Artículo original

Evaluación de las actividades genotóxicas y antigenotóxicas de solución acuosa de *Caesalpinia ferrea* (tul.) Martius (Fabacae)

Assessment of the genotoxic and antigenotoxic activities of the aqueous solution of *Caesalpinia ferrea* (tul.) Martius (Fabacae) fruit

Kênia dos Santos Oliveira[1]
Susy Ricardo Lemes[2*] https://orcid.org/0000-0001-5303-6910
Mariah de Barros Tondato[1]
Raphaela Matsuola Alves[1]
Maria Alice Montes de Sousa[1] https://orcid.org/0000-0002-3279-4533
Lilhian Alves de Araújo[1] https://orcid.org/0000-0001-6758-4774
Cristiene Costa Carneiro[3] https://orcid.org/0000-0002-6346-2179
Lee Chen Chen[2] https://orcid.org/0000-0002-0386-247X
Paulo Roberto Melo Reis[1] https://orcid.org/0000-0001-9660-2572

[1]Pontifícia Universidade Católica de Goiás. Brasil
[2]Universidade Federal de Goiás. Brasil
[3]Universidade Federal de Goiás. Brasil

[*]Autor para la correspondencia: susynzr@gmail.com

ABSTRACT

Introduction: the inner bark of *Caesalpinia ferrea* (tul.) Martius (Fabacae), *C. ferrea)*, popularly known as jucá, has been used in alternative medicine to treat wounds, bruises, asthma and chronic cough. Furthermore, the fruits of this species are used as antidiarrheals, decongestants and in healing, and their roots as antipyretics.

Objective: to assess the possible genotoxic and antigenotoxic activities of the aqueous solution of the *C. ferrea* fruit.

Methods: this study used the Ames test in Salmonella typhimurium strains and the micronucleus test in mouse bone marrow.

Results: the Ames test results for the *C. ferrea* solution were not mutagenic in the Salmonella typhimurium TA100 strain in any of the doses tested. However, a protective effect against the action of sodium azide was shown in the TA100 strain at all the doses used. The micronucleus test indicated that the *C. ferrea* aqueous solution showed no mutagenic or antimutagenic effect.

Conclusions: it was possible to conclude that the aqueous solution of the *C. ferrea* fruit showed no mutagenic effect in bacteria and mice, but an antimutagenic effect in bacteria.

Key words: *Caesalpinia ferrea;* genotoxicity; antigenotoxic; micronucleus; Ames.

RESUMEN

Introducción: la corteza interna de *Caesalpinia ferrea* (tul.) Martius (Fabacae), *C. ferrea*, popularmente conocida como jucá, se ha utilizado en medicina alternativa para tratar heridas, hematomas, asma y tos crónica. Además, los frutos de esta especie se usan como antidiarreicos, descongestivos y en curación, y sus raíces como antipiréticos.

Objetivo: evaluar las posibles actividades genotóxicas y antigenotóxicas de la solución acuosa del fruto de C. ferrea.

Métodos: se utilizó la prueba de Ames en cepas de Salmonella typhimurium y la prueba de micronúcleo en médula ósea de ratón.

Resultados: los resultados de la prueba de Ames para la solución de *C. ferrea* no fueron mutagénicos en la cepa TA100 de Salmonella typhimurium en ninguna de las dosis probadas. Sin embargo, se demostró un efecto protector contra la acción de la azida sódica en la cepa TA100 en todas las dosis utilizadas. La prueba de micronúcleos indicó que la solución acuosa de *C. ferrea* no mostró efecto mutagénico o antimutagénico.

Conclusiones: la solución acuosa del fruto de *C. ferrea* no mostró efecto mutagénico en bacterias y ratones, sino un efecto antimutagénico en bacterias.

Palabras clave: Caesalpinia ferrea; genotoxicidad; antigenotóxico; micronúcleo; Ames.

