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Chemotherapeutical effects of the herbal medicine *Uncaria tomentosa* (Willd.) DC.

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Genet. Mol. Res. 16 (3): gmr16039782
Received July 21, 2017
Accepted August 30, 2017
Published September 27, 2017
DOI http://dx.doi.org/10.4238/gmr16039782

ABSTRACT. The use of medicinal plants dates back to the beginning of humanity, and today their application as complementary therapy has been widely disseminated as an alternative to conventional therapy. The medicinal plant named *Uncaria tomentosa* (Willd.) DC. (known as cat's claw) is a common woody vine of the Amazon forest that has traditionally been used in the treatment of arthritis because of its anti-inflammatory properties. This study aimed to evaluate the cytotoxic, mutagenic, and antimutagenic potentials of this medicinal plant. The biological activities of *U. tomentosa* were determined on bone marrow cells of Wistar rats that were treated *in vivo*. For the cytotoxic and mutagenic analyses, aqueous plant extract solutions were administered by gavage (1, 2, or 3 mg/mL) for 24 h (an acute treatment) or 7 days (a subchronic treatment). For the antimutagenic analyses, aqueous plant extract solutions (1 mg/mL) were administered by gavage before (pretreatment), simultaneous to (simultaneous treatment), or after (post-treatment), the administration of cyclophosphamide (1.5 mg/mL). *U. tomentosa* did not show any cytotoxic or mutagenic effects in any of the cytological or chromosomal analyses. Besides, the antimutagenic tests

showed that the plant extracts displayed antimutagenic activities, which significantly reduced the percentages of the chromosomal aberrations that were induced by cyclophosphamide at 53.91, 58.60, and 57.03%, respectively, for the simultaneous treatment, pretreatment, and post-treatment. The results suggested a safe use of this herbal medicine that is available free of charge from the Brazilian Public Health System for the treatment of arthritis. This medicinal plant can also effectively contribute to improving the quality of life and the recovery of people undergoing chemotherapeutical treatments.

Key words: Alternative therapies; Cat's claw; Chromosome aberration; Medicinal plant; Protective effects; Wistar rats

INTRODUCTION

Arthritis is a rheumatic, inflammatory, and systemic disease that is characterized by the following symptoms: joint pains, synovial inflammations with stiffness and swelling, fatigue, together with a limitation of physical functions (Choy and Panayi, 2001). Among the diseases that are denoted as being arthritis, there is rheumatoid arthritis, systemic lupus erythematosus, juvenile idiopathic arthritis, seronegative spondyloarthropathies, and osteoarthritis (Walsh et al., 2005), with rheumatoid arthritis being the most common and reaching about 1% of the world's population (Yang et al., 2013). The current treatments for these diseases are the use of some drugs, especially non-steroidal anti-inflammatory ones, which have a range of adverse events, such as gastrointestinal perforations and bleedings that require additional medical visits, diagnostic procedures, treatment, and hospitalizations. As a result, any alternative therapies for arthritis are of great interest (Smalley et al., 1995; MTSU - Medical Treatment Schedule Utilization, 2009).

Since ancient times, a widespread practice that has been adopted by the world's population is the use of medicinal plants as an alternative treatment for the prevention of various diseases, as they are believed to be effective, safe, and have been used in several cultures to treat patients for thousands of years (Dutra et al., 2016). In Brazil, species of the *Uncaria* genus have been used for more than 2000 years by the indigenous tribes to treat degenerative illnesses, inflammation, ulcers, and other harmful processes. *Uncaria tomentosa* (Willd.) DC., of the Rubiaceae family, is commonly used in commercial preparations (Valente, 2006; NIH - National Institute of Health, 2016). *U. tomentosa*, also known as cat's claw, is a common woody vine found in the Amazonian region that has traditionally been used for the treatment of arthritis (Valente, 2006; Caon et al., 2014). Many chemical constituents have already been identified in extracts of *U. tomentosa*, predominantly the alkaloids. Among these alkaloids are the tetracyclic oxindole and the indole alkaloids, as well as the oxindole glycosides and the triterpenes of quinovic acid, together with the flavonoids and the polyphenols (Lock et al., 2016). The oxindole glycosides and the triterpenes of quinovic acid have demonstrated anti-inflammatory and antiviral actions, together with the antioxidant compounds of tannins, catechins, polyphenols, and the procyanidins A, B1, B2 and B4, as well as the plant sterols that are also related to their anti-inflammatory properties (Williams, 2001; Heitzman et al., 2005; Cheng et al., 2007; Lock et al., 2016). Several studies have indicated that the *Uncaria* genus has a high

