Averrhoa carambola L., Syzygium cumini (L.) Skeels and Cissus sicyoides L.: medicinal herbal tea effects on vegetal and animal test systems

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ABSTRACT. Since folk medicine has been greatly appreciated for centuries, many researchers decided to study more deeply the curative qualities of plants. In the present study, meristematic cells of Allium cepa L. were used as vegetal test system and bone marrow cells of Wistar rats as animal test system. Both were treated in vivo to evaluate whether the plants Averrhoa carambola L., Syzygium cumini (L.) Skeels and Cissus sicyoides L. presented cytotoxic and mutagenic effects and whether they resulted in cell alterations in their morphology, chromosomes or cell cycle division. Herbal teas were prepared as normally done by the population, albeit in two different concentrations, the usual concentration and a concentration ten times higher. Rats were treated with only one concentration of teas. Results showed that teas did not alter the cell cycle of Allium cepa L., with the exception of the 24 hours analysis after suspension of treatment (recovery of treatments), with a lower concentration of Averrhoa carambola. The latter had a low mitotic index when compared to control and to the post-treatment analysis, showing an inhibition of cell division. The three herbal teas neither induced an increase in the number of chromosomal damage in bone marrow cells of Wistar rats nor altered the cell division cycle. Results are important in so far as these plants are used as therapeutic agents.

Key words: medicinal herbs, chromosome damage, mutagenicity test.

RESUMO. Averrhoa carambola L., Syzygium cumini (L.) Skeels e Cissus sicyoides L.: efeitos dos chás de plantas medicinais sobre os sistemas-teste vegetal e animal. As plantas medicinais têm sido muito estudadas devido aos seus efeitos curativos. Neste estudo foram utilizados o sistema teste vegetal em células meristemáticas de Allium cepa L. e o sistema teste animal em células da medula óssea de ratos Wistar tratados in vivo para avaliação dos efeitos citotóxicos e mutagênicos das plantas Averrhoa carambola L., Syzygium cumini (L.) Skeels e Cissus sicyoides L., analisando-se o ciclo de divisão celular, morfologia e cromossomos. Os chás das plantas foram preparados da mesma maneira usada pela população e em duas diferentes concentrações, a usual e outra dez vezes maior. Os chás foram aplicados em doses únicas nos ratos. A avaliação demonstrou que os chás não alteram o ciclo celular de Allium cepa L., exceto na análise 24 horas após a retirada do tratamento (recuperação dos tratamentos) com a menor concentração de Averrhoa carambola L., o qual apresentou um baixo índice mitótico quando comparado ao controle e à análise imediatamente após o tratamento, mostrando uma inibição da divisão celular. Os três chás não induziram aumento do número de alterações cromossômicas em células da medula óssea de ratos Wistar e não alteraram o ciclo de divisão celular. Os resultados são importantes pelo fato de que essas plantas são usadas como agentes terapêuticos pela população.

Palavras-chave: plantas medicinais, aberração cromossômica, teste de mutagenicidade.

Medicinal herbs have been popularly used in tea form all over the world, although nearly all of them have never been scientifically tested. The teas and plant infusions may have substances with toxic or even mutagenic effects. On the other hand, the consumption of teas can suppress the effects of certain powerful mutagenic agents in human beings. Averrhoa carambola L., Syzygium cumini (L.) Skeels and Cissus sicyoides L. are examples of medicinal plants used as teas and whose cytotoxic and mutagenic effects are unknown.
The star fruit tree (*Averrhoa carambola*) is a small, shrub-like ornamental tree with eatable fruit, rich in vitamins, phosphorus and oxalic acid. The plant belongs to the Oxalidaceae family and is native to India. The star fruit has an antidiabetic and high blood tension lowering-effect, an appetite stimulator, and an anti-diarrhea, anti-scavity, antipyretic attributes (Moreira, 1985). It is used topically on poisonous bites and stings (Iamoni, 1997), and its fruit is prescribed against eczemas. It is a diuretic and combats kidney and bladder diseases.