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Introduction

C. ferrea, a tree that belongs to the family Facabeae, grows throughout Brazil and is widely distributed in the North and Northeastern regions, primarily in the states of Pernambuco and Ceará.[1,2,3] It is popularly known as pau-ferro, jucá, ibirá-obi, imirá-itá, muirá-obi, and muiré-itá.[4]

In popular medicine, the inner bark of the *C. ferrea*, is used to heal wounds, treat bruises, asthma and chronic cough.[5] The fruits are also used as antidiarrheals, decongestants (anticatarrhals) and cicatrizants, and the roots as antipyretics.[6,7] The effect of crude aqueous extract on the treatment of gastric ulcers also was described antiinflammatory and analgesic activities were reported in 1996.[8] Cardiotonic, antimicrobial, analgesic, antiinflammatory, antihistamine, antiallergic, anticoagulant and hepatotoxic activities were also characterized.[8,9]

Few studies describe the genotoxic and antigenotoxic potential of *C. ferrea*. Therefore, there is a need for investigations that assess the efficacy and safety of the aqueous solution. Identifying the mutagenic compounds in the photochemical composition of medicinal plants may determine the risks inherent to ingesting these natural compounds in traditional treatments.

Since *C. ferrea* has been widely used by the population, this study aimed at assessing the genotoxic and antigenotoxic effects of the aqueous solution of the *C. ferrea* fruit on bacteria and mice applying the Ames and micronucleus tests, respectively.

Methods

Aqueous solution of the *Caesalpinia ferrea* (tul.) Martius (Fabacae)

The plant was collected in the city of Várzea Grande in Mato Grosso state (Latitude: -15.647551 and longitude: -56.1046863).

The exsiccate of the material containing the stem, leaves, flowers and fruits was deposited and registered under no. 41.204 at the Herbarium of Universidade Federal do Estado de Mato Grosso - UFMT. The aqueous solution was prepared using 500 ml of boiling water and 10 g of fruit.

The Ames Mutagenicity Test

Bacterial Strains

Were used mutant bacterial strains of *Salmonella typhimurium* TA 100, deficient in the synthesis of histidine amino acid.

These included the following: Top-agar; 0.5% sodium chloride solution; Histidine/Biotine (0.5mM) solution; Nutrient broth; Glyphosate Minimal Medium (GMM) containing Vogel-Bonner 50X salts (heptahydrate magnesium sulphate, monohydrate citric acid, dibasic potassium phosphate, sodium phosphate and ammonium) and dextrose solution (40%).

The positive controls are specific for each strain. For the TA 100 strain, sodium azide (1.5µg/plate) was used as positive control, and sterile distilled water as negative control.[10]

The *S. typhimurium* TA 100 strains were incubated in nutrient broth at 37ºC, with constant agitation and aeration, until the stationary growth phase. To assess mutagenic activity, aliquots of bacterial strain cultures were incubated in tubes in triplicate using different doses of *C. ferrea* tea (1, 2, 3 and 5 mg/plates), for 25 minutes, with constant agitation and aeration. To assess antimutagenic activity, the positive control was co-administered with the different does of *C. ferrea* tea (1, 2, 3 and 5 mg/plates).

After incubation, liquefied glycosate agar (top-agar) was added at a temperature of 45ºC, containing a solution of histidine/biotin (0.5 mM). The contents were poured in triplicate into plates containing glyphosate minimal medium, and incubated at 37º C for 48 hours in a BOD oven. The prototrophic revertant colonies were counted for histidine, considering the mean results of the plates.[10] The results were expressed as the mean number of prototrophic revertants obtained in independent experiments conducted in triplicate.

To assess mutagenicity after counting the number of revertants, the mutagenicity ratio (MR) was calculated for each dose used. The following expression was used to calculate the mutagenicity ratio:

$$MR = \frac{\text{Mean number of revertants per test plate (spontaneous + induced)}}{\text{Mean number of revertants per test plate of negative control (spontaneous)}} \quad (1)$$

Mutagenicity was considered positive when the number of revertant colonies in the plates was greater than or equal to twice the number of spontaneous revertant colonies from the negative control.[11] The results were also assessed by the ANOVA statistical test and a p-

value of $p<0.05$ was considered significant. Furthermore, the percent inhibition (PI) of mutagenicity was calculated using the following equation:

$$PI(\%) = \left[1 - \left(\frac{\text{Number of revertants per test plate (doses } + \text{ C+)}}{\text{Number of revertants per test plate of the positive control}}\right)\right] \times 100 \quad (2)$$

Micronucleus test in mouse marrow

The present study was approved by the Animal Ethics Committee (CEUA) of Pontifícia Universidade Católica de Goiás, under protocol n°. 0002/1.