antimicrobial potential in pathogens such as *Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*, as well as in the yeasts of the *Candida* genus (Tay et al., 2014; Navarro-Hoyos et al., 2017). Furthermore, different extracts of *U. tomentosa* have presented antioxidant, anti-inflammatory, immunomodulating, antitumor, cytotoxic, genotoxic, and apoptotic activities (Pilarski et al., 2010; Caon et al., 2014; Kaiser et al., 2016).

Phytotherapy is the only therapeutical resource for a portion of the Brazilian population and also for more than two-thirds of the planet's population (Pinto et al., 2002) Currently, the World Health Organization has stimulated the use of medicinal plants in primary health care (WHO, 2013). In addition to these facts, the new National Policy of Medicinal and Phytotherapeutical Plants of the Brazilian Public Health System (SUS) that was introduced in the year 2006 can be added. Moreover, ever since 2010, this policy has allowed for the distribution of *U. tomentosa* tablets for the treatment of arthritis, free of charge to the Brazilian population. Therefore, it is of great interest to determine if the usages of this medicinal plant can be an alternative therapy that brings changes at cytological or chromosomal levels while aiming at the safety of the consumer for this particular product.

Studies involving the mutagenic and the antimutagenic potentials of medicinal plants are important, because these plants can be used as complementary treatments, without interfering with a normal lifestyle, or resulting in the ingestion of an excessive number of synthetic drugs (Düsman et al., 2013). Besides, the use of natural products is a growing trend in today's society. Thus, this study aimed at evaluating the cytotoxic, mutagenic, and antimutagenic potentials for a commercial presentation of an aqueous solution of *U. tomentosa* against the clastogenic effects of cyclophosphamide, a mutagenic chemotherapeutical agent, when using the bone marrow cells of Wistar rats, *in vivo*. Results presented here demonstrated the antimutagenic/protective effect of *U. tomentosa.*

MATERIAL AND METHODS

Treatment solutions

The phytotherapeutic Unha de Gato® was purchased from the Herbarium® Laboratory (Curitiba, Brazil) and is presented in capsules of 100 mg *U. tomentosa* dry extract (equivalent to 5.0 mg of total alkaloids calculated as mitraphylline). Its contents were extracted and diluted in water at three different concentrations (1, 2, and 3 mg/mL). Concentrations were chosen based on an extrapolation of the daily dose consumed by humans. The clastogenic drug cyclophosphamide (CP) was diluted with water at a concentration of 1.5 mg/mL and used as a positive control.

Wistar rat bone marrow cells

Six Wistar rats (*Rattus norvegicus*), three males and three females for each group, were obtained from the Central Vivarium of the State University of Maringá. Experiments were carried out using 35-day-old rats weighing approximately 0.1 kg body weight (bw).

Ethics statement

During the experimentation period, the animals remained under a controlled

temperature of approximately 25°C, with humidity at approximately 50% and with a photoperiod of a 12-h light/dark cycle. Furthermore, all Ethical Principles, Protocols, and Regulations on Experimentation with Laboratory Animals were used according to the standards established internationally and by the approved project by the Institutional Ethics Committee of State University of Maringá, the Ethics Committee on Animal Use in Experimentation (process No. PRO 520/2003), following the Ethical Principles for Animal Experimentation established by the Brazilian College of Animal Experimentation (COBEA), as well as the specific treatment and collection protocols made to chromosomal aberration tests.

