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Aqueous extract of *Strychnos pseudoquina* exhibits no mutagenicity but shows increased mutagenicity upon interaction with sodium azide

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Abstract
The infusion of *Strychnos pseudoquina* leaves and bark has been used in folk medicine to treat fevers, malaria, liver and stomach conditions, and anemia. Despite its popular use, the mutagenic effects of *S. pseudoqunia* are unclear. It is important to note that species of this same genus are generally classified as both toxic and mutagenic. The present study evaluated the mutagenic and antimutagenic potential of an aqueous extract of *S. pseudoquina* bark using the Ames test in *Salmonella typhimurium*. The concentrations of 0.62, 1.25, 2.5, 5, and 10 mg/plate of *S. pseudoquina* were evaluated. The results revealed the absence of mutagenicity and antimutagenicity for all tested concentrations of *S. pseudoquina*, suggesting the potential medicinal value for this plant. Importantly, *S. pseudoquina* solution can interact with other drugs. We observed that the aqueous extract of *S. pseudoquina* bark increased the mutagenicity of sodium azide in *S. typhimurium* by as-yet unknown components and mechanisms. Thus, despite not having mutagenic or antimutagenic actions, the use in association with other drugs may bring unwanted effects. Further scientific data are required to guide clinical practice and safeguard the welfare of users.

Keywords: Genotoxicity, quina-do-cerrado, ames test, *salmonella typhimurium*, cerrado, synergistic effect

Introduction
Many plant species have been used in folk medicine for the treatment and prevention of various human diseases (Al-Asmari *et al*. 2014) [1]. Despite their therapeutic advantages, the toxic potential of medicinal plants and their dosages are not always recognized by the general public or by many professional groups in traditional medicine (Soetan and Aiyelaagbe 2009) [23]. Thus, mismanagement during therapy could be harmful to health.

The genotoxic, mutagenic, and carcinogenic potential of medicinal plants has been a subject of great concern in the scientific community, especially regarding bioactive components found in natural products. In contrast, many plant extracts also have antimutagenic activity against the mutagenic influences of endogenous or exogenous agents and guarantee the integrity of DNA (Burcham 1999; Ribeiro and Salvadori 2003) [6, 19]. This process takes place through DNA repair mechanisms, damage tolerance, cell cycle checkpoints, and cell death pathways (Jackson and Bartek 2009) [13].

A widely used species in folk medicine is *Strychnos* pseudoquina A. St.-Hil., popularly known as falsa-quina, quina-branca, and/or Quina-do-cerrado. The plant is native to the Brazilian Cerrado. Ethnobotanical evidence has indicated the use of *S. pseudoquina* to treat intermittent fevers, malaria, liver and stomach conditions, and anemia (Brandão and Rapini 2018). The leaves and stem bark are main plant parts used in traditional medicine, either through infusions or as powder (De Saint-Hilaire 2009) [8]. However, their use is based only on popular knowledge and traditions, with no scientific basis on the efficacy and safety of the preparations (De Saint-Hilaire 2009) [8].

The genus *Strychnos*, to which *S. pseudoquina* belongs, has phytocompounds with powerful toxicity and mutagenicity (Bonamin *et al*. 2011) [4]. This toxicity is mainly due to the presence of some alkaloids. However, despite their genotoxic properties, antioxidant and antimutagenic substances, such as flavonoids, are also present (Jurado *et al.* 1991) [14], and, even though rare, their toxicity and mutagenic capacity are also reported in the literature (Moreira *et al.* 2002) [16].

Most species of the genus *Strychnos* are genotoxic and data on their biological properties and chemical compositions is limited. Thus, data are needed to ensure efficacy and safety when using *S. pseudoquina* (Silva *et al*. 2005) [3, 4, 22, 24].

This study evaluates the mutagenic and antimutagenic potential of an aqueous extract of *S. pseudoquina* by using the indicator species *Salmonella typhimurium* in the Ames test bioassay. The main advantages of this bioassay are its simplicity, cost-effectiveness, flexibility, and large database of validated data. The test also allows the evaluation of various concentrations and determination of the dose-response relationship (Mortelmans and Zeiger 2000) [17, 26]. This assay is used to provide safety data in the use of medicinal plants (Sturbelle *et al.* 2010) [25]. This is important, since the use of mutagenic substances can increase or accelerate the mutation rate, leading to the development of neoplasms (Ribeiro *et al.* 2003) [19].

