# Assessment of mutagenicity and cytotoxicity of *Solanum paniculatum* L. extracts using in vivo micronucleus test in mice

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## Abstract

*Solanum paniculatum* L. is a plant species widespread throughout tropical America, especially in the Brazilian Savanna region. It is used in Brazil for culinary purposes and in folk medicine to treat liver and gastric dysfunctions, as well as hangovers. Because of the wide use of this plant as a therapeutic resource and food, the present study aimed at evaluating the mutagenic and cytotoxic effects of *S. paniculatum* ethanolic leaf and fruit extracts using the mouse bone marrow micronucleus test. Our results indicate that neither *S. paniculatum* ethanolic leaf extract nor its ethanolic fruit extract exhibited mutagenic effect in mice bone marrow; however, at higher doses, both extracts presented cytotoxic activity.

*Keywords:* mutagenicity, cytotoxicity, *Solanum paniculatum* L., micronucleus, mice.

## Avaliação da mutagenicidade e citotoxicidade de extratos de *Solanum paniculatum* L. usando o teste do micronúcleo in vivo em camundongos

## Resumo

*Solanum paniculatum* L., popularmente conhecida como jurubeba, ocorre em toda a América tropical, especialmente no Cerrado. No Brasil, é utilizada para fins culinários e na medicina popular para o tratamento de distúrbios gástricos e hepáticos, além de ressacas. Devido à grande utilização desta planta pela população como recurso terapêutico e alimentício, o presente estudo teve como objetivo avaliar as atividades mutagênica e citotóxica dos extratos etanólico das folhas e frutos de *S. paniculatum* utilizando o teste do micronúcleo em medula óssea de camundongos. Os resultados indicam que os extratos etanólicos tanto das folhas quanto dos frutos de *S. paniculatum* não apresentaram ação mutagênica em medula óssea de camundongos, porém, em doses mais elevadas, ambos os extratos exibiram atividade citotóxica.

*Palavras-chave:* mutagenicidade, citotoxicidade, *Solanum paniculatum* L., micronúcleo, camundongos.

## 1. Introduction

The medicinal use of plants for treating various disorders in humans and in their animals has been a tradition for centuries in many cultures (Vermani and Garg, 2002). The possible benefit of plant-derived medications constitutes a rewarding area of research, particularly in countries such as Brazil, which have a rich biodiversity of natural plant resources coupled with a high prevalence and variety of infectious diseases (Brandão et al., 2008). According to their traditional use, natural compounds are often assumed to be safe. However, several studies have reported that a great number of plant species used as food ingredients or in traditional medicine present mutagenic, carcinogenic, or toxic properties (Ferreira and Vargas, 1999; Déciga-Campos et al., 2007; Mohd-Fuat et al., 2007). For this reason, the identification and characterisation of these compounds and the definition of their mutagenic and carcinogenic effects can lead to important strategies to reduce the risk of cancer in human beings. Therefore, plants exhibiting clear mutagenic properties should be considered as potentially unsafe and they certainly require further testing before being recommended (Verschaeve and Van Staden, 2008).

A variety of in vitro and in vivo tests are available for evaluating early genetic damage induced by xenobiotics, among which we can cite mainly the micronucleus assay in rodents (Morita et al., 1997). The in vivo mice micronucleus test is widely used for detection of cytogenetic damage (Morita et al., 1997) and this assay also presents some

advantages compared to other methods, such as low cost and high reliability. This test also identifies several plant extracts with clastogenic and/or aneugenic activity (Schmid, 1975; Hayashi et al., 1990). Plants with genotoxic activity detected by the micronucleus test, e.g. *Cochlospermum regium* ( Schrank) Pilg. (Andrade et al*.,* 2008), or cytotoxic activity, e.g. *Annona crassiflora* Mart. (Vilar et al*.,* 2008), should be considered with some circumspection.