For the micronucleus test, we used 20 healthy male mice (*Mus musculus*) from Swiss Webster outbred strains, weighing between 30 and 40 grams and aged between 45 and 60 days on the day of the experiment.

For the mouse test the animals were divided into four groups of five animals. Ten animals were used for the genotoxicity and antigenotoxicity tests, 5 for the negative control and 5 for the positive control.

To assess the genotoxic activity of the aqueous solution of the *C. ferrea* fruit, 5 animals were treated intraperitoneally (ip) with a dose of 200.0 mg.kg-1 of animal body weight. To evaluate the antigenotoxic activity of the *C. ferrea*, another 5 animals were injected ip with a dose of 200.0 mg.kg-1 of *C. ferrea* concomitantly with the administration of 4 mg.kg-1 of mitomycin C (MMC). The negative control group was treated ip with sterile distilled water, while the positive controls received a single dose (ip) of 4 mg.kg-1 of mitomycin C, corresponding to 80% of DL50.

After 24 hours, the treated animals were euthanized by cervical dislocation and the femurs removed. The epiphyses of the femur were cut and the marrow washed in 1mL of fetal bovine serum. The test tubes containing the marrow and serum were centrifuged at 3000 rpm for five minutes. The surfactant was partially discarded and the cellular precipitate was transferred to the glass slide where the cell smear was made.[12,13]

After the blood smear was dried, all the slides were fixed in absolute methanol for 5 minutes in a Giemsa solution for 15 minutes.[13] Next, the slides were washed under running water and left to dry at ambient temperature.

The slides were analyzed under an optical microscope in order to detect possible changes and/or chromosomal losses (micronuclei) in the polychromatic erythrocytes (PEC) of the

marrow of animals submitted to different treatments. A total of 1000 PEC per animal were assessed.

Análisis estadística

To assess cytotoxicity normochromatic erythrocytes (NEC) were counted and the PEC/NEC ratio was determined. The means of the frequencies of micronucleated polychromatic erythrocytes (MNPEC) per 1000 PEC from the group treated with *C. ferrea* and the group treated concomitantly with MMC were compared with the negative and positive control group, respectively, using the student's t-test, with p-values < 0.05 considered significant. The PEC/NEC ratios of each treated group were compared with the respective negative and positive control groups by the chi-squared test, and p-values < 0.05 were considered significant.[14]

Results

Ames Mutagenicity Test

The results of genotoxicity and antigenotoxicity activities, as determined by the Ames test showed that the aqueous solution of the *C. ferrea* fruit was not mutagenic in *Salmonela typhimurium* strain TA 100, at any of the doses tested. However, there was a protective effect against the action of sodium azide in the strain TA 100 at all doses used, but only at the lowest doses (1 and 2 mg/plate) was there a significant difference in the number of histidine revertants from the test plates compared to the positive control plates ($p<0.05$).

Micronucleus Test in Mouse Bone Marrow

In the assessment of genotoxic and antigenotoxic activities of *C. ferrea*, as measured by the micronucleus test of mouse bone marrow, the results obtained for the frequency of micronucleated polychromatic erythrocytes (mean +/- standard deviation) and the PEC/NEC ratio are demonstrated in tables 1 and 2, respectively.

Table 2 shows mild cytotoxic activity in the aqueous solution of *Caesalpina ferrea*. However, the dose of the solution analyzed (200.0 mg.kg-1 body weight) exhibited no difference ($p>0.05$) at a frequency of MNPEC in relation to the negative control group. With respect to the positive control group, the differences were significant ($p <0.05$).

The PEC/NEC ratio for the solution of 200.0 mg.kg-1 of *C. ferrea* plus 4 mg.kg-1 of mitomycin C showed no statistical difference when compared to the positive control ($p>0.05$). However, there was a significant difference for this treatment when compared to the negative control ($p<0.05$).