Materials and Methods

S. pseudoquina

The *S. pseudoquina* barks were purchased from Chá e Cia - Ervas Medicinais (Jacareí, Brazil). The product is, produced and distributed by J T F Produtos Naturais (São José dos Campos, Brazil; batch: 022021). An aqueous extract of the barks of Quina-do-cerrado (*S. pseudoquina*) was prepared according to the manufacturer's directions for popular use.

To prepare the aqueous extract, 60 g of macerated bark was added to 1 l of water, which is equivalent to the manufacturer's recommended dose of two tablespoons in 1 l of water. The mixture was placed in a container and heated to the boiling point. The container was then covered for 10 min.

The mixture was then ready for use. For the tests, five concentrations were used: 0.62, 1.25, 2.5, 5, and 10 mg/ml.

Bacterial strain

S. typhimurium TA-100 was the bacterial strain used. The strain was provided by the Laboratory of Radiobiology and Mutagenesis, Federal University of Goiás, Campus Samambaia, Goiânia-Goiás, Brazil.

Experimental procedure

Evaluation of mutagenic and antimutagenic activities

S. typhimurium TA100 was inoculated in sterile nutrient broth and incubated for 12 h at 37 °C in a water bath under constant agitation until reaching the stationary phase of growth. To evaluate the mutagenic activity, aliquots of 100 µl of the bacterial strain cultures were incubated with 0.62, 1.25, 2.5, 5, and 10 mg/plate of *S. pseudoquina* for 25 min at 37 °C in triplicate test tubes under constant agitation. Negative (20 µl autoclaved distilled water) and positive (20 µl sodium azide) controls were also included in the experiments. To evaluate the antimutagenic activity, the positive control was co-administered with the different doses of *S. pseudoquina*. After incubation, liquid glucose agar (top-agar) containing a histidine/biotin solution (0.5 mM) was added at a temperature of 45 °C. The content was vortexed and then poured into petri dishes containing solid medium (minimal glucose agar). Triplicate preparations of each sample and control were incubated at 37 °C for 48 h in a biochemical oxygen demand incubator. After this period, revertant colonies were enumerated using an electronic counter. The mean of the triplicate values was reported (Maron and Ames 1983; Mortelmans and Zeiger 2000) [15, 17, 26].

Evaluation parameters

Mutagenesis and antimutagenesis

For evaluating the mutagenicity of *S. pseudoquina*, the mutagenicity ratio (MR) was calculated for all tested doses, using the following equation:

$$\mathrm{MR} = \frac{\text{number of revertants/test sample plate}}{\text{number of revertants/negative control plate}}$$

The test is considered positive for mutagenicity when the number of revertant colonies on the test plates is equal to or greater than twice the number of spontaneous revertant colonies of the negative control (Maron and Ames 1983) [15]. The percent inhibition (PI) was calculated using the following equation:

$$\mathrm{PI}(\%) = \left[1 - \left(\frac{\text{number of revertants on test plate} - \mathrm{RE}}{\text{number of revertants on positive control plate} - \mathrm{RE}}\right)\right] \times 100$$

Where test plate refers to plates incubated with mutagen and compound, number of revertants of test plate refers to histidine positive revertants, number of revertants on positive control plate refers to plates incubated with mutagen only, and RE: spontaneous revertants refer to test strains incubated in the absence of compound and mutagen.

Statistical analyses

Statistical analyses of the data from the Ames test were performed using BioEstat version 5.3 software (Ayres *et al*. 2007) [2]. The Shapiro-Wilk normality test was used to determine the best statistical analysis method to be applied, considering $p>0.05$ and samples with a normal distribution. Analysis of variance (ANOVA) test was then used to determine possible significant differences between means. Finally, the Tukey's post-hoc test was performed to identify groups with significant differences ($p<0.05$). For the Ames test, after counting the number of revertants and calculating the MR for each dose and PI, the mutagenicity and antimutagenicity parameters were considered ($p<0.05$ compared to the negative and positive control, respectively).

Results

Table 1 presents the results of the evaluation of mutagenic and antimutagenic activity of *S. pseudoquina* by the Ames test.

The different doses of *S. pseudoquina* showed no significant difference ($p>0.05$) compared to the negative control in mutagenic activity. The result indicated the lack of mutagenic potential of *S. pseudoquina* aqueous extract at doses of 0.62, 1.25, 2.5, 5, and 10 mg/plate.

