

**MANGIFERA INDICA L.: EVALUATION OF THE CYTOTOXIC AND GENOTOXIC POTENTIAL OF THE AQUEOUS EXTRACT BY THE ALLIUM CEPA TEST**

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**ABSTRACT**

The mango tree, *Mangifera indica* L., is a large tree belonging to the family Anacardiaceae. Its bark, roots and leaves are used in folk medicine in virtually all regions where it grows. In particular, the leaves are used to treat various ailments, such as pain, diarrhea, fever, flu, coughing and skin rashes and wounds. This study examined the cytotoxic and genotoxic potential of aqueous extracts from *M. indica* leaves, employing the *Allium cepa* test. For this purpose, *A. cepa* bulbs were separated into two control and two treatment groups, each with five onions. The negative control group remained in distilled water, the treatment groups were placed in contact with the plant extract at two concentrations (15 mg mL<sup>-1</sup> and 30 mg mL<sup>-1</sup>) and the positive control was immersed in a solution containing 25 mM of EMS. The results obtained demonstrate that the leaf aqueous extracts of *M. indica* do not have genotoxic potential, but significant cytotoxic and antiproliferative

activities were detected at the two concentrations analyzed, indicating good potential because of the presence of components that can be used as anticancer agents.

**KEYWORDS:** *Allium cepa*, cytotoxicity, genotoxicity, *Mangifera indica*.

## INTRODUCTION

The mango tree, *Mangifera indica* L., is a large tree belonging to the family Anacardiaceae, which grows widely in tropical and subtropical regions. Its fruits are widely appreciated by the population.<sup>[1]</sup> Furthermore, the bark, roots and leaves of this tree are widely used in folk medicine in the regions where it grows.<sup>[1]</sup> In particular, the leaves are used to treat various ailments, such as pain, diarrhea, fever, flu, coughing and skin rashes and wounds.<sup>[2,3,4]</sup>

Besides beneficial effects, however, plant extracts can also contain substances that pose health risks, including mutagenic effects.<sup>[5]</sup> Therefore, it is necessary to investigate these risks versus the potential benefits of the use of plant extracts.

Various studies have analyzed extracts from the bark of *M. indica*, including a commercial formulation that was developed in Cuba (Vimang®) made from the aqueous extract of *M. indica* bark.<sup>[6]</sup> Some of these studies have indicated mild cytotoxic activity of Vimang using cell cultures and micronucleus tests in mice.<sup>[7,8]</sup> However, studies are scarce related to aqueous extracts obtained from *M. indica* leaves, including their genotoxic and cytotoxic potential.

To fill this gap, the objective of this study was to assess the cytotoxic and genotoxic potential of aqueous extracts obtained from *M. indica* by applying the *Allium cepa* test.

## MATERIALS AND METHODS

### Sample collection and identification

The mango tree leaves were collected in the town of Seropédica, Rio de Janeiro state, in May 2014. The botanical material was identified at the Botany Department of Rio de Janeiro Federal Rural University (UFRRJ). A voucher specimen was deposited in the university's herbarium under number RBR 36380.

### Preparation of the aqueous extract of *M. indica* leaves

Immediately after collection, the leaves were taken to the Laboratory for Plant Genotoxic Activity (LAGEP) of UFRRJ, where they were spread on a table and left at room temperature in an area protected from sunlight. The naturally dried leaves were then placed in a flask containing silica gel to remove all remaining moisture, until the moment of preparing the crude aqueous extracts. Analysis of the crude extract is important because this is the form

generally employed in folk medicine. The aqueous extracts were obtained by infusion of ground leaves, at concentrations of 15 mg mL<sup>-1</sup> and 30 mg mL<sup>-1</sup>. Then the extracts were cooled to room temperature and filtered through cotton cloth to remove residues. Fresh extracts were prepared daily, moments before use in the experiments.

### *Allium cepa* assay

The onions, organically grown, with diameter of approximately 2.0 cm were obtained from a local produce market. The outer layer of each bulb was removed with a paring knife without damaging the root buds.