*Solanum paniculatum* L. (Solanaceae) is a neotropical weed very common in the Brazilian Cerrado, used in folk medicine and for culinary purposes. Many species of the genus *Solanum* are known by the local people as "jurubeba", but the species *S. paniculatum* L. is described as the true "jurubeba" (Corrêa, 1984). The tea prepared with the leaves of "jurubeba" is a very common household remedy used throughout Brazil for hangovers (Sabir and Rocha, 2008), and the extracts of all parts of this plant are mentioned in Brazilian phytomedicine formularies as traditionally employed to treat bronchitis, coughs, arthritis, anemia, hepatitis, intestinal parasites, and stomach disorders (Coimbra, 1958; Silva, 1977; Corrêa, 1978; Di Stasi and Hiruma-Lima, 2002; Mesia-Vela et al*.,* 2002). *S. paniculatum* has been extensively studied mainly because of its protective effects on the liver and anti-secretory gastric properties (Mesia-Vela et al*.,* 2002); also of its chemical constituents including steroidal glycoalkaloids and steroidal saponins (Ripperger et al*.*, 1967; Ripperger and Schreiber, 1968; Mesia-Vela et al*.;* 2002; Botion et al*.*, 2005).

As far as we know, to date, no studies have been published on the relationship between the use of *S. paniculatum* and the frequency of micronucleated cells in mice bone marrow. Due to the widespread use of this plant in folk medicine by Brazilian people, as well as for culinary purposes, this research aimed at evaluating the mutagenic and cytotoxic activities of *S. paniculatum* ethanolic leaf extract (ELE) and ethanolic fruit extract (EFE) using the in vivo mouse bone marrow micronucleus test.

## 2. Material and Methods

### *2.1. Plant material*

Leaves and fruits of *S. paniculatum* were collected in Goiânia, in the state of Goiás, Brazil, in September 2006. A voucher specimen was deposited at the Federal University of Goiás Herbarium under the number 30430/UFG. The leaves and fruits were dried at 45 °C in a forced ventilated stove and exhaustively extracted with one litre of 95% aqueous ethanol at room temperature for 3 days. The resultant alcohol solutions were filtered and then concentrated under reduced pressure at 40 °C to dryness. The crude ethanolic extracts (yield of ELE = 12% w/w and yield of EFE = 14.28% w/w) were transferred to glass flasks filled to the top and kept at 5 °C until the moment of use. *S. paniculatum* ELE and EFE used in the experiments were dissolved in water (1, 2 or 3 $mg.mL^{-1}$) just before use and the volume was administrated according to the mice weight.

### *2.2. Experimental procedure*

Healthy, young male adult outbred mice (*Mus musculus* – Swiss Webster), obtained from the Central Animal House of the Federal University of Goiás, were randomly allocated to treatment groups. All animals were brought to the laboratory five days before the experiments and housed in plastic cages (40 × 30 × 16 cm), in groups of five animals, in air-conditioned rooms at 22 ± 2 °C and 50 ± 10% of relative humidity, with a 12-hours light-dark natural cycle. Food (appropriate commercial rodent diet Labina, Ecibra Ltda.) and water were given ad libitum. On the day of dosing, the animals were approximately 7-9 weeks old and weighed 25-35 g.

Groups of five animals were orally treated with three different doses (100, 200, 300 $mg.kg^{-1}$) of *S. paniculatum* ELE or EFE. A positive (4 $mg.kg^{-1}$ i.p. mitomycin C (MMC), $C_{15}H_{18}N_4O_5$, Bristol-Myers Squibb, lot nº 237AEL) and a negative control group (distilled sterile $H_2O$) were included. The animals were euthanised 24 and 48 hours after the administration of the extracts by cervical dislocation and their bone marrow cells were flushed from both femurs in fetal calf serum (FCS) (Laborclin, lot nº 30721063). After centrifugation (300 × g, 5 minutes) the bone marrow cells were smeared on glass slides, coded for blind analysis, air-dried, and fixed on absolute methanol ($CH_4O$, Synth, lot nº 55026) for 5 minutes at room temperature. The smears were stained with Giemsa (Doles, lot nº 1081), dibasic sodium phosphate ($Na_2HPO_412H_2O$, Vetec, lot nº 982162) and monobasic sodium phosphate ($NaH_2PO_4H_2O$, Vetec, lot nº 983831) to detect micronucleated polychromatic erythrocytes (MNPCE). For each animal, three slides were prepared and a minimum of 2,000 polychromatic erythrocytes (PCE) were counted to determine the frequency of MNPCE. Cytotoxicity was evaluated by the PCE and normochromatic erythrocytes (NCE) ratio (PCE/NCE). The slides were analysed by microscopy (Olympus BH-2 10 × 100). The micronucleus test and MNPCE scoring were carried out according to Schmid (1973).

