Cytotoxic and genotoxic effects of two medicinal species of Verbenaceae

Sousa[1]* Saulo M., Pâmela S. Silva[2], José Marcello S. Campos[1], Lyderson F. Viccini[1]

[1]Departamento de Biologia, Instituto de Ciências Biológicas, Universidade Federal de Juiz de Fora (UFJF), 36036-900. Juiz de Fora, Minas Gerais, Brazil.
[2]Departamento de Farmacologia, Faculdade de Medicina, Universidade Estadual de São Paulo (USP), 14.049-900. Ribeirão Preto, São Paulo, Brazil.

Abstract — *Lantana camara* L. and *Lippia alba* (Mill.) N. E. Br. are two important species of Verbenaceae family and are commonly used in folk medicine in many countries of Central and Southern America. The aim of this study was to investigate, for the first time, the cytotoxic and genotoxic effects of aqueous extracts from leaves of both species on *Lactuca sativa* (lettuce) root tip meristem cells using a cytogenetic approach. Seeds of lettuce were separately treated during 72h with different concentrations of *L. camara* and *L. alba* aqueous extracts (5, 10, 20 and 30g/L). The percentage of germination, root development and cellular behavior were analyzed and the results showed that the highest concentration of aqueous extracts reduced the mitotic index, the seeds germination and the root development of lettuce. The extracts also induced chromosome aberrations and cellular death in roots cells of *L. sativa*. The cytogenotoxicity of *L. camara* and *L. alba* extracts was comparatively described.

Key words: cellular death, chromosome aberrations, cytogenotoxicity, *Lantana camara*, *Lippia alba*, mitotic index, mutagenesis.

INTRODUCTION

Plants have always been used as a common source of medicinal substances, both in traditional remedies and in industrialized products. In Brazil, many people use traditional natural preparations derived from plant material for treating various diseases (Lorenzi and Matos 2002). As a consequence it is extremely important the employment of genotoxicity tests to identify their possible mutagenic potential (Camparoto *et al.* 2002; Pugliesi *et al.* 2007; Lubini *et al*. 2008).

Genotoxic effects of extracts, infusions, essential oils and fractions of extracts of many plants have been widely evaluated using cytogenetic approaches (Sobita and Bhagirath 2005; Zanoni *et al*. 2005; Çelik 2006; 2007). Among the various tests available for this purpose, those that use roots tips are extremely useful and are relatively inexpensive and can easily be handled. More yet, plant cytotoxic bioassays have a good correlation with mammalian test systems, validating their application for genotoxic assays (Fiskesjö1985; Jovtchev *et al.* 2002; Yi and Meng 2003; Çelik and Aslantürk 2006; 2007; Lubini *et al.* 2008).

Regarding plant bioassays *Allium cepa*, *Lactuca sativa*, *Zea mays* and *Vicia faba* have been the most common species used for cytogenotoxicity evaluation. This can be explained mainly by the great number of seeds produced by these species, the easy handling and by the great contact surface proportioned by bulbs and/or seeds in the aqueous extracts administrated. In addition, these species also show high sensibility to toxic compounds and they don't have small chromosomes, increasing their application for cytogenetic studies (Singh and Das 2002; Sobita and Bhagirath 2005; Campos *et al.* 2008; Lubini *et al.* 2008).

Using cytogenetic approach, the mitotic index (indicator of cytotoxicity), the micronuclei formation and chromosomes aberrations (indicators of genotoxicity) are the most common parameters evaluate. The micronuclei can be interpreted as a consequence of clastogenic (chromosome breakage) or aneugenic (chromosome lagging and interference on the spindle behavior) effects, and chromosomes aberrations can be originated by chromosome breakage and/or chromosome ex-

* Corresponding author: e-mail: saulo_marcal@yahoo.com.br.

change (CHACON *et al.* 2002; ZANONI *et al.* 2005; PUGLIESI *et al.* 2007).