The bulbs were initially placed in a container holding distilled water for 48 hours to allow the roots to grow, with daily water exchange. Then the bulbs were separated into two control groups and two treatment groups, each with five onions. The negative control group remained in the distilled water and the positive control group was immersed in a solution containing ethyl methanesulfonate (EMS, 25 mM). EMS is a highly efficient mutagenic agent, which acts directly on the DNA molecule through its alkylation activity. The treatment groups were immersed in two mango leaf extract solutions (concentrations of 15 mg mL<sup>-1</sup> and 30 mg mL<sup>-1</sup>). The solutions were exchanged daily for all groups and the temperature was maintained at 25°C.

For each treatment, the root apical meristems, between 2 and 2.5 cm in length, were removed from the bulbs after 48 hours of exposure to the respective solutions and used to prepare slides according to the method described by Iganci et al. (2006), with some modifications.<sup>[9]</sup> Five meristems were removed from each bulb. After removal, the meristems were fixed in an ethanol: glacial acetic acid solution at 3:1 (V/V) and stored at 4°C until the moment of preparing the slides. Five slides were prepared for each bulb, using five different meristems (one slide for each meristem). The meristems were washed with distilled water twice for 5 minutes, hydrolyzed in HCl 5N for 30 minutes, washed again twice in distilled water for 5 minutes and then placed on the slides with tweezers. The meristem regions were fragmented with a scalpel, stained with 2% acetic orcein, and covered with a coverslip.

The slides were observed under a common optical microscope with 100 X magnification. The parameters used to determine the genotoxic and cytotoxic potential of the extracts were the presence of chromosome and cell alterations as well as changes in the mitotic index. A total of 1,000 cells were analyzed per bulb, or 5,000 cells for each group. With the exception of

the mitotic index, expressed as a percent, the results were expressed in absolute terms. The most frequent anomalies are shown in the micrographs.

### Statistical design

The data were analyzed by the Chi-square ( $\chi^2$ ) test with probability  $<0.05$ .

## RESULTS

The results obtained from the *A. cepa* test demonstrated that the aqueous extracts of *M. indica* leaves do not present genotoxic potential, but cytotoxic activity was detected at the two concentrations used ( $15 \text{ mg mL}^{-1}$  and  $30 \text{ mg mL}^{-1}$ ).

Tables 1 and 2 report the alterations found in the meristem cells of *A. cepa* treated with the extracts. There was a significant decline in the number of cells with changes in the number and size of nucleoli and nuclear buds in relation to the positive and negative control groups. Those changes are characteristic of cells exposed to genotoxic agents (Fig. 1B and 1C). These results, besides demonstrating the extracts are not genotoxic, indicate they have a protective effect. However, the extracts did have a cytotoxic effect, based on observation of a large number of cells undergoing apoptosis (Fig. 1E) and with nuclear alterations (Fig. 1F) related to cell death processes. The cells submitted to the concentration of  $15 \text{ mg mL}^{-1}$  also presented a significant increase in necrosis. At both concentrations, a sharp reduction was observed in the mitotic index (Tables 1 and 2) in comparison with the positive and negative controls.

**Table 1: Cells changes and mitotic index in *A. cepa* roots submitted to different treatments.**

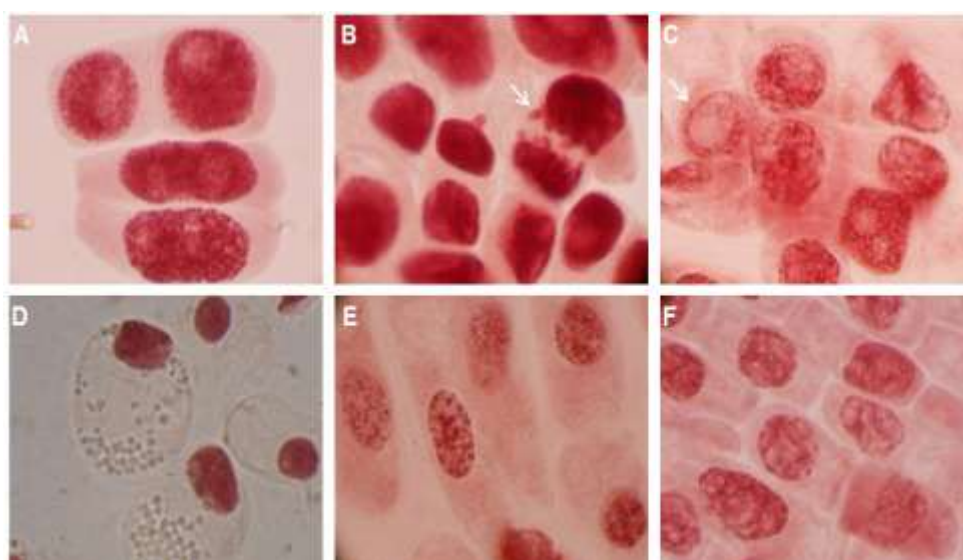
Treatment	Conc.	LN	BN	NEC	MN	AP	AN	IM (%)
Neg control	Distilled water	321 <sup>(b)</sup>	151	22	57 <sup>(b)</sup>	9 <sup>(b)</sup>	16	3,08
EMS	25 mM	750 <sup>(a)</sup>	178	19	107 <sup>(a)</sup>	21 <sup>(a)</sup>	25	2,1 <sup>(a)</sup>
MI extract	$15 \text{ mg mL}^{-1}$	163 <sup>(a)(b)</sup>	74 <sup>(a)(b)</sup>	50 <sup>(a)(b)</sup>	6 <sup>(a)(b)</sup>	132 <sup>(a)(b)</sup>	985 <sup>(a)(b)</sup>	0,26 <sup>(a)(b)</sup>

