibited a statistically lower MN frequency in relation to the positive control group [F(7/40)= 24.762; p<0.001]. Groups treated with *P. niruri* and cyclophosphamide showed an increase in MN frequency in relation to negative controls [F(7/40)= 24.762; p<0.001] and a decrease in this parameter in comparison to groups treated only with *P. niruri* [F(7/40)= 24.762; p<0.001]. This behavior is similar to

groups treated only with cyclophosphamide, which display an increase in MNPCE frequency in relation to negative controls [F(7/40)= 24.762; p<0.001] (Figure 4).

Cytotoxicity evaluation of bone marrow erythrocytes showed animals treated with the extract exhibited a statistically higher PCE/NCE ratio when compared with positive controls (Figure 5). This is similar to the negative control group, indicating a lack of cytotoxicity in the extract.

DISCUSSION

Toxicological assessment of plant extracts is a mean of investigating possible collateral effects and establishing their safe use by the population.

According to Barros *et al*. (2005), there is great concern about the safe use of plant extracts. Freitas (2007) underscores that natural products traditionally consumed by the population need further study, since they may

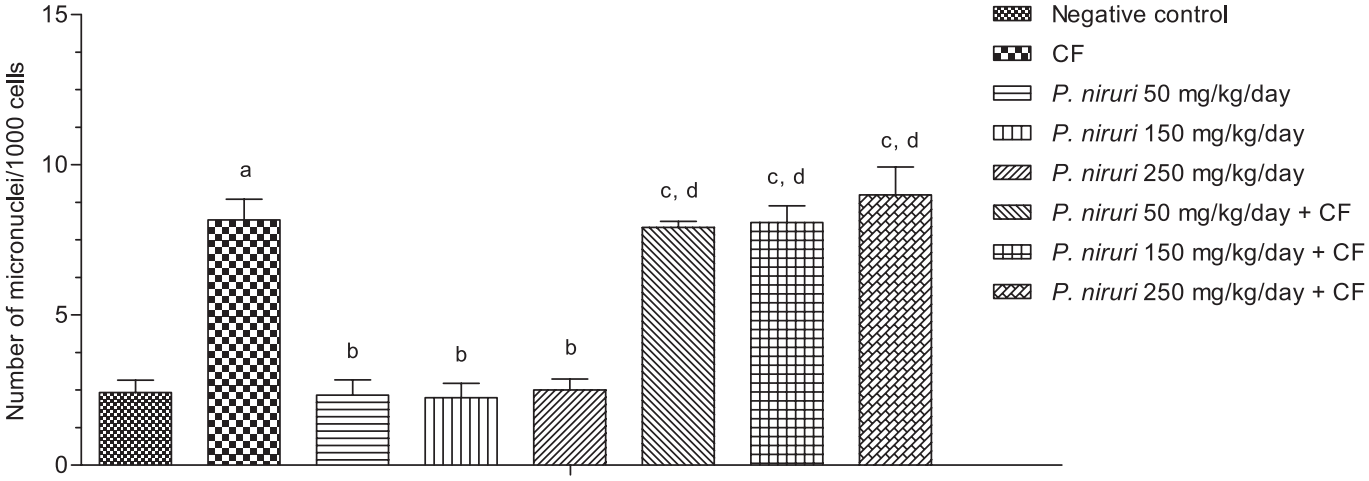

FIGURE 4 - Evaluation of micronuclei frequency in polychromatic erythrocytes (PCE) in the bone marrow of rats (n=6/group) exposed to different doses of dry *P. niruri* extract (50, 150 or 250 mg/kg/day; p.o.). The positive control group received a single dose of cyclophosphamide (CF) (50 mg/kg; i.p.). The above values represent the mean and standard error for each group [a, b, c, d] (p<0.001, ANOVA followed by the Tukey-Kramer test). (a) positive control *vs* negative control; (b) *P. niruri vs* positive control; (c) *P. niruri* + cyclophosphamide *vs* negative control; (d) *P. niruri* + cyclophosphamide *vs P. niruri*.