The species *Lantana camara* L. and *Lippia alba* (Mill.) N. E. Br., both Verbenaceae species, are commonly used in folk medicine in Brazil and another countries of Central and Southern America. According to ethnobotanic relates, the syrup obtained from fresh or dry leaves and flowers of *L. camara* is generally used as tonic, sudorific, antireumatic, and for respiratory disease, while the infusions of *L. alba* leaves are used as sedative, carminative, spasmolitic, emanogoge, sudorific, antidysenteric, antireumatic, antihypertensive and expectorant (LORENZI and MATOS 2002; ZÉTOLA *et al.* 2002; HENNEBELLE *et al.* 2008).

Previous studies reported cytotoxic effects of *L. alba* extracts using rats cells strains (COSTA 1996), but genotoxic studies using cytogenetic approach were not described. Similarly, the toxic potential of *L. camara* was also described but not using cytogenetic parameters (LORENZI and MATOS 2002; ZÉTOLA *et al.* 2002).

The present work was done in order to evaluate the cytotoxic and genotoxic effect of aqueous extracts from fresh leaves of *L. alba* and *L. camara* on *Lactuca sativa* (lettuce), by cytogenetic approach. In addition, these results can also contribute to better understand the mechanism of the toxic effects previously reported.

MATERIAL AND METHODS

Plant material and extracts preparation - Fresh leaves of *Lantana camara* and *Lippia alba* were collected at the Botanical Experimental Area of Universidade Federal de Juiz de Fora (UFJF). Voucher specimens of each species were deposited at the CESJ Herbarium of the UFJF. Four aqueous extracts were prepared (5, 10, 20 and 30g, respectively, in 1000ml of distilled water). After 24h, at room temperature, the extracts were filtered in filter paper before the application in the seeds bioassays.

Germination and root growth - The treatments were arranged in a completely random design with 4 repetitions at 28°C. Each repetition corresponding to 60 seeds of lettuce placed in a Petri dish. As a control, we used lettuce seeds exposed to distilled water. The germination percentage and root growth were evaluated after 12, 24, 36, 48, 60 and 72h of exposition time. The germination percentage was obtained by the counting of seeds with apparent roots in each one of the time of evaluation as described previously. The root growth were obtained by the aid of one caliper, the measure were obtained in each one of the time of evaluation as described previously.

Cytogenetics studies - After 72h, 10 roots were collected of each repetition (40 roots for treatment). These roots were fixed in a fresh cold methanol/glacial acetic acid solution 3:1 (v/v) during 24h. Following fixation, the roots were submitted to an enzymatic maceration (Pectinex NOVO NORDISK™) at 34°C for 1:45h. Subsequently, the roots were hydrolyzed in 5N HCl for 11 minutes. By air dry technique (CARVALHO and SARAIVA 1993), five slides were prepared for each repetition (20 slides for treatment) and stained with Giemsa 10% during 3 minutes. Mitotic index was determined for each treatment and the presence of chromosomes abnormalities was also evaluated.

Statistical analyses - The percentage of germinated seeds, root size, mitotic index and percentage of chromosome aberrations were evaluated. The data were submitted to one-way analysis of variance (ANOVA) and comparisons among the average of each treatment with the average of control were carried out using Tukey test ($p < 0.05$).

RESULTS

The germination test exhibited different behavior when compared extracts obtained from leaves of *L. camara* and *L. alba*. The germination of *Lactuca* seeds submitted to *L. camara* extract decreased as the concentrations of the extract increased. Significant difference were observed for all concentrations Lac1 (5g/L), Lac2 (10g/L), Lac3 (20g/L) and Lac4 (30g/L) when they were compared to the control. In addition, the germination time was also influenced by different extracts concentration. Seeds submitted to Lac1 and Lac2 only initiated their germination after 24h of the exposition and those submitted to Lac3 and Lac4 only initiated their germination after 36h (Table 1). Differently, the lettuce seeds submitted to *L. alba* extracts showed few differences from the control at Lia1 and Lia2 concentration. Nevertheless, the treatment Lia3 (20g/L) showed a significant decrease in the percentage of germination and Lia4 (30g/L) impeded seeds germination (Table 1).