(a) and (b) Significant difference compared to negative and positive control respectively ( $P < 0,05$ ) according to the  $\chi^2$  test; **MI extract** – *M. indica* extract; **Conc** – concentration; **LN** – one or more large nucleoli; **BN** – nuclear bud; **NEC** – necrotic cells; **MN** – multiple nucleoli; **AP** – apoptosis; **AN** – nuclear abnormalities; **IM** – mitotic index. 5000 cells for each treatment were analyzed.

**Table 2: Cells changes and mitotic index in *A. cepa* roots submitted to different treatments.**

Treatment	Conc.	LN	BN	NEC	MN	AP	AN	IM (%)
<b>Neg control</b>	Distilled water	339 <sup>(b)</sup>	66	12 <sup>(b)</sup>	24 <sup>(b)</sup>	4	61 <sup>(b)</sup>	3,52
<b>EMS</b>	25 mM	1000 <sup>(a)</sup>	76	77 <sup>(a)</sup>	91 <sup>(a)</sup>	2	12 <sup>(a)</sup>	2,04 <sup>(a)</sup>
<b>MI extract</b>	30 mg mL <sup>-1</sup>	55 <sup>(a)(b)</sup>	20 <sup>(a)(b)</sup>	1 <sup>(a)(b)</sup>	21 <sup>(b)</sup>	576 <sup>(a)(b)</sup>	879 <sup>(a)(b)</sup>	0,2 <sup>(a)(b)</sup>

(a) and (b) Significant difference compared to negative and positive control respectively ( $P < 0,05$ ) according to the  $\chi^2$  test; **MI extract** – *M. indica* extract; **Conc** – concentration; **LN** – one or more large nucleoli; **BN** – nuclear bud; **NEC** – necrotic cells; **MN** – multiple nucleoli; **AP** – apoptosis; **AN** – nuclear abnormalities; **IM** – mitotic index. 5000 cells for each treatment were analyzed.



**Fig. 1: *A. cepa* meristematic cells submitted to different treatments: (A) negative control; (B) and (C) positive control: nuclear buds and nucleoli with altered sizes can be observed (arrows); (D) necrotic cells; (E) apoptosis; (F) nuclear abnormalities.**

## DISCUSSION

Tea made from the leaves of *M. indica* is used around the world for treatment of many ailments and conditions, such as pain, diarrhea, fever, flu, coughing and skin rashes/wounds<sup>[2,3,4]</sup>, but no studies have been published assessing the cytotoxic and genotoxic potential of this type of extract. The majority of studies of *M. indica* extracts have analyzed preparations made from the bark, particularly Vimang®, a natural product made in Cuba, where it is widely used to enhance the quality of life of patients suffering from stress.<sup>[10,11]</sup>

Our results demonstrate that the aqueous extracts of *M. indica* leaves do not have genotoxic potential and even show a possible protective effect, indicated by the significant decline in the number of cells with alterations in the buds and nucleoli. However, we found cytotoxic effects at the two concentrations tested, shown by the significant increase in the number of cells undergoing apoptosis and with nuclear alterations.

