Genotoxicity assessment of *Schinopsis brasiliensis* Engl. (Sapindales: Anacardiaceae) in somatic cells of *Drosophila melanogaster* (Meigen, 1830) (Diptera: Drosophilidae)

Silmara de Moraes Pantaleão¹, Jéssica Adele dos Santos¹, Thalyta Linacher¹, Clisiane Carla de Souza Santos², Charles dos Santos Estevam² and Neila Coelho da Costa³

¹Universidade Federal de Sergipe. Centro de Ciências Biológicas e da Saúde, Laboratório de Genética e Conservação de Recursos Naturais, Departamento de Biologia, São Cristóvão, Sergipe, Brasil. Email: spleao51@gmail.com.
²Universidade Federal de Sergipe, Centro de Ciências Biológicas e da Saúde, Departamento de Fisiologia, Universidade Federal de Sergipe, São Cristóvão, Sergipe, Brasil.
³Universidade Federal de Goiás, Departamento de Ciências Biológicas, Campus Catalão, Catalão, Goiás Brasil.

**Abstract.** *Schinopsis brasiliensis* Engl. (Sapindales: Anacardiaceae) has been used in the Brazilian folk medicine to treat several illnesses. However, the phytochemical profile of *S. brasiliensis* as well as its genotoxic potential are poorly understood, which compromises population safety regarding the medicinal use of this plant species. In this study, we analyzed the genotoxic effects of *S. brasiliensis* using the Somatic Mutation and Recombination Test (SMART) of *Drosophila melanogaster*. Larvae from both standard cross (ST) and high bioactivation capacity cross (HB) were exposed to different concentrations of the hydroethanolic extract and ethyl acetate fraction of *S. brasiliensis*. We analyzed wings from *D. melanogaster* according to the type and number of mutant hair. Ours results suggested no genotoxic activity of *S. brasiliensis* in *D. melanogaster* somatic cells.

**Keywords:** Genotoxicity; Baraúna; Caatinga; Tradicional medicine; SMART.
Introduction

Many plant species from Brazilian Caatinga have shown pharmacological potential, such as *Erythrina velutina* (Vasconcelos et al., 2007), *Tabebuia aurea* (Reis et al., 2014), and *Ziziphus joazeiro* (Ribeiro et al., 2013). That fact has raised a lot of attention for plants used in the folk medicine regarding their properties and safety (Varanda, 2006; Albuquerque and Oliveira, 2007; Silva et al., 2012). However, when considering the number of native species from Caatinga that have been used by local communities, there are still few studies concerned with the composition and biological activity of most species (Vicentini et al., 2001; Liporacci et al., 2017). The genotoxic effects of medicinal plants are usually related to the concentration used by the individual (Corrêa et al., 2001; Sponchiado et al., 2016). Moreover, other aspects have to be considered when analyzing the genotoxic activity of plant species, such as the part of the plant, the age, and the presence of contaminants in the plant tissues (Saad et al., 2006).

*Schinopsis brasiliensis* Engl. (Anacardiaceae) is an endemic tree from Brazilian Caatinga, which occurs from the State of Bahia to the State of Paraíba (Engler, 1879). The popular common names for this species include "baraúna", "braúna", and "quebracho" (Prado et al., 1995; Cardoso et al., 2015).

*S. brasiliensis* is used in the folk medicine to treat disturbs of the nervous system (hysteria and anxiety), inflammation, pain, and infection (Albuquerque et al., 2007; Lorenzi, 2008; Saraiva et al., 2011). Phytochemical studies suggested that some species from the genus *Schinopsis* have a high concentration of phenolic compounds such as tannins, flavonoids, flavanones, and gallic acid (Marín-Martínez et al., 2009; Sánchez-Martína et al., 2010; Saraiva et al., 2011). Analyzing the phytochemical profile of *S. brasiliensis*, Almeida et al. (2010) noticed the presence of tannins, quinones, and triterpenes, while Santos et al. (2014) showed that the ethyl acetate fraction from the bark of *S. brasiliensis* contains aurones, catechins, chalcones, and saponins (Santos et al., 2014). On one hand, studies demonstrated that some of those compounds have protective and antigenotoxic effect (Rodríguez-García, 2013; Manzolli et al., 2015). On the other hand, compounds such as saponins were noticed to have genotoxic effect on human lymphocytes (Kalachaveedu et al., 2014). In addition, the extract of *S. brasiliensis*’ bark demonstrated larvicide activity against *Aedes aegypti* and toxic potential on *Biomphalaria glabrata* (Santos et al., 2014).

