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DNA DAMAGE IN PHYLLOSTOMIDAE BATS FROM A MINING AREA IN CENTRAL CERRADO, BRAZIL

DANO AO DNA EM MORCEGOS PHYLLOSTOMIDAE DE UMA ÁREA DE MINERAÇÃO NO CERRADO CENTRAL, BRASIL

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Abstract

The micronucleus (MN) test is widely used for detecting DNA damage resulting from pollutant exposure. However, studies involving wild mammals, particularly bats, one of the most diverse groups on the planet, are still scarce. In this context, the use of bioindicator organisms, such as bats, is essential for assessing environmental health. This study aimed to analyze the frequency of MN in peripheral blood samples from bats inhabiting an area impacted by ferronickel mining in Central Cerrado, Brazil. Three species were investigated: *Artibeus lituratus* (n = 5), *Platyrrhinus lineatus* (n = 6), and *Lophostoma silvicola* (n = 6). A total of 1000 cells were quantified for each animal. Overall, the mean MN frequency was 6.40 ± 2.70 for *A. lituratus*, 5.17 ± 2.64 for *P. lineatus,* and 9.50 ± 4.42 for *L. silvicola*, with no statistically significant differences among the species (F = 2.23; p = 0.143). Despite this result, *L. silvicola,* an insectivorous specimen, exhibited a slightly higher mean than the frugivorous species analyzed. The results reinforce the importance of the MN test as a valuable tool for ecotoxicological assessment and environmental health monitoring in areas subjected to anthropogenic activities, providing baseline data for the species investigated.

Keywords: Biomarker, Ecotoxicology, Chiroptera.

Resumo

O teste de micronúcleo (MN) é um método amplamente utilizado para detectar danos no DNA resultantes da exposição a poluentes. No entanto, ainda são escassos os estudos envolvendo mamíferos silvestres, especialmente os morcegos, um dos grupos mais diversificados do planeta. Nesse contexto, a utilização de organismos bioindicadores como os morcegos revela-se fundamental para avaliar a saúde ambiental. O presente estudo teve como objetivo analisar a frequência de MN em amostras de sangue periférico de morcegos provenientes de uma área impactada pela mineração de ferroníquel no Cerrado Central, Brasil. Três espécies foram investigadas: *Artibeus lituratus* (n = 5), *Platyrrhinus lineatus* (n = 6) e *Lophostoma silvicola* (n = 6). Para cada animal foram quantificadas 1000 células. De forma geral, a frequência média de MN foi de 6,40 ± 2,70 para *A. lituratus*, 5,17 ± 2,64 para *P. lineatus* e 9,50 ± 4,42 para *L. silvicola*, não sendo observada diferença estatisticamente significativa entre as espécies (F = 2,23; p = 0,143). Embora não tenha sido identificada diferença significativa entre as espécies, destaca-se que o insetívoro *L. silvicola* apresentou uma média levemente superior em comparação às espécies frugívoras analisadas. Os resultados reforçam a importância do teste de micronúcleo como ferramenta relevante para avaliação ecotoxicológica e monitoramento da saúde ambiental em áreas sujeitas a atividades antrópicas, cujos dados servem como nível basal para essas espécies investigadas.

Palavras-Chaves: Biomarcador, Ecotoxicologia, Quirópteros.

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Introduction

The current growth of human society is strongly related to the progressive industrialization and increased exploitation of natural resources (MONTALVÁN-OLIVARES et al., 2021). Among these resources, mineral exploration stands out. Mining is a crucial sector in the Brazilian economy, and the country is the second-largest producer of iron ore worldwide (BANSAL et al., 2023). Despite its economic relevance, open-pit mining (i.e., the focus of this study) can also cause substantial changes to the landscape, including habitat fragmentation and environmental pollution (THEOBALD et al., 2020; BENVINDO-SOUZA et al., 2023), factors that may impair local fauna and human health. In this context, studies using bioindicator organisms are essential for assessing the health of ecosystems. Bats emerge as effective bioindicators among the groups of organisms, especially due to their ability to true flight, differentiating them from other mammals.

Bats are a highly diverse group of mammals that perform essential ecosystem services, including pest suppression, pollination of economically important plants, and seed dispersal (RUSSO et al., 2021). These services underscore the environmental and economic importance of bats (VILCHES-PIÑONES et al., 2024; TUNEU-CORRAL et al., 2024). However, considering the environmental pressures, these animals may be exposed to multiple stressors, including heavy metal pollution (ZUKAL et al., 2015), pesticides (BAYAT et al., 2014), and urban xenobiotics (RUSSO; ANCILLOTTO, 2015; BENVINDO-SOUZA et al., 2019). Bat responses as bioindicators encompass DNA damage (SOTERO et al., 2022), immunological changes, and bioaccumulation (ZUKAL et al., 2015; FREITAS et al., 2024), aspects often investigated in this group.