In the antimutagenic evaluation, *S. pseudoquina* did not significantly reduce ($p>0.05$) the number of *S. typhimurium* colonies when compared to the positive control (20 µg/plate

of sodium azide) in any of the tested doses. The result indicating that aqueous extract of *S. pseudoquina* bark does not provide protection to bacterial DNA.

Table 1. Evaluation of mutagenic and antimutagenic activity of an aqueous extract of *Strychnos pseudoquina* in *Salmonella typhimurium* TA100 by the Ames test.

TA100			
Mutagenicity			
Treatment	**Mean ± SD**	**MR (%)**	**PI (%)**
Negative control	322 ± 24	1.00	-
Positive control	2961 ± 535*	9.19	-
S. pseudoquina 0.62 mg/plate	560 ± 68	1.74	-
S. pseudoquina 1.25 mg/plate	356 ± 41	1.11	-
S. pseudoquina 2.5 mg/plate	360 ± 60	1.12	-
S. pseudoquina 5 mg/plate	339 ± 77	1.05	-
S. pseudoquina 10 mg/plate	367 ± 44	1.14	-
Antimutagenicity			
Treatment	**Mean ± SD**	**MR (%)**	**PI (%)**
Negative control	344 ± 21§	-	-
Positive control	1867 ± 135	-	-
S. pseudoquina 0.62 mg/plate + PC	1852 ± 141	-	1
S. pseudoquina 1.25 mg/plate + PC	2302 ± 197	-	-28
S. pseudoquina 2.5 mg/plate + PC	2242 ± 557	-	-24
S. pseudoquina 5 mg/plate + PC	2308 ± 524	-	-28
S. pseudoquina 10 mg/plate + PC	2268 ± 351	-	-26

ANOVA; Tukey test

Negative control: 20 µl of distilled water; positive control (PC): 20 µg/plate of sodium azide. Mean ± standard deviation (SD) of revertant colonies.

MR: mutagenicity ratio; PI: percent inhibition

*Significant difference (p<0.05) compared to the negative control

§Significant difference (p<0.05) compared to the positive control

Discussion and Conclusion

The x`present study evaluated the mutagenic and antimutagenic activity of *S. pseudoquina*. Our results reveal the lack of mutagenic activity for *S. typhimurium* TA100. Corroborating this data, Nunes (2008) [11, 18] tested the ethanolic extract of the bark in the Ames mutagenicity test using *S. typhimurium* TA98 and TA100, and reported no mutagenic activity at the tested doses. In contrast, Gontijo *et al.* (2020) [10, 11] evaluated the mutagenicity of leaves and bark of *S. pseudoquina* by the Ames test, and found that both displayed mutagenic potential.

The absence of *S. pseudoquina* mutagenic activity and toxicity on *S. typhimurium* at the tested doses provide evidence of the potential medicinal value of aqueous extracts for humans. However, knowledge of the effect of *S. pseudoquina* on eukaryotic cells is still unknown. Studies with models that more closely mimic mammals, especially humans, are needed. Before being indicated for use, it is also necessary to determine the mechanisms of action of the bioactive compounds present in the aqueous extract of *S. pseudoquina* and the appropriate dosage for safe and effective use in humans.

Another concern regarding the use of natural products is their drug interactions. Many potentially non-harmful substances can increase or decrease the activity of other drugs. Our results showed a potentiation of the action of the sodium azide mutagen in the presence of *S. pseudoquina*. The negative PI indicated increased mutagenic activity at the highest doses. Although the aqueous extract of *S. pseudoquina* barks lacked mutagenic and antimutagenic actions, the mutagenicity of sodium azide for *S. typhimurium* was increased. Similar results have been described for *Vernonanthura polyanthes*, *Piper cubeba*, *Lycopersicon esculentum*, and *Tabebuia impetiginosa* in different experimental models (Dutra *et al.* 2009 [9]; Sousa *et al.* 2009 [12, 24]; Rezende *et al.* 2011 [24]; Barbosa *et al.* 2012 [3]; Guerra-Santos *et al.* 2016) [12].