Acute treatment

Wistar rats were treated via gavage with 1 mL of the solution treatment (1, 2, and 3 mg/mL per 0.1 kg bw). The control group (CO) received 1 mL water/0.1 kg bw, via gavage and the positive control received 1.5 mg/mL CP/0.1 kg bw, intraperitoneally. The animals were euthanized 24 h after the treatments with an intraperitoneal dose of 0.5 mL Thionembutal (1g sodium thiopental/25 mL distilled water).

Subchronic treatment

Wistar rats were submitted to subchronic treatment for 7 days. The control group received 1 mL water daily via gavage, and treatment groups received the same amount of *U. tomentosa* (1, 2, and 3 mg/mL). The rats were kept in their cages; food and water were replaced daily at the same time. On the eighth day, the rats were euthanized.

Antimutagenicity treatment

The rats used in the antimutagenic analysis received 1 mg/mL *U. tomentosa*/0.1 kg bw, via gavage, before (2 h pretreatment, PRE), simultaneously (simultaneous treatment, SIM), or after (2 h post-treatment, POST) the administration of 1.5 mg/mL CP/0.1 kg bw, intraperitoneally. The control group received 1 mL water/0.1 kg bw. Cyclophosphamide was administered as a positive control. All animals were euthanized 24 h after the treatments.

Chromosomal aberration test

The chromosomal aberration test was performed on the bone marrow cells from Wistar rats using the Ford and Hamerton (1956) method, with some modifications. The mitotic cells were interrupted in metaphase with the intraperitoneal administration of 0.5 mL colchicine/0.1 kg bw (0.16%), 90 min before euthanasia. The analysis of the slides was performed by a light microscope, analyzing 100 cells in metaphase per animal, totaling 600 cells each for the control and treatment groups. Chromosomes were assessed for the appearance of alterations, such as gaps, breaks, acentric fragments, and others. The mitotic index for the cytotoxicity evaluation was calculated from 5000 cells from each sex, totaling 10,000 cells per group. The mitotic index was calculated as a percentage, as follows: the number of dividing cells divided by the total number of cells present in the fields. The statistical calculation was performed using the chi-square test ($N = 6$, $\alpha = 0.05$).

RESULTS

In this study, an experimental evaluation of the cytotoxic effects for an aqueous solution of *U. tomentosa* on the bone marrow cells of Wistar rats that were treated *in vivo* was not observed in the mutagenicity test (acute: [1] $\chi^2 = 0.18$, [2] $\chi^2 = 0.00$, [3] $\chi^2 = 0.08$; subchronic: [1] $\chi^2 = 0.00$, [2] $\chi^2 = 0.02$, [3] $\chi^2 = 0.02$) or in the antimutagenicity test ([1] $\chi^2 = 0.06$, SIM $\chi^2 = 0.00$, PRE $\chi^2 = 0.07$, POST $\chi^2 = 0.00$). No statistically significant differences were observed between the treatments and the control group (Figure 1).