### *2.3. Statistical analyses*

In order to analyse the mutagenic activity of *S. paniculatum* ELE and EFE, the frequency of MNPCE in the treated groups was compared to the results of the negative control group by one-way ANOVA, and a value of $P < 0.05$ was taken as the criterion for statistical significance.

To evaluate the cytotoxicity of the extract, the PCE/NCE ratio of all treated groups was compared to the result of the negative control. A non-parametric Qui-square test ($\chi^2$) was applied to determine the statistical significance of the results, and a value of $P < 0.05$ was considered significant.

## 3. Results

Table 1 summarises the frequency of MNPCE and PCE/NCE ratio in bone marrow cells of mice treated with *S. paniculatum* ELE and EFE.

**Table 1.** Frequencies of MNPCE and PCE/NCE ratio in the bone marrow of mice treated with *S. paniculatum* ELE and EFE.

| Treatment | Time (hours) | MN/2000 EPC (individual data) | MN/2000 EPC ($\bar{x} \pm s$) | PCE/NCE ($\bar{x} \pm s$) |
|---|---|---|---|---|
| Negative control* | 24 | 6, 4, 3, 4, 4 | $4.2 \pm 1.09^{a}$ | $0.93 \pm 0.09^{c}$ |
| Positive control** | 24 | 32, 23, 20, 16, 16 | $21.4 \pm 6.61^{b(p < 0.05)}$ | $0.55 \pm 0.10^{d(p < 0.05)}$ |
| | 48 | 10, 12, 11, 12, 14 | $11.8 \pm 1.48^{b(p < 0.05)}$ | $0.70 \pm 0.06^{d(p < 0.05)}$ |
| 100 mg.kg$^{-1}$ ELE | 24 | 3, 3, 4, 3, 2 | $3 \pm 0.70^{a(p > 0.05)}$ | $0.92 \pm 0.04^{c(p > 0.05)}$ |
| | 48 | 3, 4, 2, 4, 5 | $3.6 \pm 1.14^{a(p > 0.05)}$ | $0.88 \pm 0.04^{c(p > 0.05)}$ |
| 200 mg.kg$^{-1}$ ELE | 24 | 5, 4, 5, 4, 6 | $4.8 \pm 0.83^{a(p > 0.05)}$ | $0.62 \pm 0.09^{d(p < 0.05)}$ |
| | 48 | 4, 3, 4, 5, 4 | $4 \pm 0.7^{a(p > 0.05)}$ | $0.92 \pm 0.06^{c(p > 0.05)}$ |
| 300 mg.kg$^{-1}$ ELE | 24 | 2, 2, 4, 3, 3 | $2.8 \pm 0.83^{a(p > 0.05)}$ | $0.75 \pm 0.03^{(p < 0.05)}$ |
| | 48 | 2, 3, 3, 1, 1 | $2 \pm 1.0^{a(p > 0.05)}$ | $0.96 \pm 0.09^{c(p > 0.05)}$ |
| 100 mg.kg$^{-1}$ EFE | 24 | 1, 3, 4, 3, 3 | $2.8 \pm 1.09^{a(p > 0.05)}$ | $0.91 \pm 0.06^{c(p > 0.05)}$ |
| | 48 | 5, 4, 5, 5, 3 | $4.4 \pm 0.89^{a(p > 0.05)}$ | $0.87 \pm 0.02^{c(p > 0.05)}$ |
| 200 mg.kg$^{-1}$ EFE | 24 | 3, 2, 3, 3, 4 | $3 \pm 0.70^{a(p > 0.05)}$ | $0.74 \pm 0.07^{d(p < 0.05)}$ |
| | 48 | 6, 4, 7, 6, 5 | $5.6 \pm 1.14^{a(p > 0.05)}$ | $0.65 \pm 0.06^{d(p < 0.05)}$ |
| 300 mg.kg$^{-1}$ EFE | 24 | 6, 5, 7, 5, 5 | $5.6 \pm 0.89^{a(p > 0.05)}$ | $0.93 \pm 0.08^{c(p > 0.05)}$ |
| | 48 | 2, 3, 3, 4, 3 | $3 \pm 0.70^{a(p > 0.05)}$ | $0.80 \pm 0.04^{d(p < 0.05)}$ |