Despite the efficacy of *S. brasiliensis* in the folk medicine, there are few studies describing its composition and activity on organisms (Saraiva et al., 2009; Santos et al., 2014). The lack of scientific knowledge about this species implies on risks to people who use *S. brasiliensis* as an alternative treatment for several diseases. Considering the safety involving the consumption of medicinal plants, we found important to conduct genotoxic studies, in order to validate their use by local communities. For that finality, the Somatic Mutation and Recombination Test (SMART) in *Drosophila*
Genotoxic assessment of *S. brasiliensis* Engl. is an indicated test due to its capacity and sensibility in the identification of mutations and recombination events, besides the detection of promutagens and procarcinogens (Graf et al., 1984; Graf and Van Schaik, 1992; Panchal and Tiwari, 2017).

This study aimed to evaluate the genotoxic effects of two extracts of *S. brasiliensis* on somatic cells of *D. melanogaster* by using the Somatic Mutation and Recombination Test (SMART).

**Materials and methods**

**Plant collection**
We collected samples of *S. brasiliensis* in Piranhas, AL, Brazil (09° 37' 26" S e 37° 45' 25" W). The species was identified and deposited at Federal University of Sergipe's herbarium, São Cristóvão, SE, Brazil.

**Extracts preparation**
The preparation of extracts consisted on keeping the plant's bark at room temperature until the material was completely dried. In sequence, we submitted the material to maceration with ethanol (90%) for five days. We obtained the hydroethanolic extract by filtering and concentrating the material under vacuum in rotatory evaporator (LS LOGEN) at 45 °C. To obtain the ethyl acetate fraction, we diluted the hydroethanolic extract in methanol-water (MeOH:H₂O, 2:3). The extraction was completed by adding ethyl acetate to the solution.

**Somatic Mutation and Recombination Test (SMART)**
The use of the SMART in *Drosophila melanogaster* to evaluate genotoxicity of medicinal plants and drugs is an efficient tool due to its sensibility and the genetic similarity between *D. melanogaster* and mammals (Graf et al., 1984).

Here, we used three mutant lineages of *D. melanogaster* (1) multiple wing hairs (mwh): mwh/mwh; (2) flare-3 (flr³/In (3LR)TM3, ripsep l(3)89Aabx³ε e Bd³; and (3) ORR; flare-3 (ORR/ORR; flr³/In (3LR)TM3, ripsep l(3)89Aabx³ε e Bd³). Considering a proportion 2:1 for females and males, we made two different crosses: the standard cross (ST), in which flr³ individuals were females and *mwh* individuals were males (Graf et al., 1989); and the high bioactivation cross (HB), in which *ORR*; flr³ individuals were females and *mwh* individuals were males (Graf and Van Schaik, 1992).

After the eggs posture (72 h ± 4 h), we placed 3rd instar larvae in plastic recipes containing food solution (1.5 g of potato powder and 5 mL of one of the four extract concentrations (0.5, 1.0, 2.0 e 4.0 mg/mL)). The controls consisted of ultrapure water (negative control) and 0.891 mg/mL of urethane (positive control). For each treatment, we made four replicates. The larvae were submitted to chronical treatment, which consisted of exposing the larvae to food solution containing the plant extract for 48 h.