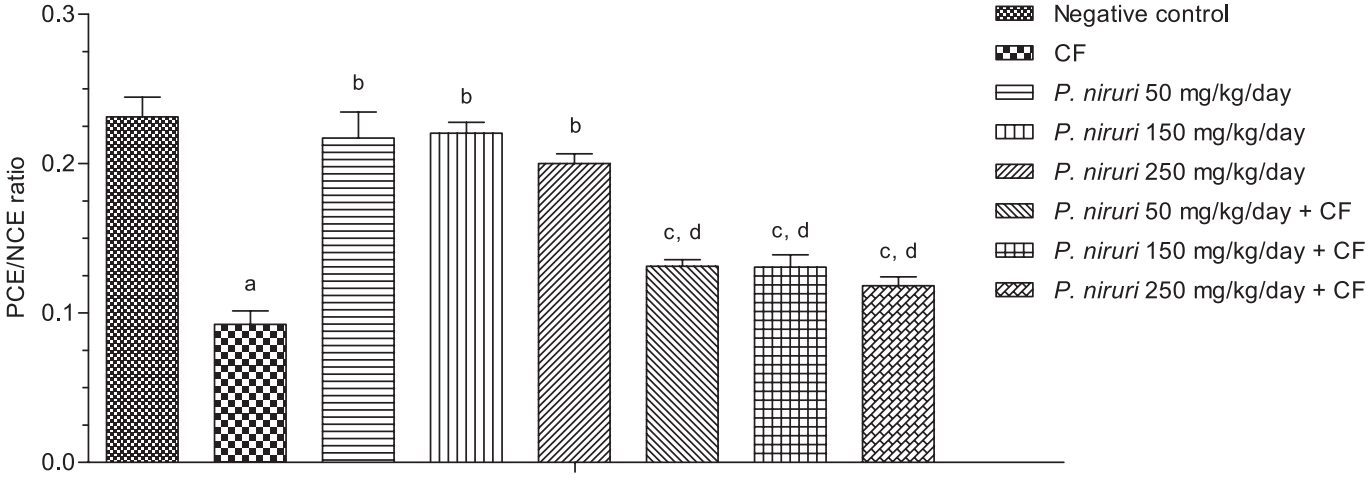

FIGURE 5 - Evaluation of the polychromatic/normochromatic ratio (PCE/NCE) in the bone marrow of rats (n=6/goup) exposed to different doses of dry *P. niruri* extract (50, 150 or 250 mg/kg/day; p.o.). The positive control group received a single dose of cyclophosphamide (CF) (50 mg/kg; i.p.). The above values represent the mean and standard error for each group [a, b, c, d] (p<0.001, ANOVA followed by the Tukey-Kramer test). (a) positive control *vs* negative control; (b) *P. niruri vs* positive control; (c) *P. niruri* + cyclophosphamide *vs* negative control; (d) *P. niruri* + cyclophosphamide *vs P. niruri*.

be mutagenic. In order to ensure the quality and safety of phytomedicines, the Brazilian National Agency of Sanitary Vigilance (ANVISA) published resolution no. 17 (Brazil, 2000) on 24 February 2000, decreed that they must be submitted to preclinical and clinical toxicological trials.

In this study general bone marrow toxicity, genotoxicity and cytotoxicity were assessed, using the micronucleus test in rats fed dry *P. niruri* L extract.