Root development of lettuce was also influenced by the extracts. In general, the extract of *L. camara* caused the reduction of lettuce roots size as the concentration increased. On the other hand, in the minor concentration (5g/L) it was observed a recuperation of the development of lettuce roots around 48h, once the average observed were very

Table 1 — Germination of lettuce seeds after 12, 24, 36, 48, 60 and 72h of exposition to aqueous extracts of *Lantana camara* and *Lippia alba*.

	Exposition (h)					
Treatments	12	24	36	48	60	72
Lantana camara						
Control	81.23 (± 1.23)	87.54 (± 2.21)	90.12 (± 3.43)	94.34 (± 3.98)	96.21 (± 2.13)	98.75 (± 4.25)
Lac1	—	43.12 (± 3.84)*	48.56 (± 2.39)*	52.17 (± 3.12)*	56.82 (± 4.17)*	58.14 (± 3.41)*
Lac2	—	39.23 (± 2.54)*	42.76 (± 2.56)*	46.04 (± 2.12)*	48.23 (± 1.19)*	49.12 (± 2.95)*
Lac3	—	—	23.12 (± 2.21)*	25.98 (± 3.65)*	27.13 (± 3.12)*	28.45 (± 2.28)*
Lac4	—	—	18.93 (± 1.23)*	21.12 (± 1.49)*	21.97 (± 2.01)*	23.42 (± 1.75)*
Lippia alba						
Control	78.98 (± 3.28)	82.34 (± 2.87)	87.90 (± 4.89)	91.23 (± 4.17)	95.43 (± 3.21)	97.12 (± 3.95)
Lia1	73.24 (± 4.23)	75.67 (± 3.65)	81.34 (± 3.76)	87.98 (± 3.21)	91.11 (± 2.34)	93.12 (± 2.09)
Lia2	69.80 (± 2.36)	72.56 (± 2.54)	77.23 (± 3.54)	78.90 (± 3.04)	82.87 (± 3.23)	85.98 (± 3.67)
Lia3	38.12 (± 1.97)*	42.11 (± 2.32)*	45.13 (± 2.28)*	46.91 (± 2.03)*	48.98 (± 1.87)*	50.12 (± 2.65)*
Lia4	—	—	—	—	—	—

Lac_1: *L. camara* aqueous extract (5g/L), Lac_2: *L. camara* aqueous extract (10g/L), Lac_3: *L. camara* aqueous extract (20g/L), Lac_4: *L. camara* aqueous extract (30g/L). Lia_1: *L. alba* aqueous extract (5g/L), Lia_2: *L. alba* aqueous extract (10g/L), Lia_3: *L. alba* aqueous extract (20g/L), Lia_4: *L. alba* aqueous extract (30g/L). *Significantly different from the control ($p < 0.05$) (Tukey test).

Table 2 — Size (cm) of lettuce roots during 12, 24, 36, 48, 60 and 72h of exposition in *Lantana camara* and *Lippia alba* aqueous extracts.

	Exposition (h)					
Treatments	12	24	36	48	60	72
Lantana camara						
Control	0.58 (± 0.10)	0.74 (± 0.12)	1.02 (± 0.15)	1.47 (± 0.22)	1.68 (± 0.31)	2.48 (± 0.26)
Lac1	—	0.47 (±0.10)*	0.72(± 0.12)*	0.97 (± 0.11)	1.28 (± 0.21)	1.97 (± 0.34)
Lac2	—	0.35 (± 0.08)*	0.39 (± 0.08)*	0.46 (± 0.13)*	0.54 (± 0.18)*	0.68 (± 0.10)*
Lac3	—	—	0.26 (± 0.03)*	0.37 (± 0.08)*	0.45 (± 0.09)*	0.56 (± 0.07)*
Lac4	—	—	0.12 (± 0.02)*	0.18 (± 0.04)*	0.23 (± 0.08)*	0.34 (± 0.05)*
Lippia alba						
Control	0.66 (± 0.13)	0,79 (± 0.15)	0.97 (± 0.11)	1.51 (± 0.23)	1.72 (± 0.36)	2.35 (± 0.31)
Lia1	0.58 (± 0.12)	0.62 (± 0.15)	0,84 (± 0,12)	1.27 (± 0.41)	1.36 (± 0.27)*	1.52 (± 0.22)*
Lia2	0.49 (± 0.23)	0.54 (± 0.12)	0.79 (± 0.21)	1.08 (± 0.29)*	1.29 (± 0.34)*	1.37 (± 0.31)*
Lia3	0.12 (± 0.08)*	0.18 (± 0.09)*	0.23 (± 0.08)*	0,39 (± 0,07)*	0.42 (± 0.08)*	0.47 (± 0.05)*
Lia4	—	—	—	—	—	—