Table 3 shows no antigenotoxic activity in the aqueous solution of the *C. ferrea* fruit administered concomitantly with mitomycin C. There was no significant difference between the MNPEC frequency of the treatment dose and that of the positive control ($p>0.05$). There was a significant difference in relation to the negative control group ($p <0.05$).

Discussion

To assess the mutagenic and antimutagenic activities of the *C. ferrea* solution, micronucleus tests of mouse bone marrow were carried out as well as the Ames mutagenicity test in *Salmonella typhimurium* strain TA100.

The results of the micronucleus test indicate the absence of mutagenic activity and mild cytotoxicity of the *C. ferrea* solution at a dose of 200.0 mg.kg-1 of body weight, since there was no increase in the frequency of micronuclei in polychromatic erythrocytes, or significant decrease in the PEC/NEC ratio when compared to the results obtained in the negative control group.

These findings corroborate with some reported, where the aqueous extract of the *C. ferrea* seed showed no acute toxicity in mice even when the maximum dose (0.3mL.10g-1 of body weight) was administered.[15] Other research found that the mixture of compounds contained in the extracts did not cause a significant increase in the number of cells with micronuclei or chromosomal aberrations, when administered at doses of 500, 1000 and 1500 mg / kg of body weight in mice, respectively.[16]

There are few studies describing which secondary metabolites are present in *C. ferrea.* Were detected flavonoids, saponins, tannins, sterols and phenolic compounds in the leaves and bark of *C. ferrea*.[17,18]

According researches, saponins are present in the crude aqueous extract of *C. ferrea* fruits. Thus, it is suggested that a possible reduced concentration of this metabolite contributed to not detecting the genotoxic activity of the *C. ferrea*.[19,20]

A study found a low concentration of tannins and other phenolic compounds (less than 1%) in the stem of *C. ferrea*.[21] In addition to saponins, tannins are also associated with

genotoxic activity.[22,23] These compounds capture free radicals by intercepting active oxygen, thereby allowing the formation of stable radicals. However, there are few literature reports on tannins and their intervention in pathological processes.[24]

It is important to underscore that the genotoxic effect, when observed in the different studies, is often dependent on the dose of the plant product tested as well as the interaction between its active compounds.[25] The dose of 200.0 mg.kg-1 of body weight tested in the present study did not result in genetic damage to the mice.

Also in this study, the group of animals treated simultaneously with the solution of *C. ferrea* and MMC exhibited no significant difference in the frequency of micronuclei in polychromatic erythrocytes when compared to the positive control group ($p>0.05$), indicating the absence of a protective effect against DNA damage caused by MMC. I*n vitro* experimental studies of colon cancer cell lines indicated the protective activity of saponins against the risk of developing this cancer. This attribution is due to the reduction in iNOS, prostaglandins and COX-2, decline in nuclear translocation of NF-KB p50 and p65 subunits and induction of apoptosis by suppression of Bcl-2, in addition to the increased expression of the BAX protein.[26,27] Thus, it is suggested that a low concentration of genoprotective dose-dependent compounds (such as saponins) may have prevented detection of the antigenotoxic effect of *C. ferrea,* since saponins and tannins are primary constituents of this plant.[17,18]

In addition to the micronucleus test, the Ames mutagenicity test was also carried out in *S. typhimurium* strain TA100 in order to assess the mutagenic and antimutagenic activities of the *C. ferrea* solution. The results show that the *C. ferrea* solution was not mutagenic in *S. typhimurium* strain TA100 at any of the doses tested. However, a protective effect against sodium azide was detected in strain TA100 at all the doses used.

Others results described that the extract of *C. pyramidalis* and *C. ferrea* exhibited a zone of inhibition greater than 11mm and 17mm respectively against bacterial strains of *Enterobacter gergoviae, Escherichia coli* and *Staphylococcus aureus*.[28] This effect has been attributed primarily to the presence of phenolic compounds (such as tannins) present in the genus *Caesalpinia,* which are capable of inhibiting the activity of gram-positive and gram-negative bacteria.[29]

Given the findings of the present study it was possible to conclude that in the experimental conditions used, the aqueous solution of the *C. ferrea* fruit showed no mutagenic effect in bacteria and mice, but an antimutagenic effect in bacteria.

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Conflict of interest

The authors declare that they have no conflict of interest.