Assessment of the mutagenic and antimutagenic activity of *Synadenium umbellatum* Pax latex by micronucleus test in mice

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Received November 10, 2009 – Accepted March 9, 2010 – Distributed February 28, 2011

Abstract

Synadenium umbellatum Pax, popularly known as "cola-nota", is a medicinal plant that grows in tropical regions. The latex of this plant is used against various diseases, such as diabetes mellitus, leprosy, tripanosomiasis, leukemia, and several malignant tumors. The mutagenic, antimutagenic, and cytotoxic effects of the latex of this plant were investigated by measuring the frequency of micronuclei in mice bone marrow cells. To evaluate mutagenicity, the animals were treated with four doses of latex (10, 30, 50, and 100 mg/kg body weight). To study the antimutagenic activity, the animals were simultaneously treated with latex and mitomycin C (4 mg/kg). The cytotoxicity was evaluated by polychromatic and normochromatic erythrocytes ratio. Our results showed a significant increase of frequency of micronucleated polychromatic erythrocytes (MNPCE) compared to the negative control group ($p < 0.05$). Concerning antimutagenicity, the doses of 10 and 30 mg/kg co-administered with mitomycin C showed significant decrease in MNPCE frequency compared to the positive control group ($p < 0.05$). However, no significant reduction in MNPCE frequency ($p > 0.05$) was detected at the doses of 50 and 100 mg/kg. Under our experimental conditions, the results obtained indicate strong mutagenic and cytotoxic activity of *S. umbellatum* latex except the dose of 10 mg/kg and moderate antimutagenic effect at lower doses.

Keywords: medicinal plant, genotoxicity, antigenotoxicity, cytotoxicity, bone marrow, in vivo test.

Avaliação da atividade mutagênica e antimutagênica do látex de *Synadenium umbellatum* Pax pelo teste do micronúcleo em camundongos

Resumo

Synadenium umbellatum Pax, popularmente conhecida como "cola-nota", é uma planta medicinal que cresce em regiões tropicais. O látex desta planta tem sido utilizado no tratamento de várias doenças, como diabetes mellitus, hanseníase, tripanossomíases, leucemia e vários tumores malignos. Os efeitos mutagênico, antimutagênico e citotóxico do látex dessa planta foram investigados pela mensuração da frequência de micronúcleos em células de medula óssea de camundongos. Para avaliar a mutagenicidade, os animais foram tratados com quatro doses do látex (10, 30, 50 e 100 mg/kg). Para o estudo da atividade antimutagênica, os animais foram tratados com o látex e mitomicina C (4 mg/Kg) simultaneamente. A citotoxicidade foi avaliada pela razão de eritrócitos policromáticos e normocromáticos. Nossos resultados mostraram um aumento significativo da frequência de eritrócitos policromáticos micronucleados (EPCMN) em relação ao grupo controle negativo ($p < 0,05$). Na avaliação da atividade antimutagênica do látex, as doses de 10 e 30 mg/kg coadministradas com mitomicina C mostraram uma diminuição significativa na frequência de EPCMN comparada com o grupo controle positivo ($p < 0,05$). Entretanto, não houve redução significativa na frequência de EPCMN ($p > 0,05$) detectada nas doses de 50 e 100 mg/kg. Nas nossas condições experimentais, os resultados obtidos indicaram forte atividade mutagênica e citotóxica do látex do *S. umbellatum*, com exceção na dose de 10 mg/kg e moderado efeito antimutagênico nas doses mais baixas.

Palavras-chave: planta medicinal, genotoxicidade, antigenotoxicidade, citotoxicidade, medula óssea, teste in vivo.

1. Introduction

Brazilian traditional or popular medicine has long been making use of plants and plant-derived drugs or phytomedicines to treat a wide range of health problems. In conformity with this common knowledge, some plants have demonstrated interesting properties, such as *Annona crassiflora* Mart. (Vilar et al., 2008), *Solanum lycocarpum* St. Hil. (Costa et al., 2005), *Phaseolus vulgaris* (Ribeiro and Salvatori, 2003), and *Stryphnodendron adstringens* (Mart.) Coville (Andrade et al., 2006), since they were able to reduce the incidence of DNA damage. Nevertheless, herbal medicines can be potentially toxic to human health. Recent investigations have revealed that many plants used in folk medicine are potentially genotoxic, such as *Cochlospermum regium* (Schrank) Pilg. (Castro et al., 2004; Andrade et al., 2008), *Ocotea duckei* Vatt. (Marques et al., 2003), *Copaifera langsdorfii* Desfon (Chen-Chen and Sena, 2002), *Paullinia cupana* Kunth (Fonseca et al., 1994), and the authors suggest that their use must be well established.