*Syzgium cumini* is a big shrub from the Myrtaceae family, native to southern Asia. The bark of the tree is used against dysentry, hemorrhage and leukorrea (Moreira, 1985). It is also used to treat noninsulin-dependent type II diabetes, because it lowers the blood glucose level to normal (Moreira, 1985; Conceição, 1987; Iamoni, 1997). It has also a diuretic effect (Silva-Netto et al., 1987). It is used to treat diarrhea and infections from the upper-respiratory-tract since it has an antimicrobial property (Corrêa et al., 1998). Its chemical composition consists of tannins, resins (gambol), terpans (α-pigeon, β-pigeon, limonene), acids (gallic, palmitic, stearic, oleic), steroids (phytosterol), saponinic glycosides (antimelin) and flavanols (Albuquerque, 1989; Corrêa et al., 1998).

*Cissus sicyoides* belongs to the Vitaceae family. It is used against rheumatism, abscesses, muscle inflammation, epilepsy, stroke and convulsions. Also, it is a hypotensor and bloodstream activator, and causes deep sleep and perspiration (Santana, 1984). Its leaves are largely used for treating diabetes and are known as “similar to insulin” because of their hypoglycemic effect (Martins et al., 1995). The tea is diuretic and good for the kidney. It expels impurities and also stones from the bladder and kidney. It also balances the blood pressure and ensures healing of wounds. Some of its components are α- and β-carotenenes.

To evaluate the cytotoxic and mutagenic effects of these three herbal teas the meristematic cells of *Allium cepa* L. root were used as vegetal test system; bone marrow cells of Wistar rats were used as animal test system; both were treated *in vivo*. Our aim was to evaluate whether the plants caused morphological cell alterations, as chromosome aberrations and in the cellular division cycle.

**Material and methods**

**Medicinal herbs.** *Averrhoa carambola*, *Syzgium cumini* and *Cissus sicyoides* were obtained from the Irenice Silva Medicinal Herb Garden of the State University of Maringá. The infusions were prepared in the same way as usually done by the population, by adding boiling water to the leaves *in natura* and then leaving them to stand for cooling before straining. Teas were prepared in two different concentrations, one corresponding to that normally used by the population (0.07 mg/ml) and another ten times higher (0.70 mg/ml).

**Allium cepa root-tip cells.** The bulbs were placed in flasks with aerated water to root at room temperature. We considered the control group as 0 hour until the first root sample was obtained to serve as a control of the bulb (Co). These were then placed for 24h either into the three plant teas prepared in the two different concentrations or into the water in the control group. Next, some more roots were removed (Tr) and the bulbs returned to water for 24h to observe whether there was any recovery (Re) from possible damage. Roots were fixed and stained with Feulgen reaction and mounted on permanent slides.

The slide analysis was done in a “blind” test under a 40x lens optical microscope. One thousand cells per bulb were analyzed, adding up 6,000 per control, treatment and respective recovery. Cells with morphological structural alterations were analyzed and the Mitotic Index (MI) determined. Statistical analysis was done by the Chi-square test ($\alpha = 0.05$).

**Bone marrow cells of Wistar rat.** Wistar rats, *Rattus norvegicus*, were obtained from the Central Vivarium of the Maringá State University, weighing approximately 100 g (b.w. - body weight). Three males and three females were used for both the control and the treatment groups. Rats were put down 24h by intraperitoneal injection, after only treatment with 1ml of the tea solutions with the two different concentrations (0.07 mg/ml and 0.7 mg/ml) of the three plants.

One hour and a half before being killed, a dose of 0.5 ml/100g b.w. of colchicine 0.16% was injected in the animals. Bone marrow cells were obtained according to Ford and Hamerton (1956), with some modifications. Chromosomal analysis of the slides was done under a 100x immersion lens optical microscope. One hundred metaphases per animal were analyzed in a “blind” test, adding up 600 metaphases per control and treatment group. For each sex, the MI was calculated for the 5,000 cells, adding up 10,000 cells per group. The statistical analysis was done by the Chi-square test ($\alpha = 0.05$). The animal positive controls were treated with 1.5 mg cyclophosphamide (CP)/100 g body weight.
Results

Allium cepa root-tip cells. Table 1 shows the results of total mean of the Mitotic Index (MI), total number of analyzed cells and the cell number at the different cell cycle phases (interphase, prophase, metaphase, anaphase and telophase). They were obtained for each group of six onions: the control-control, the control and the treatment, with the teas from Averrhoa carambola L., Syzygium cumini and Cissus sicyoides plants, with the two different concentrations and respective recoveries.

