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CARALUMA FIMBRIATA AND CASSIA NOMAME EXTRACTS EVALUATION: MUTAGENICITY OR ANTI-MUTAGENICITY?

***Natani Ribeiro Demarco, Maruhen Amir Datsch Silveira, Thayná Assakawa dos Santos, and Luciana Paula Grégio d'Arce**

Department of Genetics, Universidade Estadual do Oeste do Paraná, Brazil

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ABSTRACT

Caraluma fimbriata and *Cassia nomame* are native plants in India and Japan, respectively, and are very used in extract form to prevent obesity: loss of appetite and satiety, and also forcing fat reserves to be burned. The *Allium cepa* test has great correlation with mammalian test, being very efficient to analyze mutagenic and anti-mutagenic effects of substances, exposed in direct contact with its roots, through of mitotic index (MI) the micronuclei frequency (MN) and chromosomal aberrations (CA) analyzes. Few studies was made with these plants, thus, this test was used to evaluate the extracts of *C. fimbriata* and *C. nomame* as its mutagenicity and anti- mutagenicity through the *Allium cepa test*. This study showed that *C. nomame* extract was anti-mutagenic, with differences in MN and MI frequencies. Already *C. fimbriata* did not presented to be mutagenic or anti-mutagenic in the test system.

INTRODUCTION

Caraluma fimbriata is a asclepiadácea family plant commonly found in India, Africa and Europe (Gujjala *et al.,* 2016; Kuriyan *et al.,*2007). Known for its ability to decrease appetite and quench thirst, this cactus is regularly consumed by the native population of India, mostly to quench their thirst on long hunting (Hadadare and Salunkhe, 2013). It also acts in the appetite control mechanism, and blocks the activity of several enzymes that form fat blocks, forcing fat reserves to be burned (Kuriyan *et al.* ,2007). Listed as one of the main plants of this country, *C. fimbriata* is also known by the Indian Ministry of Health as one of their medicinal plants. Its components include sitosterol, Hexadecanoic acid, Oleic acid, pregnane glycosides, flavone glycosides, megastigman glycosides, bitter principles, alkaloids, saponins, flavonoids. etc (Hadadare and Salunkhe, 2013; Naingade *et al.,* 2013). In turn, the Cassiolamina is a vegetable standardized extract of *Cassia nomame*, which contains in its fruits five flavonoid compounds, which have the ability to inhibit lipase enzyme, responsible for digest and absorb fat in the body (Elkins, 2000).

****Corresponding author: Natani Ribeiro Demarco,***
Department of Genetics, Universidade Estadual do Oeste do Paraná, Brazil

Found on the banks of rivers across Japan, it is a drink conventionally to reduce levels of cholesterol, uric acid and sugar in blood (Elkins, 2000). The *Allium cepa* test has great correlation with the results obtained from mammalian test (Grant, 1982; Chaparro *et al.,* 2010), with an 82% greater sensitivity compared to rodents (Rank and Nielsen, 1994), is relatively inexpensive, and has a wide analytical range. This assay demonstrates alterations in all phases of the cell cycle, which are considered evidence for mutagenic effects induced by clastogenic or aneugenic agents (classified according to the type of alteration induced) (Vidakovié-cifrek *et al.,*2002). Some of these alterations, such as chromosomal breaks and asynchronous micronuclei (MN), are chromosomal aberrations (CA) used to evaluate mutagenicity (Sobral *et al.,* 2013). In addition, its roots remain in direct contact with the material tested, showing the mutagenic and anti-mutagenic effects of the substance being tested through their meristematic cells (Bagatini *et al.,* 2007). Due to few studies in *C. fimbriata* and *C. nomame*, this work evaluated the mutagenicity and anti-mutagenicity of extract of these plants in the *A. cepa* test.