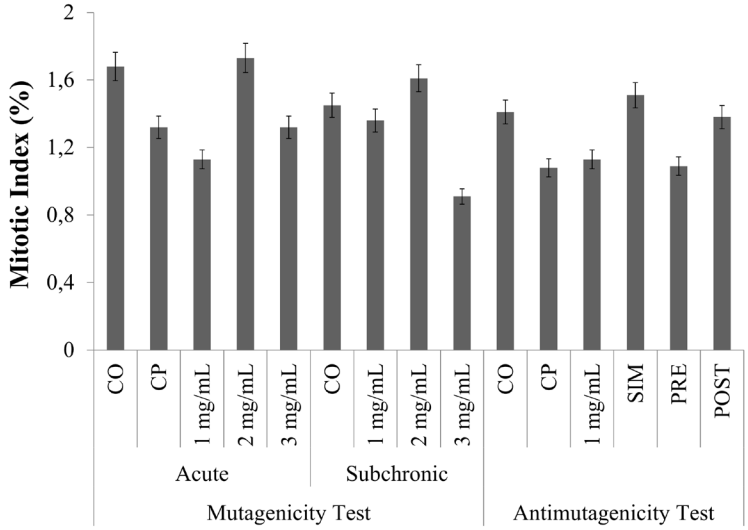

Figure 1. Mean percentage and standard deviation of the mitotic index for the mutagenicity and antimutagenicity tests with *Uncaria tomentosa* aqueous solution in Wistar rat bone marrow cells. Mutagenicity test - CO: control group (1 mL water/0.1 kg bw); CP: cyclophosphamide (1.5 mg/mL); *U. tomentosa* (1, 2, or 3 mg/mL *U. tomentosa*/0.1 kg bw). Antimutagenicity test - CO: control group (1 mL water/0.1 kg bw); CP: cyclophosphamide (1.5 mg/mL); and *U. tomentosa* (1 mg/mL *U. tomentosa*/0.1 kg bw), in simultaneous treatment (SIM), pretreatment (PRE), and post-treatment (POST) cyclophosphamide. Acute treatment: 24-h exposure; subchronic treatment: 7-day exposure.

In the mutagenicity test, the potential for an aqueous solution of *U. tomentosa* to induce chromosomal aberrations was also evaluated. Additionally, in the antimutagenicity test, the antimutagenic and protective or reductive effects of this aqueous solution on the cyclophosphamide-induced clastogenicity on bone marrow cells of Wistar rats were also evaluated. The major chromosomal abnormalities that were found in analyses of the metaphases were chromatic (*cb*) or isochromatic (*ib*) breaks, chromatic (*cg*) or isochromatic (*ig*) gaps, acentric fragments (*af*), together with minutes (*mn*) and double minutes (*dm*).

The chemotherapeutical drug cyclophosphamide, which is highly clastogenic and pro-oxidant, despite the lack of cytotoxicity that was found in this study (mutagenicity and antimutagenicity: CP $\chi^2 = 0.08$; Figure 1), was effective in inducing chromosomal damage in both tests when compared to control (mutagenicity: CP $\chi^2 = 499.59$; antimutagenicity: CP $\chi^2 = 1{,}336.36$; Figure 2). The aberrations that were observed in these treatments were: 6 *cg* + 46 *cb* + 27 *af* for the mutagenicity test and 29 *cg* + 4 *ig* + 16 *cb* + 14 *ib* + 20 *af* + 30 *mn* + 9 *dm* for the antimutagenicity test.