*Negative control group = distilled sterile $H_2O$; **positive control group = 4 mg.kg$^{-1}$ MMC; all the results were compared to the negative control group; MNPCE: one-way ANOVA, $P < 0.05$ was considered statistically significant; PCE/NCE: Qui-square test ($\chi^2$), $P < 0.05$ was considered statistically significant.

The results indicate that the positive control (MMC) ($C_{15}H_{18}N_4O_5$, Bristol-Myers Squibb, lot nº 237AEL) caused a significant ($P < 0.05$) increase in MNPCE compared to the negative control, confirming the sensitivity of the test. The results obtained showed no significant increase in MNPCE either 24 or 48 hours after the administration of any of the three tested doses of *S. paniculatum* ELE ($P > 0.05$) compared to the negative control.

In relation to cytotoxicity, no significant reduction of PCE/NCE ratio was observed at the dose of 100 mg.kg$^{-1}$ *S. paniculatum* ELE either 24 or 48 hours after the administration compared to the negative control ($P > 0.05$). However, a decrease in this ratio ($P < 0.05$) was observed at the doses of 200 and 300 mg.kg$^{-1}$ 24 hours after the administration, while after 48 hours no significant difference was observed in relation to the negative control group ($P > 0.05$). Thus, *S. paniculatum* ELE showed no mutagenic activity, although at higher doses (200 and 300 mg.kg$^{-1}$) this extract exhibited cytotoxicity 24 hours after administration.

The results obtained with *S. paniculatum* EFE demonstrated that neither 24 nor 48 hours after the administration the three doses tested increased MNPCE frequency compared to the negative control ($P > 0.05$). These data indicate that *S. paniculatum* EFE showed no mutagenic activity at any doses and any exposition time tested.

As to the cytotoxic activity of *S. paniculatum* EFE, the results showed a significant reduction in the PCE/NCE ratio at the doses of 200 mg.kg$^{-1}$ (24 and 48 hours) and 300 mg.kg$^{-1}$ (48 hours) compared to the negative control ($P < 0.05$). Nonetheless, for other doses and times employed, no significant difference of this relationship compared to the negative control ($P > 0.05$) was demonstrated. Therefore, cytotoxic action of *S. paniculatum* EFE was observed at higher doses.

## 4. Discussion

Plants produce a great diversity of substances that can have therapeutic significance for maintaining human health and improving the quality of human life, thus justifying their use in traditional medicine (Ravikumar et al., 2008). However, many plant extracts may have particular effects with regard to mutagenicity, indicating that careful use in some instances is advisable and important (Cardoso et al., 2006).

In the present work, we aimed to evaluate the mutagenic and cytotoxic activities of *S. paniculatum* ELE and EFE using the mice bone marrow micronucleus test. This assay is an in vivo short-term test developed by Heddle (1973) and Schmid (1975) and it is useful to investigate compounds with clastogenic (leading to chromosome breakage) and aneugenic (resulting in chromosome loss) activities (Schmid, 1975; Hayashi et al., 1990). Studies have already demonstrated that many mutagenic components exhibit carcinogenic effects (Chandra et al., 2006; Suzuki et al., 2008).

Micronuclei separated from and in addition to the main nucleus of a cell are the results of acentric fragments or lagging chromosomes that fail to incorporate into either of the daughter nuclei during telophase of the mitotic cells. The micronuclei frequency in mouse bone marrow PCE is a very sensitive index of damage produced by ionizing radiation and chemical mutagens (Suzuki et al., 2008).

In this study, the results of the mutagenic evaluation of *S. paniculatum* ELE and EFE using the mice bone marrow micronucleus (Table 1) indicate that these extracts did not present any mutagenic (clastogenic and/or aneugenic) effects in mouse bone marrow PCE.