MATERIAL AND METHODS

The extracts of *C. nomane* and *C. fimbriata* were obtained at pharmacy of manipulation in powder form. Seeds of *A. cepa*

were germinated at room temperature (25º C) in petri dishes with filter paper soaked in distilled water and, after growth of 1 cm, the roots were submitted to different treatments, each of which was performed in triplicate. Two control groups were used: negative and positive, respectively treated with distilled water (for 72 and 96 hours) and Methyl Methanesulfonate (MMS, ACROS, Geel, Belgium) for 72 hours at a concentration of 4.10-4M. For the mutagenicity evaluation, the roots were submitted for 72 hours to the extract of the plants only, in three different concentrations. Was used the concentrations of: 0.75 g/ml, 0.50 g/ml and 0.25 g/ml of both extracts: *C. frimbriata* and *C. nomame*, diluted in distilled water to evaluate the antimutagenic potential, three different protocols were used:

- Pretreatment: when the root were treated with MMS for 72h and then more 72 hours with the extract (72h), in order to evaluate the bio-antimutagenic potential;
- Simultaneous treatment: the roots were simultaneously submitted to MMS and the extract for 72h, to evaluate the desmutagenic potential;
- Posterior treatment: the roots were treated with the extract for 72 hours and then more 72 hours with MMS, in order to evaluate the desmutagenic effects.

Fixation was accomplished with Carnoy for 24 h after which the slides were stained based on protocol of Grant (1982), with modifications: using Shiff´s reagent for 1 hour and Acetic Carmine in the slides for counter staining.

aid of an Olympus DP 71 camera mounted onto an Olympus BX 60 using the DP manager software (version 3.1.1.208).

Statistical Analyze

The data were analyzed by One Way ANOVA test. When differences were detected at a significance level of 0.05 between the groups, the Dunett test was followed. Statistical analysis was performed with the software SigmaPlot 3.5 (Systat Software, Inc.).

RESULTS

No statistically significant changes were observed in AC frequencies, MI and MN in *C. fimbriata*, and *C. nomame*. Some of the cellular changes found during the analysis were chromosomal breaks in metaphase and/or anaphase, lagging chromosomes and bridges in anaphase or telophase, micronucleus, metaphase multi-polar division with chromosomal loss (Figure 1). Regarding anti-mutagenicity, in *C. nomame* the frequencies of AC and MN were presented statistically lower ($p < 0.05$). For AC, concentrations of 0.75 g / ml in concurrent treatment, 0.50g / ml and 0.75 g / ml in the pretreatment and all three concentrations (0.25g / ml 0.50g / ml and 0.75 g / ml) in posterior treatment were lower when compared to the positive control (MMS) ($p < 0.05$). For MN, all three concentrations in the three different treatments showed lower frequencies of MN ($p < 0.05$) when compared to positive control.