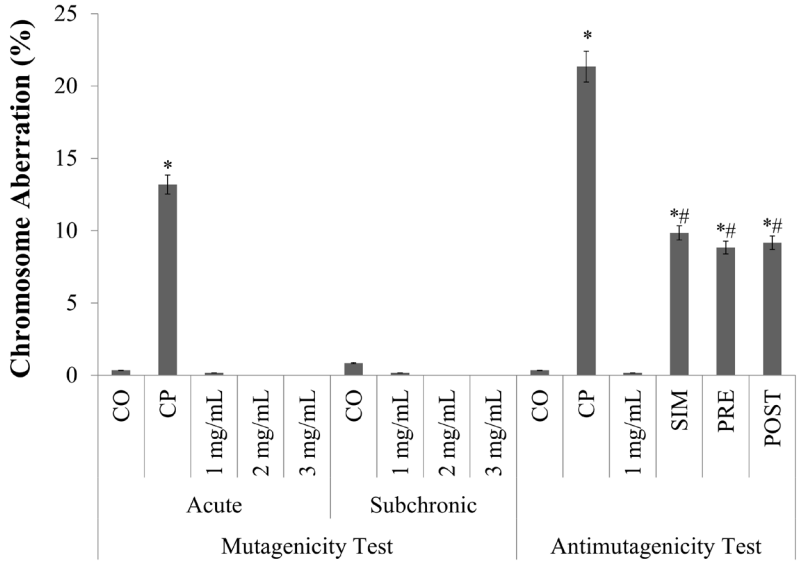

Figure 2. Chromosomal aberration (mean percentage and standard deviation) for the mutagenicity and antimutagenicity tests with *Uncaria tomentosa* aqueous solution in the bone marrow cells of Wistar rats. Mutagenicity test - CO: control group (1 mL water/0.1 kg bw); CP: cyclophosphamide (1.5 mg/mL); *U. tomentosa* (1, 2, and 3 mg/mL *U. tomentosa*/0.1 kg bw). Antimutagenicity test - CO: control group (1 mL water/0.1 kg bw); CP: cyclophosphamide (1.5 mg/mL); and *U. tomentosa* (1 mg/mL *U. tomentosa*/0.1 kg bw, in simultaneous treatment (SIM), pretreatment (PRE), and post-treatment (POST) cyclophosphamide. Acute treatment: 24-h exposure; subchronic treatment: 7-day exposure. ∗Results were statistically significant compared to CO ($P < 0.05$). #Results were statistically significant compared to CP ($P < 0.05$).

Figure 2 also shows the percentages of the chromosomal aberrations that were found for those animals that were treated with the three concentrations of *U. tomentosa*. These concentrations showed no mutagenic potentials and were statistically similar to control (acute: [1] $\chi^2 = 0.09$, [2] $\chi^2 = 0.33$, [3] $\chi^2 = 0.33$; subchronic: [1] $\chi^2 = 0.54$, [2] $\chi^2 = 0.83$, [3] $\chi^2 = 0.83$). In the antimutagenicity test, the results showed that the plant solutions significantly reduced the percentages of damage that were induced by cyclophosphamide (SIM $\chi^2 = 6.20$; PRE $\chi^2 = 7.32$; POST $\chi^2 = 6.94$). Cyclophosphamide-induced damage with *U. tomentosa* was reduced by 53.91% for the simultaneous treatments, 58.60% for the pretreatments and 57.03% for the post-treatments. However, none of the treatments were statistically equivalent to control (SIM $\chi^2 = 273.48$; PRE $\chi^2 = 218.94$; POST $\chi^2 = 236.27$). The chromosomal aberrations that were found when using the *U. tomentosa* treatments were represented by SIM: 12 *cg* + 2 *ig* + 11 *cb* + 8 *ib* + 13 *af* + 11 *mn* + 3 *dm*; PRE: 8 *cg* + 4 *ig* + 16 *cb* + 5 *ib* + 8 *af* + 8 *mn* + 4 *dm*; and POST: 12 *cg* + 4 *ig* + 14 *cb* + 7 *ib* + 7 *af* + 7 *mn* + 4 *dm*.

DISCUSSION

When considering the different biological effects that are exerted by medicinal plants, the present study aimed at identifying the cytotoxic, mutagenic, and antimutagenic properties of an aqueous solution of *U. tomentosa* administered via gavage in Wistar rats. The mitotic

index was adopted as the toxicity reference, and the different results showed no cytotoxic effects. Corroborating with these results are the studies of Santa Maria et al. (1997) who did not observe cytotoxic activities of *U. tomentosa* aqueous extracts (10-100 mg/mL) in prokaryotic (*Photobacterium phosphoreum*) and eukaryotic cells (Chinese hamster ovary cells) when using different tests. Likewise, Sheng et al. (2000) investigated the toxic effects of aqueous extracts of *U. tomentosa* bark in female W/Fu rats and found that these extracts represented no chronic toxicity signs with LD_{50} of 48 g/kg after oral administrations of the extract at the dosages of 10-80 mg/kg *U. tomentosa*/day for 8 weeks. Moreover, no body weight, food consumption, organ weight, together with kidney, liver, spleen, and pathological heart changes, were found to be associated with the aqueous extract treatments in a human clinical study (Lamm et al., 2001; Sheng et al., 2001). However, Kaiser et al. (2016) found cytotoxicity for cultured human lymphocytes that were treated with different extracts of *U. tomentosa*. In this particular case, the authors suggested that the cytotoxicity was related to the presence of alkaloids, quinovic acid glycosides, and polyphenols, among the separate components.