In the previous study realized in our laboratory to evaluate the genotoxic potencial of *S. paniculatum* ethanolic leaf extract by Inductest SOS in bacterial strains, the results were in accordance with those obtained by this micronucleus assay in mice (Curado-Rezende et al., 2008).

The micronucleus test used in this study also detects cytotoxic effects by the PCE/NCE ratio. When the normal proliferation of bone marrow cells is affected by a toxic agent, there is a decrease in the number of immature erythrocytes (PCE) in relation to the number of mature erythrocytes (NCE) and the PCE/NCE ratio may decrease (Rabello-Gay et al., 1991).

Our results (Table 1) demonstrate that ELE and EFE exhibited cytotoxic activity at higher doses. The cytotoxic action indicates that they probably contain toxic substances that may inhibit cellular division. However, this study did not exhibit an increase of cytotoxicity in dose-response at 200 and 300 $mg.kg^{-1}$.These results are in agreement with earlier studies realized with plant extracts that also did not show dose-response relationship (Suffredini et al., 2004; Tan et al., 2005).

Several species of *Solanum* produce steroidal glycoalkaloids that have close structural and configurational relationships with steroidal sapogenins (Dinan et al., 2001).The alkaloids of the genus *Solanum* include solanine, solasonine, and solamargine (Eltayeb et al., 1997; Cherkaoui et al., 2001; Weissenberg, 2001). The steroidal glycoalkaloids identified in *S. paniculatum* are known to possess a variety of biological activities, including teratogenic (Blankemeyer et al., 1998), cytotoxic, and antitumor properties (Kuo et al., 2000; Liu et al., 2004; Shiu et al., 2007; Smith et al., 2008). Many chemotherapeutic drugs are cytotoxic to cancer cells by inducing apoptosis, and research has shown that solasonine and solamargine cause apoptosis due to membrane disruption (van der Most et al., 2006). It has also been reported that solanine, solasonine, and solamargine are capable of inhibiting the growth of breast cancer and inducing apoptosis in tumor cells (Kuo et al., 2000; Berek et al., 2001; Lee et al., 2004; Liang et al., 2004; Liu et al., 2004; Shiu et al., 2007). Moreover, the inhibitory effect of solanine on tumors of the digestive system has been observed in vitro, and the role of solanine in inducing apoptosis in the sensitive tumor cell line and its effect on Bcl-2 protein has already been described (Lee et al., 2007; Ji et al., 2008).

Steroidal saponins is another class of chemical constituents present in *S. paniculatum* that have in their structure one or two sugar chains attached by glycoside linkages to the aglycone, a non-saccharide portion of the molecule called sapogenin (Kohara et al., 2007). These molecules have long been thought to have pharmacological value, and researchers have increasingly been more interested in their potential pharmacological activities, especially as anti-cancer agents (Hernandez et al., 2004; Trouillas et al., 2005; Kohara et al., 2007). Recently, a number of saponins have been found to exhibit cytotoxic properties against several strains of human cancer cells by inducing apoptosis (Cheng et al., 2008).

In our study, we observed the cytotoxic action of both *S. paniculatum* extracts and this result is basically in accordance with earlier studies carried out by several researchers on the properties of *Solanum* constituents. A number of species of the genus *Solanum* have been shown to contain steroidal glycoalkaloids and steroidal saponins with significant cytotoxic and antitumour activities (Silva et al., 2007). Thus the cytotoxic activity of *S. paniculatum* may be attributed, at least partially, to steroidal alkaloid and steroidal saponin substances. However, the complexity of plant extracts cannot be overlooked, as the final response of a treatment using them is likely to be the result of synergistic, antagonistic, and other interactive effects among their biologically active components. The complexity can possible explain the different cytotoxic activities between ELE and EFE as well as between different doses of the same extract.

In summary, our results indicate that *S. paniculatum* ELE and EFE did not exhibit mutagenic effect in mice bone marrow using the micronucleus test. Nonetheless, cytotoxicity was evidenced specially at higher doses of both extracts.

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