Plant extracts have multiple components that can exert mutagenic or antimutagenic effects alone or synergistically (Cai *et al.* 2004; Romero-Jiménez *et al.* 2005) [7, 20]. Thus, the co-administration of herbal medicines and therapeutic drugs may pose clinical risks to patients and needs to be further investigated (Barbosa *et al.* 2012) [3]. The components and mechanisms used by plant species to potentiate the effect of mutagens have been poorly investigated.

This study shows that the aqueous extract of *S. pseudoquina* bark can potentiate the mutagenic action of sodium azide by as-yet unknown components and mechanisms. Further studies are required to determine the substance(s) involved in this potentiating effect. These data are important, since understanding the components and mechanisms involved in herbal-drug interactions is essential for clinical risk assessment (Barbosa *et al.* 2012) [3]. Further studies are also needed to confirm the efficacy and/or risks of the use of this medicinal plant by humans and to discover alternatives for the rational use of this natural resource.

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The authors have no conflicts of interest to declare.

References

1. Al-Asmari AK, Al-Elaiwi AM, Athar MT, Tariq M, Al Eid A, Al-Asmary SM. A review of hepatoprotective plants used in Saudi traditional medicine. Evid Based

Complement Alternat Med. 2014:890842. DOI:10.1155/2014/890842.
2. Ayres M, Ayres JM, Ayres DL, Santos AAS. BioEstat: aplicações estatísticas na área de ciências biomédicas. 4. ed. Belém: Sociedade Civil Mamirauá; c2007:324p.
3. Barbosa LC, Dias de Morais M, De Paula CA, Da Silva Ferreira MC, Jordao AA, Andrade e Silva ML, *et al*. Mikania glomerata Sprengel (Asteraceae) influences the mutagenicity induced by doxorubicin without altering liver lipid peroxidation or antioxidant levels. J Toxicol Environ. 2012;75(16-17):1102-1109. http://dx.DOI.org/10.1080/152 87394.2012.697842.
4. Bonamin F, Moraes TM, Kushima H, Silva MA, Rozza AL, Pellizzon CH, *et al*. Can a Strychnos species be used as antiulcer agent? Ulcer healing action from alkaloid fraction of Strychnos pseudoquina St. Hil. (Loganiaceae). J Ethnopharmacol. 2011;138(1):47-52. DOI:10.1016/j.jep. 2011.08.020.
5. Brandão EK de S, Rapini A. Flora of Bahia: Loganiaceae. Sitientibus sér. Ci Biol; c2018. https://DOI.org/10.13102/scb2648.
6. Burcham PC. Internal hazards: baseline DNA damage by endogenous products of normal metabolism. Mutat Res. 1999;443(1-2):11-36. DOI:10.1016/s1383-5742(99)00008-3.
7. Cai Z, Tsung EF, Marinescu VD, Ramoni MF, Riva A, Kohane IS. Bayesian approach to discovering pathogenic SNPs in conserved protein domains. Hum Mutat. 2004;24(2):178-84. DOI:10.1002/humu.20063.
8. De Saint-Hilaire A. Plantas usuais dos Brasileiros. Código, Dataplamt. 2. ed. Belo Horizonte: Fino Traço; c2009. p. 348.
9. Dutra ES, Dias CD, Araújo BC De, Castro AJS, Nepomuceno JC. Effect of organic tomato (*Lycopersicon esculentum*) extract on the genotoxicity of doxorubicin in the Drosophila wing spot test. Genet Mol Biol. 2009;32(1):133-137. DOI:10.1590/S1415-47572009005000008.
10. Gontijo DC, Brandão GC, Gontijo PC, De Oliveira AB, Diaz MAN, Fietto LG, *et al*. Identification of phenolic compounds and biologically related activities from Ocotea odorifera aqueous extract leaves. Food Chem. 2017;230:618-626. DOI:10.1016/j.foodchem.2017.03.087.
11. Gontijo DC, Nunes LG, Farias LM, Duarte MGR, Carvalho AF, Fietto LG, *et al*. Assessment of the phenolic content, mutagenicity and genotoxicity of ethanolic extracts of stem bark and leaves from Strychnos pseudoquina A. St.-hil. Drug Chem Toxicol. 2020;43(5):539-545. DOI:10.1080/01480545.2018.1515218.