Nwanjo (2006) evaluated acute toxicity of aqueous extract of *P. niruri* leaves in Wistar rats after treatment with doses between 250 and 1,500 mg/kg of body weight (i.p.), obtaining a DL50 of 516.2 mg/kg. Asare *et al*. (2012) observed, in a study developed with adult Sprague Dawley rats, that an ethanolic extract obtained from *P. niruri* was not acutely toxic at 3,000 mg/kg. Studies in mice revealed low toxicity in the aqueous extract of *P. niruri*, intraperitoneally administered at a dose of 1.8 mg/kg/day, showing no body weight loss after three days of treatment and a slight weight gain after seven days of treatment (Venkateswaran, Millman, Blumberg, 1987). Tona *et al*. (2001) demonstrated that aqueous and ethanol extract of *P. niruri* orally administered to mice at a dose of 500 mg/kg of body weight, once a week, for 4 weeks, caused no toxic effects or deaths. In our study, we observed a slight reduction of water intake (Figure 3) in rats treated with doses of 150 and 250 mg/kg/day of *P. niruri* when compared to the control group. However, we found no alterations in body weight (Table I), in body weight gain (Figure 1) or food consumption (Figure 2) in animals treated with *P. niruri* extract, corroborating the data of the abovementioned studies. These data are insufficient to confirm or not the toxicity of chemical substances; however, basic parameters allow us to suggest or not their toxicity. Several other tests, such as the serum and/or plasma investigation of biochemical parameters, as well as histopathological, genotoxic, mutagenic and behavioral studies, are important for a more consistent determination of chemical compound toxicity. A study recently developed (Asare *et al*., 2012) observed that an ethanolic extract obtained from *P. niruri* whole plant was not toxic to adult rats treated during 90 days with 30 and 300 mg/kg. Hematological, biochemical or histopathological alterations were not observed in rats treated with the higher dose. However, the lower dose promoted lymphocyte reduction and drastic reduction was observed in most liver function test parameters (bilirubin, AST and ALT), suggesting some degree of hepatoprotection. Otherwise, the authors conclude that the *P. niruri* whole plant ethanolic extract is generally non-toxic. This data corroborates with the findings in the actual study where the three doses (50, 150 and 250 mg/kg) of the aqueous extract obtained from *P. niruri* dry leaves were sufficient to promote mild toxicity to rats treated during 30 days.

Antioxidant activity of *P. niruri* was reported by Harish and Shivanandappa (2006). Aqueous and methanol extracts at a dose of 100 mg/kg of body weight were cited as potent inhibitors of Fe^{2+} and ascorbate-induced microsomal lipid peroxidation; inhibitors of superoxide radical formation; and DPPH radical scavengers in *in vitro* studies. According to the authors, this antioxidant potential was responsible for inhibiting carbon tetrachloride-induced lipid peroxidation in rat livers. Shyamasundar e*t al*. (1985) demonstrated that chemical components such as phyllanthin and hypophyllanthin could protect against carbon tetrachloride (CCL_4) cytotoxicity in primary cultures of isolated hepatocytes.

The cytotoxicity of a substance can be assessed by the PCE/NCE ratio. According to Mavournin *et al*. (1990), the ratio between PCE and NCE frequency decreases when replacement of PCEs originating in erythroblasts is lowered. Results show that there was no statistically significant decrease in the PCE/NCE ratio in the groups treated only with dry extract of *P. niruri* compared to negative control in all doses ($p>0.05$). The PCE/NCE ratio obtained for each dose of extract was similar to that found for the negative control group and quite different from that observed in the positive control group (Figure 5). Moreover, dry extract demonstrated no protective action against cyclophosphamide-induced cytotoxicity in rat bone and the PCE/NCE ratio fell in groups treated with *P. niruri* and cyclophosphamide. In the present study dry extract of *P. niruri* exhibited no cytotoxic activity under the experimental conditions used.

MNs appear in daughter cells, due to damage induced in parental cells (Ribeiro, 2003). The two basic phenomena that result in MN formation in mitotic cells are chromosomal breakage and nondisjunction of the mitotic apparatus. MNs are formed from acentric or chromatid chromosomal fragments that are delayed in the anaphase and are not included in daughter cell nuclei in the telophase (Ford *et al*., 1988; Lindholm *et al*., 1991; Ford, Corel, 1992; Catalán, Falck, Norppa, 1973; Falck, Catalán, Norppa, 2002).

Formation of MNs in dividing cells is the result of chromosome breakage due to loss of repair or no repair of DNA lesions, or of poor segregation owing to nondisjunction in the mitotic apparatus. These events can be induced by oxidative stress, exposure to clastogenic or aneugenic agents, genetic defects in repair genes and/or cell cycle control, as well as deficiency in nutrients required as cofactors in DNA metabolism and chromosome segregation (Kimura *et al*., 2004; Umegaki, Fenech, 2000; Rajagopa-

lan *et al*., 2004; Macgregor, 2005; Fenech *et al*., 2005). All events causing MN formation, such as chromosomal rearrangements, gene expression changes or aneuploidy, are associated with chromosome instability generally observed in cancer (Rajagopalan *et al*., 2004; Fenech, 2002; Ames, Wakimoto, 2002).