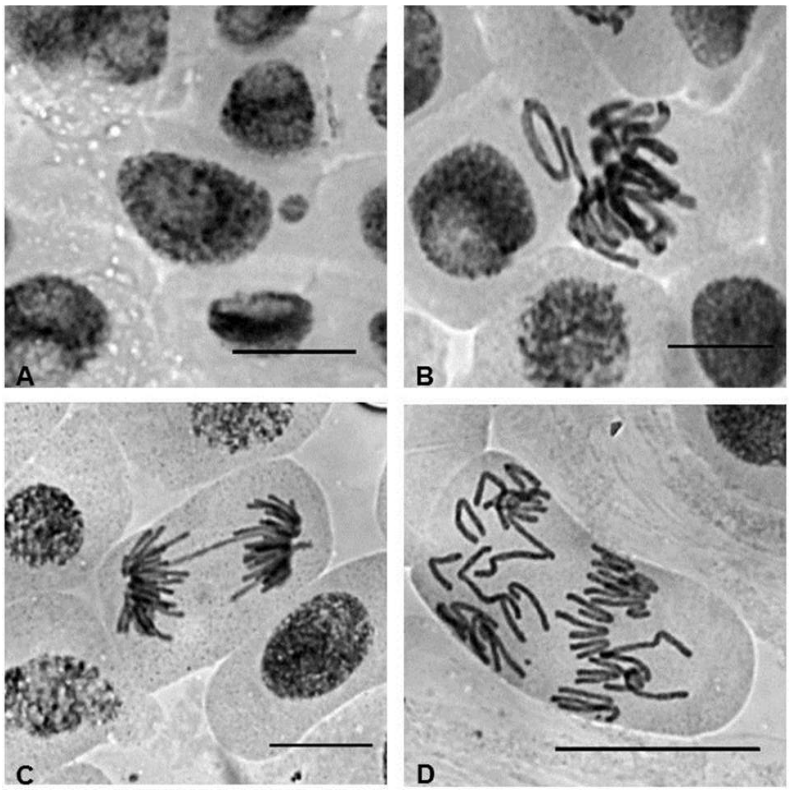

Figure 1. Some of the genetic alterations found on the blades analysis: (A) Micronuclei; (B) Metaphase with ring chromosome; (C) Bridge anaphasic; (D) Multi-polar division with chromosomal loss. All pictures with scale of 50 µm

Each treatment consisted in the analyze of 5.000 meristematic cells in 5 slides (1.000 cells per slide). Three biological replicates were evaluated, totalizing 15.000 meristematic cells analyzed per sample. Three genetic parameters we evaluated, the mitotic index (MI) the micronuclei frequency (MN) and chromosomal aberrations (CA). Images were captured with the

As for IM, it could not be assessed because MMS is not able to induce cell division, only breakages and losses of genetic material (Table 01). In *C. fimbriata* were observed no statistically significant differences when compared to the positive control ($p > 0.05$) (Table 02).

Table 01. Averages and standard deviations of the frequencies of micronuclei, chromosomal aberrations and mitotic index for 5000 cells analyzed

Treatments	Micronuclei	Cromossomal aberrations	Mitotic index
Water 72h	0.00133 ± 0.000416	0.000800 ± 0.000400	0.0373 ± 0.00239
Water 144h	0.00127 ± 0.000306	0.000800 ± 0.000200	0.0401 ± 0.00175
1ª Conc.*	0.00167 ± 0.000115	0.00120 ± 0.000400	0.0463 ± 0.00651
3ª Conc.*	0.00180 ± 0.000200	0.000733 ± 0.000115	0.0461 ± 0.00447
5ª Conc.*	0.00207 ± 0.000808	0.00147 ± 0.000503	0.0489 ± 0.00180
MMS	0.00440 ± 0.000529	0.00160 ± 0.000200	0.0379 ± 0.00716
MMS + 1ª Conc.**	0.00373 ± 0.000416*	0.001000 ± 0.000200	0.0332 ± 0.00365
MMS + 3ª Conc.**	0.00247 ± 0.000503*	0.00127 ± 0.000306	0.0410 ± 0.001000
MMS + 5ª Conc.**	0.00280 ± 0.000529*	0.000600 ± 0.000346*	0.0384 ± 0.00597
MMS > 1ª Conc.***	0.00180 ± 0.000800*	0.00113 ± 0.000231	0.0361 ± 0.00304
MMS > 3ª Conc.***	0.00213 ± 0.000757*	0.001000 ± 0.000200*	0.0427 ± 0.00503
MMS > 5ª Conc.***	0.00213 ± 0.000808*	0.000867 ± 0.000306*	0.0401 ± 0.00359
1ª Conc. > MMS****	0.00133 ± 0.000416*	0.000600 ± 0.000200*	0.0397 ± 0.00280
3ª Conc. > MMS****	0.00200 ± 0.000400*	0.000867 ± 0.000503*	0.0365 ± 0.00841
5ª Conc. > MMS****	0.00140 ± 0.000529*	0.000733 ± 0.000231*	0.0312 ± 0.00812

*Different concentrations (Conc.) of extract. ** Simultaneous treatment. *** Pretreatment with MMS. ****Posterior treatment with MMS. Different letters (*) refer to statistically relevant differences.