12. Guerra-Santos IJ, Rocha JD, Vale CR, Sousa WC, Teles AM, Chen-Chen L, *et al*. Vernonanthura polyanthes leaves aqueous extract enhances doxorubicin genotoxicity in somatic cells of Drosophila melanogaster and presents no antifungal activity against Candida spp. Braz J Biol. 2016;76(4):928-936. DOI:10.1590/1519-6984.04615.
13. Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009;461(7267):1071-1078. DOI:10.1038/nature08467
14. Jurado J, Alejandre-Durán E, Alonso-Moraga A, Pueyo C. Study on the mutagenic activity of 13 bioflavonoids with the Salmonella Ara test. Mutagenesis. 1991;6(4):289-95. DOI:10.1093/mutage/6.4.289.
15. Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. Mutat Res. 1983;113(3-4):173-215. DOI:10.1016/0165-1161(83)90010-9.
16. Moreira RRD, Santos LE, Varella SD, Varanda EA, Vilegas W. Avaliação da atividade mutagênica do extrato etanólico bruto de Paepalanthus latipes (Eriocaulaceae) e dos compostos flavonoídicos 7-metoxilados relacionados. Rev bras farmacogn. 2002;12(1):11-19. https://DOI.org/10.1590/S0102-695X2002000100002.
17. Mortelmans K, Zeiger E. The Ames Salmonella/microsome mutagenicity assay. Mutat Res. 2000;455:29-60. DOI:10.1016/s0027-5107(00)00064-6.
18. Nunes LG. Phytochemistry prospection and mutagenicity evaluate *in vitro* of tree species vegetable: Strychnos pseudoquina A. St.-Hil., Coutarea hexandra (Jacq.) K. Schum and Bathysa cuspidata (A.St.-Hil.) Hook. 114p. Dissertação (Mestrado em Bioquímica Agrícola), Mestrado em Bioquímica e Biologia molecular de plantas e Bioquímica e Biologia molecular animal, Universidade Federal de Viçosa, Viços, Brasil; c2008.
19. Ribeiro LR, Salvadori DMF, Marques EK. Mutagênese Ambiental. 1. ed. Editora da ULBRA, Canoas, RS; c2003:1-356p.
20. Romero-Jiménez M, Campos-Sánchez J, Analla M, Muñoz-Serrano A, Alonso-Moraga A. Genotoxicity and anti-genotoxicity of some traditional medicinal herbs. Mutat Res. 2005;585(1-2):147-155. https://DOI.org/10.1016/j.mrgentox.2005.05.004.
21. Shilkin ES, Boldinova EO, Stolyarenko AD, Goncharova RI, Chuprov-Netochin RN, Khairullin RF, *et al*. Translesion DNA Synthesis and Carcinogenesis. Biochemistry (Mosc). 2020;85(4):425-435. DOI: 10.1134/S0006297920040033.
22. Silva MAD, Rafacho BPM, Lima CAH, Rocha LRM, Santos LCSD, Sannomiuya M, *et al*. Evaluation of Strychnos pseudoquina St. Hil. leaves extract on gastrointestinal activity in mice. Chem Pharm Bull. 2005;53(8):881-885. DOI:10.1248/cpb.53.881.
23. Soetan KO, Aiyelaagbe OO. The need for bioactivity-safety evaluation and conservation of medicinal plants. A review. J Med Plant Res. 2009;3(5):324-328. Available at: https://academicjournals.org/article/article1380378053_Soetan%20and%20Aiyelaagbe.pdf. Accessed on: 08 Mar; c2023.
24. Sousa NC De, Rezende AAA De, Silva RMG Da, Guterres ZR, Graf U, Kerr WE, *et al*. Modulatory effects of *Tabebuia impetiginosa* (Lamiales, Bignoniaceae) on doxorubicin-induced somatic mutation and recombination in Drosophila melanogaster. Genet Mol Biol. 2009;32(2):382-388. https://DOI.org/10.1590/S1415-47572009005000042.
25. Sturbelle RT, Pinho DS De, Restani RG, Oliveira GR De, Garcias G De L, Martino-Roth M Da G. Avaliação da atividade mutagênica e antimutagênica da Aloe vera em teste de *Allium cepa* e teste de micronúcleo em linfócitos humanos binucleados. Rev bras farmacognosia. 2010;20(03):409-415. https://DOI.org/10.1590/S0102-695X2010000300019.
26. Zeiger E. The test that changed the world: The Ames test and the regulation of chemicals. Mutat Res Genet Toxicol Environ Mutagen. 2019;841:43-48. DOI:10.1016/j.mrgentox.2019.05.007.