Even among antimutagen compounds, great care should be taken because a number of substances reported to be antimutagens or anticarcinogens have been shown to be mutagenic or carcinogenic themselves (Zeiger, 2003). Due to this, it is extremely important to test the genotoxicity of these preparations in order to assess their mutagenic potential or genotoxicity modulation when associated with other substances (Silva et al., 1995; Di Stasi et al., 2002). Plants are known to contain innumerable biologically active compounds, and although they present pharmacological properties, they may also cause harm, including damage to DNA (Alade and Irobi, 1993; Marques et al., 2003). Thus, the investigation of traditionally-used medicinal plants is valuable for two main reasons: first, to use them as sources of potential chemotherapeutic drugs; and second, as a measure of safety of continuous use of medicinal plants (Verschaeve et al., 2004).

Synadenium umbellatum Pax (Euphobiaceae) (synonyms: *Euphorbia umbellata* and *Synadenium grantii* Hook), popularly known as "cola-nota", "avelós", "milagrosa", and "cancerola", is a medicinal plant that grows in tropical regions, both in the American and African continents. The latex of this plant is used against various diseases such as diabetes mellitus, Hansen´s disease, tripanosomiases, leukemia, and several malignant tumors (Ortêncio, 1997).

Phytochemical studies of members of the family Euphorbiaceae revealed the presence of flavonoids, saponins, diterpenes, and phorbol esters (Bagalkotkar et al., 2006; Jassbi, 2006). Also, phorbol esters (Kinghorn, 1980), lectins (Premaratna et al., 1981), glycoproteins (Rajesh et al., 2006), diterpene esters (Bagavathi et al., 1988), and triterpenoids (Uzabakiliho et al., 1987) were identified in SuL and have been proven to be associated with its pharmacological properties.

In popular medicine, the latex of plants belonging to the genus *Synadenium* has been considered caustic and toxic. Several studies have shown the presence of toxic substances and proteolytic enzymes in the latex of this genus *Synadenium* (Govindappa et al., 1987; Jäger et al., 1996; Menonn et al., 2002).

The mutagenic, cytotoxic, toxic, antitumoral, and antiangiogenic action of *S. umbellatum* leaves have already been shown (Nogueira et al., 2008; Oliveira et al., 2005; Valadares et al., 2007). Recently, in our studies, we have reported the angiogenic activity of the SuL (Reis et al., in press). In other species of this genus, the anti-inflammatory activity has already been identified (Jäger et al., 1996).

Due to the large utilisation of plant latex as well as the relevant pharmacological activities of this substance already described, the goal of the present work was to evaluate the possible mutagenicity, cytotoxicity and antimutagenicity of *S. umbellatum* latex (SuL) using mice bone marrow micronucleus test.

2. Material and Methods

2.1. Synadenium umbellatum latex (SuL)

SuL was collected in Goiânia (16° 37' 40.94" S and 49° 16' 13.41" W), state of Goiás, in the midwestern region of Brazil, in November 2007. A voucher specimen was deposited at the Herbarium of the Universidade Federal de Goiás under the number 40.006/UFG. We extracted the sap through incisions in the trunk, at the height of 100 cm (3.28 feet) in relation to the soil. The secretory cells drained and 1.0 mL of this latex was collected directly in a sterile plastic syringe and immediately transferred to a sterile glass container with 9 mL of sterile distilled water. This material was stocked at 4 °C and used within 30 days (Mendonça, 2004; Mrué, 1997). The density of the pure latex was 1 g/mL and it was diluted with distilled water just before use.

2.2. Animals

The experimental protocol (CEPMHA/HC/UFG no. 037/2008) was approved by the Human and Animal Research Ethics Committee of the Universidade Federal de Goiás. Male Swiss albino (*Mus musculus*) mice (8-12 week-old) weighing 35 ± 10 g were obtained from the Central Animal House of the Universidade Federal de Goiás and kept in polyethylene cages (40 cm x 30 cm x 16 cm) with husk bedding, in an air-conditioned room (24 ± 2 °C; 55 ± 5% relative humidity), with a 12-hours light-dark cycle, and free access to drinking water and food (appropriate commercial rodent diet Labina, Ecibra Ltda).

2.3. Drugs and reagents

Mitomycin C (MMC), acquired from Bristol-Myers Squibb, was used in the evaluation of antimutagenicity of the plant latex. Giemsa was obtained from Doles Reagentes e Equipamentos para Laboratórios in Goiânia. Methanol was obtained from Labsynth Produtos para Laboratórios and fetal calf serum from Laborclin Produtos para Laboratórios. Dibasic sodium phosphate and monobasic sodium phosphate were purchased from Sigma-Aldrich Chemical Company.