Regarding genetic biomarkers, the micronucleus (MN) test is one of the most widely used methods to detect DNA damage resulting from pollutant exposure. This test is versatile, cost-effective, and can be applied to a wide variety of model organisms. In mammals, it can be easily performed on peripheral blood samples since erythrocyte maturation involves the loss of the main nucleus (UDROIU, 2006). Micronuclei can form during anaphase from lagging acentric chromosomes or chromatid fragments resulting from inadequate or non-repair of DNA breaks (FENECH et al., 2011). Although MN formation is a cytogenetic outcome commonly used in genotoxicity assessment (TRIPODI et al., 2020; CHEREDNICHENKO et al., 2024; CLARO et al., 2024), few studies have focused on natural populations of mammals exposed to polluting chemical mixtures. In bats, studies using this biomarker are even scarcer.

Considering that the biota living near mining activities have a higher risk of suffering DNA damage in blood cells than those living far from these locations (CABARCAS-MONTALVO et al., 2012), this study aimed to analyze the frequency of micronuclei in three species of bats from a mining area in the Goiano Cerrado, Brazil. The present data provide baseline values for bats in a mining area.

Material and Methods

Study area

The bats were captured in a mining area in the municipalities of Niquelândia (14°22'52.88"S, 48°25'7.24"W) and Barro Alto (15° 5'18.46"S, 48°58'32.67"W), in the state of Goiás, Brazil (BENVINDO-SOUZA et al., 2023; SOTERO et al., 2023, 2024). A previous study on water samples from this mining area found Mn, Cr, Pb, and Zn at levels exceeding national, European, and American legislation standards (BENVINDO-SOUZA et al., 2023). A total of 10 mist nets (net size: 12 x 2.5 meters) were used for bat captures, which were conducted during five nights in Niquelândia and 20 nights in Barro Alto between 2021 and 2022. The collected animals were placed in cotton bags and underwent biometric identification according to the Reis et al. (2013) criteria. *Lophostoma silvicola* (n = 6) and *Artibeus lituratus* (n = 5) were sampled in Barro Alto, while two *Platyrrhinus lineatus* were recorded in Barro Alto and four in Niquelândia. These data were approved by the animal use ethics committee of the *Universidade Federal de Goiás* (n. 004/21 and 30/21) and by the *Instituto Chico Mendes de Conservação da Biodiversidade* (n. 69513–2 and 75819).

Micronucleus test in peripheral blood

The adapted MN test was performed based on Calao-Ramos et al. (2021) and Sotero et al. (2023). About 4 µL of blood was obtained from the radial artery of each bat for the smear (two slides

per animal). The slides were air-dried and fixed with methanol for 10 minutes. Then, the slides were stained with a Panoptic Kit. A total of 1000 cells were analyzed, and photographic documentation was performed using a Lab. 1001 TB optical microscope equipped with a 3.0 MP digital camera at 100x magnification. The MN frequency was calculated using the equation: total MN/1000 × 100.

Data analysis

Data are presented as mean ± standard deviation. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied to compare the species. A significance level of $p < 0.05$ was selected.

Results and Discussion

The species *A. lituratus* (Olfers, 1818), *P. lineatus* (É. Geoffroy, 1810), and *L. silvicola* (d' Orbigny, 1836) (Fig. 1) were collected from forest remnants within a mining area. Overall, the mean frequency of MN was 6.40 ± 2.70 for *A. lituratus* (n = 5), 5.17 ± 2.64 for *P. lineatus* (n = 6), and 9.50 ± 4.42 for *L. silvícola* (n = 6), with no statistically significant differences between species ($F = 2.23$; $p = 0.14$) (Fig. 2).

Although no differences in MN frequency were observed among the 128 frugivorous and insectivorous bats reported in previous studies, the overall mean values were higher than those recorded for other species in the same area, such as *Carollia perspicillata* (frugivorous), *Glossophaga soricina* (nectarivorous), *Desmodus rotundus* (hematophagous), and *Phyllostomus hastatus* (omnivorous) (BENVINDO-SOUZA et al., 2023; SOTERO et al., 2023). This difference can be attributed to the biological material used for the analysis: the present study considered peripheral blood samples, while previous studies utilized exfoliated cells from the oral mucosa. However, future studies must evaluate which method presents greater sensitivity in detecting DNA damage.

Figure 1. Bats captured in forest remnants of a mining area for micronucleus analysis in peripheral blood samples.