Total chi-square was calculated for the mitotic total mean indexes between the controls and respective recoveries, between the controls and respective recoveries and between controls and respective recoveries and, also, between these results and the one obtained for the control at the respective times for sampling. Only the result obtained between the control at 0.07 mg/ml concentration of Averrhoa carambola and its respective recovery (χ² = 6.21, α = 0.05) was statistically significant. After treatment with 0.07 mg/ml concentration of Averrhoa carambola tea, the root’s meristematic cells of the six bulbs did not respond to the recovery process, showing a mitotic total mean index of 2.3. This is equivalent to half the treatment’s mitotic index (5.6) and a quarter of the control’s mitotic index (10.3). It seems that this inhibitory effect of the treatment was cumulative because there was a maintenance or even an increase of this effect after the recovery time in water. Such significant result was repeated when this recovery result was compared to the one obtained at 48h of the control in water (MI=8.0 e χ² = 4.06, α = 0.05), confirming the lack of recovery after this treatment. In the remaining treatments with the three plants and the control and in spite of the decrease in the mitotic index after 24h which have been maintained, in most cases, after 24h of recovery in water, these differences in the mitotic total mean index were not statistically significant (α = 0.05). This fact shows that neither the treatments nor the recoveries altered the cell cycle when it is compared to controls.

Bone marrow cells of Wistar rats. Table 2 shows the results of total mean of MI, the total metaphases analyzed and the number of alterations obtained from male and female Wistar rats, non-treated control and treated with the Averrhoa carambola, Syzygium cumini and Cissus sicyoides teas, using the two different concentrations during the acute treatment.

Table 1. Total cells analyzed, total number of cells in different phases of the cell cycle (I, P, M, A and T) and total mean of Mitotic Index (MI), obtained from different groups in Allium cepa L. root-tip cells, control-0h (Co), treatments-24h (Tr) and respective recoveries-24h (Re) with the two different concentrations of Averrhoa carambola (Ac), Syzygium cumini (Sj) and Cissus sicyoides (Cs).

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Cell MI</th>
<th>Number of Cells</th>
<th>Total %</th>
<th>I</th>
<th>P</th>
<th>M</th>
<th>A</th>
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<td>(mg/ml)</td>
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<tr>
<td>Control</td>
<td>Co 6000 14.1 5157 456 155 154 78</td>
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<td></td>
<td>Tr 6000 11.6 5387 306 123 151 59</td>
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<td></td>
<td>Re 6000 8.0 5518 267 114 68 33</td>
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<tr>
<td>Ac (0.07)</td>
<td>Co 6000 10.3 5385 341 135 91 48</td>
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<td></td>
<td>Tr 6000 5.6 5662 197 76 47 18</td>
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<td></td>
<td>Re 6000 2.3 5861 82 23 25 09</td>
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<tr>
<td>Ac (0.70)</td>
<td>Co 6000 8.7 5478 326 97 68 31</td>
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<td></td>
<td>Tr 6000 6.6 5602 215 103 56 24</td>
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<td></td>
<td>Re 6000 8.3 5504 274 103 86 33</td>
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<tr>
<td>Sj (0.07)</td>
<td>Co 6000 10.5 5371 376 107 95 51</td>
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<td>Tr 6000 6.8 5593 235 81 59 32</td>
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<td>Re 6000 8.6 5486 279 105 91 39</td>
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<td>Sj (0.70)</td>
<td>Co 6000 8.2 5507 279 113 56 45</td>
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<td>Tr 6000 7.0 5579 263 96 46 16</td>
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<td></td>
<td>Re 6000 6.8 5594 211 103 59 33</td>
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<tr>
<td>Cs (0.07)</td>
<td>Co 6000 8.7 5479 330 92 50 49</td>
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<td>Tr 6000 8.6 5483 236 102 79 45</td>
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<td>Re 6000 8.0 5521 268 93 76 42</td>
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<tr>
<td>Cs (0.70)</td>
<td>Co 6000 10.4 5376 400 105 76 43</td>
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<td></td>
<td>Tr 6000 9.2 5451 306 121 78 44</td>
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<td></td>
<td>Re 6000 8.9 5468 317 118 58 39</td>
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</table>