The cytotoxicity of this plant might also be related to an induction of apoptosis. Organic extracts of *U. tomentosa* have shown high toxicity, together with inductions of apoptosis in human lymphoma cells, by an increased oxidative stress, cytochrome c release, and by caspase activations (Cheng et al., 2007). Furthermore, oxindole alkaloids isolated from this plant also showed cytotoxic and pro-apoptotic activities in promyelocytic leukemia cells (HL-60) (Kaiser et al., 2016).

The mutagenicity tests as performed in the present study also showed that an aqueous solution of *U. tomentosa* presented no mutagenic potential. These results were confirmed by Immich (2011) who did not find a mutagenic activity of crude ethanolic extracts and fractions that were isolated from *Saccharomyces cerevisiae*. Additionally, Kaiser et al. (2016) did not find mutagenicity by micronucleus tests, nor genotoxicity as revealed by the comet assay of human lymphocytes that were treated with alkaloids extracted from this herbal medicine. Previous studies performed with *U. tomentosa* preparations from stem bark also revealed a weak genotoxic potential (Romero-Jiménez et al., 2005; Immich, 2011).

When considering that no mutagenic effects of *U. tomentosa* were observed, the antimutagenic/protective potentials of this medicinal plant were verified by a reduction of the induced damage by the chemotherapeutical and clastogenic agent, cyclophosphamide. In the present study, the simultaneous treatment, the pretreatment, and the post-treatment, significantly reduced the percentages of the chromosomal aberrations that were induced by this mutagen. However, these reductions were not statistically significant when compared to control. Similarly, Caon et al. (2014) observed the protective effects of alkaloid extracts from *U. tomentosa* and *U. guianensis* by a comet assay on cultured human fibroblasts. The authors observed that simultaneous treatments and pretreatments reduced the percentages of DNA fragmentation that were induced by ultraviolet light (UV-C), whereas the post-treatments were not as effective. The protective effects were correlated to the antioxidant activities of the extracts from these plants.

Based on the mechanisms of action of the mutagen cyclophosphamide, it is possible that the protective effects that have been observed in the antimutagenicity tests were related to the antioxidant activities of *U. tomentosa*. These mechanism activities were probably responsible for the protective effects that were observed in the simultaneous treatment and the pretreatment, since the bioactive components of this plant can capture reactive oxygen species, and thus, reducing the DNA-induced damage. The antioxidant activities of *U. tomentosa*

have been described by Sandoval et al. (2000). On the other hand, in the post-treatments, it was possible that an aqueous solution of *U. tomentosa* activated the mechanisms of DNA-repair, correcting most of the induced damage. This has been supported by increments in the DNA-repair system after an exposition to hydrogen peroxide that was observed in a clinical trial in which the subjects were supplemented (350 mg/day for 8 weeks) with a commercial formulation of *U. tomentosa* (Sheng et al., 2001). It is likely that both the mechanisms of protection and repair of damages that were observed in the antimutagenicity tests, as described by Kada et al. (1978) and Kuroda et al. (1992), have contributed to the antimutagenic effects of this medicinal plant, which presents, despite the recognized anti-inflammatory activities, chemoprotective effects against the clastogenic actions of cyclophosphamide.

CONCLUSIONS

The results presented in this study at the chromosomal and cytological levels have suggested antimutagenic/protective effects of an aqueous solution of *U. tomentosa* when concerning the chemotherapeutical agent, cyclophosphamide. These outcomes have revealed a reduction of more than 50% in the DNA-induced damage as was observed for the simultaneous treatment, the pretreatment, and the post-treatment. The herbal medicine *U. tomentosa* is distributed free of charge by the public health system of Brazil, as an alternative therapy for the treatment of arthritis. This medicinal plant can also effectively contribute to improving the quality of life and the recovery of people undergoing chemotherapeutical treatments. In addition to this, we can add those who have been exposed to environmental mutagens or in assisting those looking to maintain healthy and preventive habits. Moreover, based on the data obtained here, we can suggest that the biotechnological production of drugs, based on this medicinal plant *Uncaria tomentosa*, can improve the chemoprotective activities of this plant.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors would like to thank the staff of the Mutagenesis and Environmental Monitoring laboratory and the State University of Maringá.

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