The emergent adults with the genotypes: *mwh*+/+ *flr*³ ou *mwh* +/- *TM3, BdS* were separated and fixed in ethanol (70%). The wings of *D. melanogaster* were fixed on slides using Faure solution (30 g of gum arabic, 20 mL of glycerol, 50 g of hydrate of chloral and 50 mL of water), and kept on hotplate until they were completely dry. Mutant spots were analyzed and classified according to Graf et al. (1992).

**Survivorship assessment**
We assessed survivorship after larvae treatment by counting the number of living flies per replicate. Percent survival was calculated for both ST and HB cross flies exposed to different concentrations of *S. brasiliensis* extracts (Figure 1). Percent survival was given by [120 (the total number of larvae exposed to a concentration) - the number of adult flies]/120. The data were analyzed by the chi-square test (χ²).
flies that emerged from the same concentration) x 100].

**Frequency of mutant hair and data analysis**

The results were submitted to statistical analysis, according to the model of multiple decisions described by Frei and Würgler (1988). The frequency of mutant hair found in the treatment was compared to the frequencies found in our positive and negative control, using the binomial conditional test of Kastenbaum and Bowman (1970), where \( \alpha = 0.05 \). The statistical diagnoses was positive, negative, and inconclusive (Frei and Würgler, 1988).

**Results and discussion**

Survivorship analysis of *D. melanogaster* revealed no cytotoxic potential of *S. brasiliensis* extracts on both ST and HB cross flies. Percent survival of ST cross flies varied from 90% to 82.5%, while percent survival of HB cross flies varied from 80% to 70%, for all HEE concentrations (Figure 1). Similarly, percent survival in EAF treatments varied from 89.17% to 74.17%, for ST cross flies, and from 80% to 71.67% for HB cross flies (Figure 2).

![Figure 1](image1.png)

Figure 1. Survivorship curve for *Drosophila melanogaster* (N = 120) exposed to the hydroetanolic extract of *Schinopsis brasiliensis*.

![Figure 2](image2.png)

Figure 2. Survivorship curve for *Drosophila melanogaster* (N = 120) exposed to the ethyl acetate fraction of *Schinopsis brasiliensis*. 
Results on chronic treatment of *D. melanogaster* larvae suggested no genotoxic effect of *S. brasiliensis* extracts at the tested concentrations. For both crosses, there was no significant difference in the frequency of mutant spots in flies treated with HEE, EAF, and negative control (Table 1 and 2).

**Table 1.** Summary of Results Obtained with the *Drosophila* Wing Spot Test (SMART) in the Marker-Heterozygous (MH) Progeny of the Standard (ST) Cross after Chronic Treatment of Larvae with HEE and EAF of the *S. brasiliensis*.

<table>
<thead>
<tr>
<th>Genotype Concentration (mL)</th>
<th>Number of flies (N)</th>
<th>Spots per individual (number of spots) statistical diagnosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Small single-spots (1-2 cells)</td>
<td>Large single-spots (2-5 cells)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>m = 2</td>
<td>m = 5</td>
</tr>
<tr>
<td>Negative control</td>
<td>30</td>
<td>0.57 (17)</td>
<td>0.27 (8)</td>
</tr>
<tr>
<td>Urethane</td>
<td>20</td>
<td>1.65 (33)</td>
<td>0.20 (4)</td>
</tr>
<tr>
<td>HEE 0.5</td>
<td>30</td>
<td>0.10 (2)</td>
<td>0.07 (2)</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>0.07 (2)</td>
<td>0.03 (1)</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>0.07 (2)</td>
<td>0.20 (6)</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>0.07 (2)</td>
<td>0.20 (6)</td>
</tr>
<tr>
<td>EAF 0.5</td>
<td>30</td>
<td>0.23 (7)</td>
<td>0.07 (2)</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>0.17 (5)</td>
<td>0.13 (4)</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>0.07 (2)</td>
<td>0.13 (4)</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>0.07 (2)</td>
<td>0.03 (1)</td>
</tr>
</tbody>
</table>

1 Statistical diagnoses according to Frei and Wurgler (1988): +, positive; −, negative; i, inconclusive; P < 0.05.
2 Including rare fl+ single spots.
3 Considering much clones from much single and twin spots.