Table 2. Total mean of Mitotic Index (MI), total and types of alterations and total metaphases analyzed from Wistar rats, control (Co), positive control (CP) and treated (acute treatment) with two different concentrations of Averrhoa carambola (Ac), Syzygium cumini (Sj) and Cissus sicyoides (Cs).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>MI</th>
<th>Total number of cells with alteration (%)</th>
<th>Number of types chromosomal alterations</th>
<th>Total number of metaphase analyzed</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg/ml)</td>
<td>%</td>
<td>cr ct cr ct</td>
<td>cr ct cr ct</td>
<td>cr ct cr ct</td>
</tr>
<tr>
<td>Control</td>
<td>1.32 2</td>
<td>2 (0.3)</td>
<td>0 1 1 0</td>
<td>600</td>
</tr>
<tr>
<td>CP</td>
<td>1.32 79 (13.2)*</td>
<td>6 0 46 27</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>Ac (0.07)</td>
<td>1.55 1</td>
<td>1 (0.2)</td>
<td>0 1 0 0</td>
<td>600</td>
</tr>
<tr>
<td>(0.70)</td>
<td>1.83 0</td>
<td>0 0 0 0</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>Sj (0.07)</td>
<td>1.51 1</td>
<td>1 (0.2)</td>
<td>0 0 0 0</td>
<td>600</td>
</tr>
<tr>
<td>(0.70)</td>
<td>2.21 0</td>
<td>0 0 0 0</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>Cs (0.07)</td>
<td>2.28 1</td>
<td>1 (0.2)</td>
<td>0 0 0 0</td>
<td>600</td>
</tr>
<tr>
<td>(0.70)</td>
<td>2.49 0</td>
<td>0 0 0 1</td>
<td>600</td>
<td>600</td>
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</table>

Chi-square test (α = 0.05) showed that Averrhoa carambola Syzygium cumini and Cissus sicyoides teas at 0.07 and 0.70 mg/mL did not induce a statistically significant increase in the number of the cell with chromosome alterations in bone marrow cells of Wistar rats. Compared to the results obtained for the
non-treated controls, there was no alteration in the cell division index after the treatments.

**Discussion**

Data show that teas from *Averrhoa carambola*, *Syzygium cumini* and *Cissus siyoides*, tested in concentrations used by the population and in concentrations ten times higher than usual in a twenty-four hour treatment, caused no alteration in the cellular cycle of meristematic cells of *Allium cepa* L. (except in the recovery group treated with water during 24h after 0.07 mg/mL of *Ac* tea). The *Allium cepa* L. test system is well accepted for the study of cytotoxic effects, because its roots are in direct contact with the tested substance, allowing the evaluation of different concentrations at different times of treatment. Chromosomal alterations and alterations of the meristematic cells’ cycle division of the onion root have been frequently used to warn the population about the consumption of the product. Current research presents no indication against the ingestion of the teas of the three analyzed plants. This observation seems to be corroborated by the results obtained in the animal test system.

Recent studies show that the risk of cancer formation has been reduced with the consumption of green tea (Gao et al., 1990). Among the beneficial effects of this tea are inhibition of carcinogenesis and mutagenesis (Wang et al., 1991, 1992; Yamane et al., 1991; Yang and Wang, 1993, Yamada and Tomita, 1994); prevention of arteriosclerosis (Kono et al., 1992; Stamper and Rimm, 1993); reduction of serum cholesterol (Kono et al., 1992; Stensvold et al., 1992) and inhibition of nitrosamine formation in nitrosation reactions (Jain et al., 1989; Stich, 1992). Most of these effects have been attributed to the antioxidative and free-radical scavenging properties of tea, particularly of polyphenolic compounds (Yang and Wang, 1993; Ho et al., 1992; Klausnig, 1992, Stavric et al., 1996). Several studies show that the antimutagenic and anticarcinogenic effects of green tea are due to the presence of tea polyphenols and catechins, especially epigallocatechin gallate (Yang and Wang, 1993, Fujiki et al., 1992, Mukhtar et al., 1994). Using rat erythroblastic leukemic cells, Fujie et al. (1993) verified that crude catechins extracted from green tea suppressed sister-chromatid exchanges induced by trihalomethanes, formed in the chlorination process of water. The aqueous extract from green tea showed a powerful antimutagenic effect in routinely used concentrations in human’s daily diet against the major classes of occupational carcinogens (Bu-Abbas et al., 1994a). In another work Bu-Abbas et al. (1994b) evaluated the selective induction of certain hepatic proteins and peroxisomismic proliferation by the green tea.