2.4. Experimental design

To assess SuL mutagenicity, doses of 10, 30, 50, and 100 mg/kg body weight (bw) were administered by intraperitoneal injection (ip) to groups of five animals for each treatment. A positive control (MMC) (4 mg/kg bw) and a negative control (distilled sterilised water) were also included. Mice were euthanised by cervical dislocation 24 hours after SuL administration.

To evaluate antimutagenicity, four animal groups of five animals each were co-treated by ip with 10, 30, 50, and 100 mg/kg bw of SuL and 4 mg/kg bw of MMC and euthanised by cervical dislocation 24 hours after treatment. We used the same positive control (MMC) and negative control (H_2O) groups employed in the mutagenicity evaluation.

For both experiments, after the period of treatment (24 hours), mice femurs were dissected, opened, the bone marrow was gently flushed out with fetal calf serum, and centrifuged (300 g, 5 minutes). The bone marrow cells were smeared on glass slides, coded for blind analysis, air-dried, and fixed with absolute methanol for 5 minutes. To detect MNPCE frequency, we fixed the smears with Giemsa (1:30) (Heddle, 1973), prepared two slides for each mouse, and scored 1000 polychromatic erythrocytes (PCE) per slide. The results were the average of two slides. To determine the cytotoxic activity, we simultaneously computed 1000 normochromatic erythrocytes and the polychromatic erythrocytes frequency.

2.5. Statistical analysis

In order to analyze the mutagenic activity of SuL, we compared the MNPCE frequencies obtained for the treated groups and the negative control group using one way analysis of variance (ANOVA- I), followed by a multiple comparison procedure (Tukey test). P-values lower than 0.05 ($p < 0.05$) were considered indicative of statistical significance.

To analyse SuL antimutagenicity, we compared MNPCE frequencies observed in the treated groups and the positive control group by ANOVA-I followed by the Tukey test. P values lower than 0.05 ($p < 0.05$) were considered indicative of statistical significance.

To evaluate the cytotoxicity of SuL, the polychromatic erythrocytes/normochromatic erythrocytes ratio (PCE/NCE) of all treated groups was compared to the result obtained in the mutagenic effect evaluation for the negative control group, and the results found in the antimutagenic effect evaluation for the positive control, using qui-square test (χ^2). P values lower than 0.05 ($p < 0.05$) were considered indicative of statistical significance.

3. Results

The results obtained for mice treated with different concentrations of SuL as well as SuL in combination with MMC are shown in Table 1. No significant difference in the frequency of MNPCE was observed between mice treated with 10 mg/kg of SuL and the negative control ($p > 0.05$). A high increase in the frequency of MNPCE was detected in mice treated with 30, 50, and 100 mg/kg of SuL compared to the negative control ($p < 0.05$). Also, no significant difference was observed in MNPCE induction between the group treated with 100 mg/kg of SuL and the positive control ($p > 0.05$).

Simultaneous treatments with different concentrations of SuL and MMC led to reduction in the frequency of MNPCE compared to MMC alone, which was only significant for the treatments using 10 and 30 mg/kg of SuL with the addition of MMC ($p < 0.05$), corresponding to decreases of 71.8% and 37.2% in the frequency of MNPCE, respectively. By contrast, no significant reduction was observed for the treatments combining higher concentrations of SuL (50 and 100 mg/kg) and MMC. These results indicate lack of a dose-response correlation, since the lowest concentration of SuL was found to be effective and a gradual increase in concentration did not result in a proportional increase in the reduction of mutagenicity.

No significant differences in the PCE/NCE ratio were observed when comparing mice treated with 10 mg/kg of SuL and the respective negative control ($p > 0.05$), whereas a significant reduction in this ratio was found in mice treated with 30, 50, and 100 mg/kg of SuL, indicating that these doses were cytotoxic to mice bone marrow ($p < 0.05$), as shown in Table 1. Also no significant difference was detected between the PCE/NCE ratio obtained for mice treated with 30, 50, and 100 mg/kg of SuL with the addition of MMC and those treated with SuL or MMC alone ($p > 0.05$). Nonetheless, a significant increase in the PCE/NCE ratio was observed in mice treated simultaneously with 10 mg/kg of SuL and MMC compared to those treated with MMC alone ($p < 0.05$).

4. Discussion

The present results show that the higher concentrations of SuL used (30, 50, and 100 mg/kg) provoked a mutagenic effect on erythroblasts in the bone marrow. We also detected a dose-response relation among concentration of SuL and magnitude of mutagenicity, and cytotoxity. Increased SuL concentrations resulted in increased MNPCE and reduced PCE/NCE ratios. This fact might be explained, at least partially, by the mutagenic action of SuL that resulted in cell death. The similar results were observed in the others works (Lee and Lee, 2007; Rodrigues et al., 2009).