Table 2. Averages and standard deviations of the frequencies of micronuclei, chromosomal aberrations and mitotic index for 5000 cells analyzed

Treatments	Micronuclei	Cromossomal aberrations	Mitotic index
Water 72h	0.000467 ± 0.000503	0.000733 ± 0.000306	0.0381 ± 0.0152
Water 144h	0.000333 ± 0.000306	0.000733 ± 0.000462	0.0396 ± 0.00327
1ª Conc.*	0.000333 ± 0.000306	0.001000 ± 0.000600	0.0343 ± 0.00941
3ª Conc.*	0.000600 ± 0.000529	0.000933 ± 0.000306	0.0360 ± 0.00386
5ª Conc.*	0.000400 ± 0.000346	0.00133 ± 0.000643	0.0355 ± 0.00595
MMS	0.00440 ± 0.00156	0.00280 ± 0.000917	0.0361 ± 0.0103
MMS + 1ª Conc.**	0.00113 ± 0.00163	0.00167 ± 0.00160	0.0261 ± 0.0269
MMS + 3ª Conc.**	0.00160 ± 0.00191	0.00133 ± 0.000577	0.0261 ± 0.0269
MMS + 5ª Conc.**	0.00213 ± 0.00232	0.00167 ± 0.00110	0.0377 ± 0.0103
MMS > 1ª Conc.***	0.00327 ± 0.00272	0.00180 ± 0.00160	0.0367 ± 0.00963
MMS > 3ª Conc.***	0.00340 ± 0.00342	0.00253 ± 0.00232	0.0327 ± 0.00901
MMS > 5ª Conc.***	0.00260 ± 0.00178	0.00153 ± 0.00103	0.0295 ± 0.00303
1ª Conc. > MMS****	0.00200 ± 0.00122	0.00233 ± 0.00163	0.0554 ± 0.0151
3ª Conc. > MMS****	0.00180 ± 0.00140	0.00207 ± 0.000231	0.0453 ± 0.0101
5ª Conc. > MMS****	0.00153 ± 0.000231	0.00187 ± 0.000757	0.0442 ± 0.0113

*Different concentrations (Conc.) of extract. ** Simultaneous treatment. *** Pretreatment with MMS.
****Posterior treatment with MMS. Different letters (*) refer to statistically relevant differences.

DISCUSSION

MN results from damage in the genetic material, and can be seen in all stages of cell division. When chromosomal fragments resulting from breaks or even whole chromosomes not correctly segregated are not incorporated into the main body of daughter cells after mitosis, it results in an MN (Salvadori *et al.,*2003). The AC may be structural, when parts of chromosomes rearrange, or numerical resulting from errors in the segregation of chromosomes in cell division (Snustad and Simmons, 2008). Thus, MN and AC are injuries of important indications in the genetic material, and efficient markers mutagenicity. As a lack in literature, our study is the first to evaluate both extracts in *A. cepa* test, evaluating mutagenic and anti-mutagenic effects. As observed in our study, *C. nomane* had no mutagenic effect in *A. cepa* test. It shows no genotoxic and mutagenic potential. By the way, *C. nomame* showed anti-mutagenic effects. Konishi *et al*. (2004) found that cassiolamina, the extract of *C. nomame*, showed potential anti-clastogenic, as confirmed by our study, as this extract showed to be desmutagenic. Clastogenicity is the process of breaking the genetic material and one of the pathways to formation of MN (Villela *et al.,*2003). As previously described, our results showed anti-clastogenic potential and desmutagenic potential in all three different concentrations and treatments, so, we might confirm that *C. nomame* prevents the mutagen to enter the cell, probably performing a protective effect on the plant wall of meristematic cells of *A. cepa*, and acts inactivating the mutagens that escape this protective effect, as seen in the simultaneous and posterior treatment. Despite *C. fimbriata* being one of the main supplements for weight loss and satiety (Astell *et al.,*2013), it did not show any mutagenic nor antimutagenic effects, confirming that while this plant has no protective effects against mutagens, it offered nor any mutagenic or genotoxic risk.

Conclusion

C. nomame and *C. fimbriata* showed no mutagenic effects in *A. cepa*. *C. nomame* in addition to a loss of appetite and satiety effect showed in literature, showed an anti-mutagenic effect to reverse and prevent damage. *C. fimbriata* showed no anti-mutagenic effects. This data confirm no genotoxic risk in the use of both extracts, but further studies should be made.

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