Several authors have reported the antimutagenic effect of black tea extract by various compounds such as aflatoxin B1, benzo[a]pyrene, nitrate derivatives and, most recently, the heterocyclic aromatic amines (Ito et al., 1989; Jain et al., 1989, Wang et al., 1989; Ho et al., 1992, Yamada and Tomita, 1994; Yen and Chen, 1994; Apostolides et al., 1996). This latter compound is produced in high-temperature cooking during meat preparation. It has a powerful mutagenic and carcinogetic activity in animals (Stavric et al., 1996, Felton et al., 1992). Black and green tea extracts inhibited the mutagenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, formed during cooking of high protein foods (Apostolides and Weisburger, 1995). In a study undertaken by Shiraki et al. (1994) using polyphenols and theaflavins of black tea, the authors observed that the theaflavins scavenge free radicals, producing antimutagenic and antioxidative effects *in vitro* in low concentrations. The authors suggested that these compounds could have a positive role in our daily lives as a prevention of several diseases, cancers and aging, for which lipid peroxides or active oxygen are relevant.

The suppressant effect of the crude extract of three kinds of teas was evaluated for chromosomal aberrations present in man’s daily diet, in the presence of fraction S9, together with benzo[a]pyrene or mitomycin C, in mice and Chinese hamster ovary cells (CHO) cultures: the green tea of Japan, the Po-lei tea of China and the Rooibos tea of South Africa. Studies suggest that in some cases the catechins found in some samples had an antimutagenic effect (Sasaki et al., 1993). Confirming the previously obtained results in *in vitro* test system, Horikawa et al. (1994) treated male mice subcutaneously with benzo[a]pyrene. Mice received concomitantly an aqueous extract of six Chinese medicinal teas for 50 weeks. The authors verified that the antimutagenicity of the isolated fractions in that study could be due to the active tannins. The antimutagenic properties of soluble instant tea were evaluated by Constable et al. (1996) using the Ames test. Results obtained in this study suggest that the catechins are not the sole compounds responsible for the protective and antioxidant effects of teas. The authors discuss the oxidation that occurs during tea processing, polymerizing the catechin monomers and forming other compounds.
Bu-Abbas et al. (1996) compared the antimutagenic effect of green, black and decaffeinated teas and concluded that flavanols are the major tea components responsible for this activity.

The chemioprotective benzo[a]pyrene on human lymphocytes treated with Purnark, a mixture of solvent extracts of natural products, was reported by Ghaisas and Bhide (1994). There is a confirmation on the antimutagenic effect of alcoholic extract of lemon grass plant (Cymbopogon citratus Stapf), commonly used in the tea form in diets and in medicine, over many known mutagens in Salmonella typhimurium (Vinitketkumnuen et al., 1994). Nakamura et al. (1997) evaluated the suppressant effects of clastogenicity of Tochu tea, an aqueous extract obtained from the leaves of Eucommia ulmoides, a popular beverage in Japan, in CHO and mice.

Among the plants tested in this work, only the species Syzygium cumini and Cissus sicyoides have some known chemical compounds. The tannins, gallic acid and flavanols in Syzygium jambolanum might be responsible for the non-cytotoxicity and mutagenicity verified in the vegetal and animal species.

Adverse effects of Tochu tea, an aqueous extract obtained from the leaves of Eucommia ulmoides, a popular beverage in Japan, in CHO and mice.

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