The absence of a correlation between cytotoxic and genotoxic effects is common, as observed by other authors. While cytotoxic agents can lead indirectly to changes in genetic material, they are not necessarily associated with a genotoxic effect.<sup>[12,13]</sup>

Although there was a significant increase in the number of meristem cells undergoing necrosis after treatment with the aqueous extract at the concentration of 15 mg mL<sup>-1</sup>, the extract at 30 mg mL<sup>-1</sup> did not present this effect. This also is a common observation in studies analyzing crude plant extracts, because it is known that aqueous extracts at different concentrations show variable interactions among their components, so there is not necessarily a direct correlation between the concentration and changes in effects of the extract. Results of this type have been reported by other researchers.<sup>[14,15]</sup>

The nuclear changes observed in this study are similar to karyorrhexis, in which the chromatin is irregularly distributed throughout the cytoplasm, sometimes forming clumps in the nuclear membrane. Such changes are also found in cells undergoing death by necrosis or apoptosis.<sup>[16,17,18]</sup>

Results similar to those found in this study have been reported by other authors analyzing the aqueous extract of the bark of *M. indica* (in particular Vimang®) using animal systems. In these studies, no signs of genotoxicity or acute or chronic toxicity have been observed when the extract is administered orally in rodents, although signs of cytotoxicity have been observed.<sup>[8,11]</sup> Rodeiro et al. (2007) evaluated the cytotoxicity of Vimang® on rat hepatocytes using a wide range of concentrations (50 – 1000 µg mL<sup>-1</sup>) after 24, 48 and 72 hours of treatment.<sup>[8]</sup> Although no significant cytotoxic effect was noted after exposure for 24 hours to the different concentrations, a significant reduction of cell viability was observed at the higher concentrations after incubation for longer periods. Gonzáles et al. (2007), investigating genotoxicity using the Comet assay and micronucleus formation test with bone marrow, observed no significant increase in the incidence of micronuclei and no increase in DNA damage (detected by breakage of single or double strands), indicating the aqueous bark



extract is not genotoxic.<sup>[11]</sup> However, they did observe a cytotoxic effect of the extract. There was a toxic effect on the marrow, evidenced by a 10% reduction in the proportion of polychromatic erythrocytes (PCE). The authors associated this effect with an antiproliferative activity of the extract. The cytotoxicity of Vimang® was also previously detected by Rodeiro *et al.* (2006), due to the significant reduction in the viability of human lymphocytes kept in culture after 20 h of treatment with the extract.<sup>[7]</sup>

The aqueous extracts of the *M. indica* leaves also promoted a significant reduction in the mitotic index in the meristem cells of *A. cepa* submitted to the treatments. Mitotic indices lower than in the control group can indicate that the growth and development of exposed organisms are affected by the components contained in the samples tested.<sup>[19,20]</sup> This reduction can be related to the large number of cells undergoing apoptosis at the two concentrations analyzed. Relations of this type have been detected by other authors.<sup>[21,22]</sup>

Serveri *et al.* (2009) reported that the decoction extract of *M. indica* leaves did not present acute toxicity when administered orally to rats and observed a gastroprotective effect against various ulcerogenic agents.<sup>[1]</sup> The authors demonstrated that the main bioactive molecules present in this extract are xanthanoids (mainly mangiferin) and benzophenone glucoside and associated the gastroprotective activity to the antioxidant property of these components.

The substance mangiferin, found in large quantities in the leaves, fruits and bark of *M. indica*, has various pharmacological properties, such as antiviral, anti-inflammatory, hypoglycemic, analgesic and antitumor activities, among others. Rodeiro *et al.* (2012) reported the absence of cytotoxic or genotoxic effects of mangiferin and found it to be efficient in reducing the mutagenicity caused by various agents.<sup>[23]</sup>

The presence of components such as mangiferin and benzophenone can be the cause of the apparent protective effect of the aqueous extract noted in this study, since we observed a significant reduction in the number of buds and alterations of the nucleoli in relation to the positive and negative controls, changes that indicate the presence of genotoxic agents.<sup>[17,24,25]</sup>

## CONCLUSION

The tests conducted on *A. cepa* showed that the tea made from *M. indica* leaves at the two concentrations analyzed does not pose risks regarding genotoxic activity. However, we observed a significant antiproliferative effect, probably related to the inducement of cell

death processes, indicating good potential related to the presence of components that can be used as anticancer agents.

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