As expected, our results corroborate data from the literature confirming the genotoxic effect of cyclophosphamide expressed by the increased frequency of micronuclei. Cyclophoshamide is an atineoplastic agent with alkylating properties, which indiscriminately complexes with DNA among normal and cancerous cells and is inactive until it is metabolized in the liver by mixed function oxidases of the cytochrome P-450 (Valadares, Castro, Cunha, 2007; Rang *et al*., 2003). The acute toxicity of cyclophosphamide is primarily associated with its genotoxicity (Krishna; Hayashi, 2000). In somatic cells, it provokes gene mutations, chromosomal aberrations, micronuclei and exchanges of sister chromatids in a variety of cell cultures with and without the presence of metabolic activation (Monteith, Vanstone, 1995; Elhajouji *et al*., 1994; Madle *et al*., 1986). Cyclophosphamide also damages chromosomes and micronuclei in the hematopoietic system of rats, mice and Chinese hamsters by producing highly reactive carbon ions (Moore *et al*., 1995).

Other species of the genus *Phyllanthus*, as *P. amarus* demonstrated antimutagenic properties. Sripanidkulchai *et al.* (2002) demonstrated that *P. amarus* protects against mutagenic effects of 2-aminofluorene, 2-aminoanthracene, 4-nitroquinoline 1-oxide, *N*-ethyl-*N*-nitro-*N*-nitrosoguanidine, 2-nitrofluorene and sodium azide in bacteria testing, as well as antagonize DNA damage caused by dimethylnitrosamine in hamster livers. Kumar and Kuttan (2004) reported that orally-administered aqueous extract of *P. amarus* offers radioprotection in rats by suppressing the formation of reactive oxygen species (ROS). This detoxifies species induced by radiation, increasing restoration and repair processes, accelerating hematopoietic recovery and possibly regulating the activity of response genes. The same authors (2005) also demonstrated the chemoprotective activity of 75% *P. amarus* methanolic extract induced by cyclophosphamide in mice. The extract significantly lowered myelosuppression and improved bone marrow cellularity. It also increased glutathione (GSH) and cellular glutathione-S-transferase (GST), reducing the toxic effects of cyclophosphamide metabolites in the cells. Asare *et al.* (2012) observed that an ethanolic extract obtained from *P. niruri* was not able to promote micronucleus at PCE bone marrow cells of adult rats treated with a single dose of 30 or 300 mg/kg. In this study, *N*-ethyl-*n*-nitrosourea, was employed as positive control. After 48h of treatment the animals were killed and bone marrow collected for evaluation of genotoxic and cytotoxic activities. The authors revealed that the extract adopted was not genotoxic or cytotoxic, corroborating with the data obtained in the actual study.

In all groups treated only with *P. niruri* extract, PCEMN frequency was similar to the spontaneous PCEMN rate of 3 micronuclei per 1000 PCEs (Rabello-Gay, 1991), found in the negative control group. These data suggest the extract does not exhibit mutagenic activity. Furthermore, our research with dry *P. niruri* extract in doses of 50, 150 and 250 mg/kg of body weight, with subchronic oral administration, different as observed in the studies mentioned above with *P. amarus* (Sripanidkulchai *et al.*, 2002; Kumar, Kuttan, 2004; Kumar, Kuttan, 2005) showed no protective activity in bone marrow cells against damage caused by cyclophosphamide. This was evidenced by the similar PCEMN frequency between the groups treated with the extract + cyclophosphamide and the positive control group. The different animal species employed in our study (rats) and the above mentioned studies (hamster and mice) as well as the plant species and the doses and treatment periods may explain the absence of effects against damage caused by cyclophosphamide.

CONCLUSIONS

The data revealed here suggest that subchronic oral administration of dry *P. niruri* extract to Wistar rats in the three different doses shows low toxicity and exhibits no genotoxic or cytotoxic potential in bone marrow cells, or chemoprotective properties against damage caused by cyclophosphamide. This favors the medicinal use of the plant in doses recommended for humans.

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