HEE and EAF had negative results for small single spots and large single spots. For both extracts, the results on twin spots were inconclusive, fact that is usually associated to the low frequency of twin spots found in negative controls. However, analyzing the total spots the results for either HEE or EFA were negative at the standard cross (ST), which has normal metabolization activity (Graf et al., 1984).

In Table 2, we summarized the results of the HB cross, which analyzed the activity of substances dependent on metabolization via cytochrome P450. The HB cross includes the ORR lineage, which is known by its resistance to DDT and high level of CYP450 enzymes (Pádua et
This fact enhances fly sensibility to promutagens and procarcinogens, which allows the identification of substances with indirect activity. For the HB cross our results on large single spots and twin spots were inconclusive for all concentrations of HEE and EAF. However, those results have low biological significance, considering that the frequency of total spots we found in the HB cross flies exposed to HEE and EAF were lower than the frequencies found in the positive control.

The analysis of the trans-heterozygous progeny from both ST and HB crosses exposed to uretan showed that the negative results obtained in the treatments with HEE and EAF are not false-negatives.

Machado (2012) verified that S. brasiliensis extract had on its phytochemical composition, substances that were able to inhibit development of Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa, suggesting antimicrobial properties of S. brasiliensis. Santos et al. (2014) noticed that the ethyl acetate fraction of S. brasiliensis had larvicide activity on Aedes aegypti and toxic effect on Biomphalaria glabrata. In addition, the authors classified tannins as the main phenolic compounds in the bark of S. brasiliensis. It is known that the amount of tannins found in wild plants is usually higher than the amount found in domesticated plants (Hagerman and Butler, 1991). This fact is understood as an adaptation to herbivory, seeing that tannins create a negative response to predation by promoting antinutritional and toxic effects on herbivores through the coagulation of mucoproteins and consequent inhibition of digestion (Harborne, 1988).

On the other hand, phenolic compounds have antioxidant properties, once those substances have the ability of donating hydrogen and electrons due to the presence of stable radicals that avoid the oxidation of compounds (Degáspari and Waszczynskyj, 2004). Moreover, an antigenotoxicity study regarding the use of pro-anthocyanins (condensed tannins) extracted from Vittis vinifera seeds suggested a protective effect induced by the substance on genotoxic damages caused by doxorubicin hydrochloride (an antracicline that acts on the formation of free radicals by interfering the enzyme topoisomerase II) (Rezende et al., 2009). Thus, future researches would focus on the antigenotoxic potential of S. brasiliensis.

Our results were statistically non-significant for both ST and HB crosses, which indicates that the two extracts of S. brasiliensis had neither genotoxic effect on D. melanogaster somatic cells with low metabolization activity, nor genotoxic effect on D. melanogaster somatic cells with high metabolization activity, regarding the promotion of mutation, deletion, non-disjunction and mitotic recombination.

Conclusions

The results presented in our preliminary study suggest no genotoxic effect of S. brasiliensis on D. melanogaster. However, considering other organisms is a step necessary to guarantee the safe use of S. brasiliensis by the population or even to validate its use as a medicine. Additional studies would also focus on the anti-genotoxic potential of S. brasiliensis in order to explore the pharmacological potential of this species.

Acknowledgments

CSE and CCSS coordinate the extract preparation and biological studies. NCS and SMP contributed to design the study supervised the laboratory work execution and critical reading of the manuscript. JASS, TLGSR, CCSS, MRB and PS contributed in running the laboratory work, analysis of the data and drafted the paper. JASS contributed in plant identification and herbarium confection.
Conflict of interest

The authors declare that they have no competing interests.

References


Kastenbaum, M. A.; Bowman, K. O. Tables for determining the statistical significance of mutation frequencies. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, v. 9, p. 527-


License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.