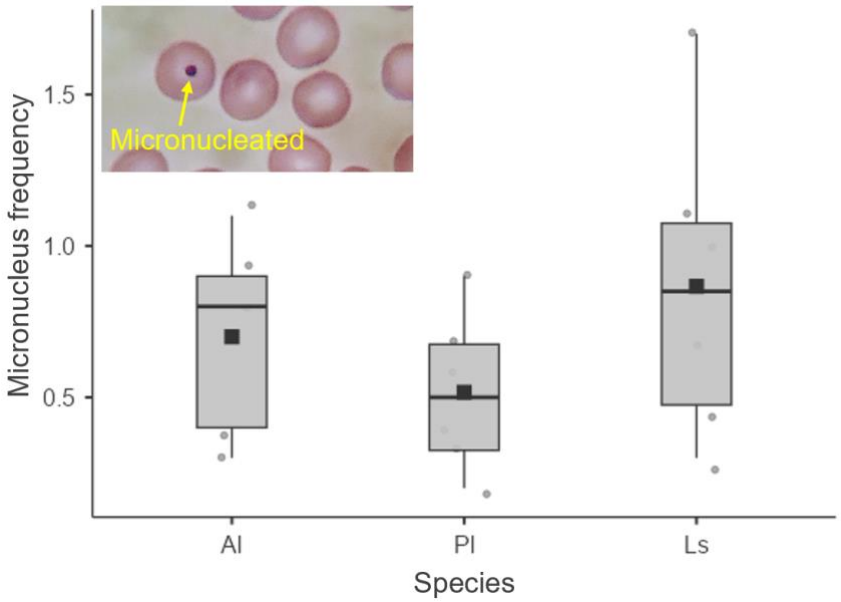

Figure 2. Frequency of micronuclei in the peripheral blood of bats. Al = *Artibeus lituratus,* Pl = *Platyrrhinus lineatus*, and Ls = *Lophostoma silvicola*.

The large fruit bat (*A. lituratus*) is a sizable species whose diet is mostly based on fruits (LAURINDO; VIZENTIN-BUGONI, 2020). In previous studies, the mean frequency of MN recorded for *A. lituratus* was 1.17 and 1.28 in agricultural areas (BENVINDO-SOUZA et al., 2022) and 0.15 in sewage treatment areas (FREITAS et al., 2024). The other frugivore (*P. lineatus)* is a bat widely distributed in South America. The species is easily recognized by a lightly colored dorsal stripe and paired facial stripes (SILVESTRE et al., 2016). *P. lineatus* presented the lowest mean of MN (1.22 ± 0.43) compared with other species, such as *A. lituratus, A. planirostris, G. soricina, Molossus molossus,* and *C. perspicillata* in agricultural areas of the Cerrado (BENVINDO-SOUZA et al., 2022). In this study, *P. lineatus* from the mining area presented a mean MN threefold higher than that observed previously. On the other hand, to our knowledge, this study was the first to evaluate the frequency of MN in the insectivore *L. silvicola*; therefore, the literature lacks prior references for the comparison of this biomarker. The high frequency of micronuclei in animals from mining areas compared with those in agricultural areas indicates a greater susceptibility to DNA damage, probably due to atmospheric pollution; however, further studies are needed. For instance, inhaling fine dust from mining can cause serious health effects, depending on the particulate load (TIAN et al., 2019). Studies have already indicated that fruit bats in mining areas accumulate metals in various tissues and exhibit more oxidative and histological damage, as observed in the state of Minas Gerais, Brazil (DESTRO et al., 2024). In southern Brazil, in a coal mining area of the Santa Catarina coal basin, insectivorous bats exhibited increased DNA damage in blood cells and metal accumulation in tissues (ZOCCHE et al., 2010). To date, this is the 15th study to employ the MN test in bats.

Previous studies have investigated spontaneous micronucleation (ZÚÑIGA-GONZÁLEZ et al., 2000), exposure to ionizing radiation (MEEHAN et al., 2004), effects of agriculture or pesticides (or both) (THIES et al., 1996; SANDOVAL-HERRERA et al., 2021; BENVINDO-SOUZA et al., 2019b; 2022; ADAM et al., 2022), urbanization (BENVINDO-SOUZA et al., 2019a) migration (OLOPADE et al., 2020), mining activities, and exposure to toxic metals (CALAO-RAMOS et al., 2021; SOTERO et al., 2023; BENVINDO-SOUZA et al., 2023), as well as the impacts of sewage treatment plants (NAIDOO et al., 2015; FREITAS et al., 2024). Given the limited number of studies on these mammals, the present study reinforces the application of the MN test as a valuable tool, as it is one of the least invasive biomarkers capable of providing relevant information about the health status of bats. Considering that a greater association between the increased frequency of MN and the presence of mining areas is already recognized (PASTOR-SIERRA et al., 2023; CRUZ-ESQUIVEL et al., 2023; ROSA MORAES et al., 2024), this study demonstrated the basal level of this mutagenic

marker for the species studied in a ferronickel mining area.

This assay is recommended for peripheral blood samples or exfoliated cells from the oral mucosa since bone marrow analysis involves the sacrifice of animals. Thus, the method used in this study is suitable for studies that do not require animal death. While the assay with blood samples quantifis only MN, the analysis of exfoliated cells also includes the scoring of binucleated cells, which indicates failures in cell division and cytotoxicity markers (e.g., karyorrhexis, pyknosis, and karyolysis) that are associated with cell death, as reported in the review by Sotero et al. (2022).

Conclusion

In summary, the MN test was applied to peripheral blood samples from *A. lituratus, P. lineatus, and L. silvicola* from a mining area in the Cerrado of Goiás. Although the observed frequency was higher than that reported for other species in previous studies, no significant difference was found between the animals analyzed in this study. Given this scenario, further studies are encouraged for these species, especially considering comparisons with reference areas (e.g., conservation units) to validate the frequency of MN in these animals. Nevertheless, the results reinforce the importance of applying the MN test in the peripheral blood of wild mammals as a valuable tool for assessing environmental health.

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