Valadares et al. (2007) demonstrated the mutagenic activity of *S. umbellatum* leaf extract using the micronucleus test in mice, in which the extract increased the frequency of MNPCE when compared to the negative control group. The authors also showed a dose-response relation, although not proportional to increase in dose.

On the other hand, in the present work, a simultaneous treatment with the lowest concentrations of SuL (10 and 30 mg/kg) and MMC revealed a protective effect against chromosome damage induced by MMC. Although, this work did not provide the exact mechanism of action against the genotoxic effects of MMC, it may be explained, at

Table 1. Frequency of MNPCE and PCE/NCE ratio in mice treated with *S. umbellatum* latex and/or MMC.

Treatment (mg/kg)	No. of animals	MNPCE				PCE/NCE
		Individual data[a]	No.	%	Mean ± SD[a]	
Distilled water	5	2-3-3-2-1	11	0.22	2.2[c] ± 0.83	0.97[c]
MMC (4 mg/kg)	5	18-13-14-15-18	78	1.56	15.6[b] ± 2.30	0.35[b]
SuL (mg/kg)						
10	5	3-2-4-2-2	13	0.26	2.6[c] ± 0.89	0.89[c]
30	5	7-6-5-9-7	34	0.68	6.8[b,c] ± 1.48	0.69[b,c]
50	5	11-8-10-10-9	48	0.96	9.6[b,c] ± 1.14	0.54[b,c]
100	5	15-11-10-12-16	64	1.28	12.8[b] ± 2.58	0.36[b]
SuL + MMC						
10 + 4	5	6-4-5-4-3	22	0.44	4.4[b,c] ± 1.14	0.73[b,c]
30 + 4	5	12-7-9-9-12	49	0.98	9.8[b,c] ± 2.16	0.41[b]
50 + 4	5	16-13-11-13-10	63	1.26	12.6[b] ± 2.30	0.36[b]
100 + 4	5	20-19-13-17-18	87	1.74	17.4[b] ± 2.70	0.32[b]

[a]Per 1000 polychromatic erythrocytes per mouse. [b]Statistically different from the negative control (distilled water) ($p < 0.05$). [c]Statistically different from the positive control (MMC) ($p < 0.05$).

least partially, by the reduction of alkylation and/or the antioxidant actions exerted by SuL, since the genotoxic action of MMC is related to its ability to alkylate DNA and produce reactive free radicals. Studies have shown that the 2-amino group of guanine is the site of alkylation by the MMC (Kang et al., 2006). Thus, SuL action might be related to the protection of the nucleophilic site in DNA.

In this study, we observed an increase in the PCE/NCE ratio compared to the positive control (MMC) at the dose of 10 mg/kg co-treated with MMC. This fact indicated that the lowest concentration of SuL with the addition of MMC reduced the cytotoxic effect of the latter. No difference in the PCE/NCE ratio was observed among the doses of 30, 50, and 100 mg/kg of SuL with the addition of MMC and MMC alone. It has already been reported that the association of MMC with other antitumoral agents cause an increase in their cytotoxic effect due to an increase in apoptosis induction (Estrem and Vanleeuwen, 2000; Kraut and Drnovsek-Olup, 1996), but in our study, this was not detected. By contrast, we observed that there was no increase in cytotoxicity when using SuL co-administered with MMC, which might be explained by a simultaneous mutagenic and antimutagenic action of SuL at the concentrations of 30, 50 and 100 mg/kg.

It is well established that many substances reported to be mutagens or carcinogens have themselves been shown to be antimutagenic or anticarcinogenic (Zeiger, 2003). Phorbol esters are substances that present these properties (Jongen et al., 1986; Emerit and Lahoud-Maghani, 1989). As the species *S. umbellatum* presents phorbol esters (Kinghorn, 1980), the mutagenic and antimutagenic effects exhibited by this plant can thus be associated, at least partially, to these components.

5. Conclusion

In the present work, the mutagenic, cytototoxic and antimutagenic activities of *S. umbellatum* latex were evaluated by the mice bone marrow micronucleus test and the results showed that this latex presents the mutagenic and cytotoxic activity in higher doses and antimutagenic action in lower doses.

Acknowledgements – We are grateful to the sponsors of this research project: Fundação de Amparo à Pesquisa do Estado de Goiás (FAPEG-GO) and Fundação de Apoio à Pesquisa da UFG (FUNAPE-UFG).

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