Lac_1: *L. camara* aqueous extract (5g/L), Lac_2: *L. camara* aqueous extract (10g/L), Lac_3: *L. camara* aqueous extract (20g/L), Lac_4: *L. camara* aqueous extract (30g/L). Lia_1: *L. alba* aqueous extract (5g/L), Lia_2: *L. alba* aqueous extract (10g/L), Lia_3: *L. alba* aqueous extract (20g/L), Lia_4: *L. alba* aqueous extract (30g/L). *Significantly different from the control ($p < 0.05$) (Tukey test).

similar when compared to the control (Table 2). Respect to the other species in study, the extract of *L. alba* only caused significant decrease of the lettuce root development in all experiment in the Lia3 concentration (20g/L), Lia2 caused decrease in the times of 48, 60 and 72h and Lia1 only in the times of 60 and 72h of exposition (Table 2).

In a similar way the extracts of the two medicinal plants also influenced the cellular division of lettuce roots. The extract of *L. camara* caused a reduction in mitotic index as the concentration increased, mainly from the Lac2 concentration (Table 3). For *L. alba*, only Lia3 (20g/L) exhibit a significant value when compared to the control (Table 3).

Chromosome aberrations were also observed in meristematic roots of lettuce exposed to the extracts. It was possible to observe chromosomes stickiness, chromosome breakage, chromosome bridge and micronuclei formations (Figure 1). Additionally, we observed communication among nucleus and cellular dead (Figure 1). Considering *L. camara* extracts, only the highest concentration provoked chromosome aberration, except for chromosome bridge that was observed in various concentrations. Differently, *L. alba* extracts induced chromosome aberration in various concentrations, with an increase of the alterations in the highest concentrations (Table 4). The percentage of cellular death was also incremented in the highest concentrations of the extracts for both medicinal plants evaluated (Table 5).

DISCUSSION

The chemical interaction among plants, including stimulatory as well inhibitory influence can be designed as allelopathy (MOLISCH 1937). This process involve the capacity of one plant species produce and liberates allelochemicals into the environment to suppress or stimulate the development of others plant species (CHRON *et al.* 2005). These potentialities play important role in natural and agro-ecosystems once the manipulation of allelochemicals produced by plants can be utilized to improve crop productivity and to protect the environment. It is possible to control weeds, pests and crop disease using novel agrochemicals based on natural products (TINNIN and MULLER 1971; CHOU and CHUNG 1974; NEWMAN and ROVIRA 1975; WU *et al.* 1976; MILLER 1996; WU and HARIVANDI 1998; ZANONI *et al.* 2005; PUGLIESI *et al.* 2007; LUBINI *et al.* 2008).

The results obtained in this work indicate that the increase of the extracts concentrations

Table 3 — Mitotic index of lettuce meristematic cells exposed to different concentrations of *Lantana. camara* and *Lippia alba* aqueous extracts after 72h.

Treatments	MI	MCD	MC
Lantana camara			
Control	5.74 (± 0.51)	635.75 (± 102.41)	11030.00 (± 1105.02)
Lac_1	5.25 (± 0.45)	595.00 (± 152.70)	11297.75 (± 2574.59)
Lac_2	3.32 (± 0.52)*	372.00 (± 36.89)	11311.75 (± 982.31)
Lac_3	1.37 (± 0.56)*	151.25 (± 46.31)	11738.50 (± 3273.27)
Lac_4	1.20 (± 0.22)*	144.75 (± 46.46)	11931.75 (± 1932.25)
Lippia alba			
Control	5.04 (± 0.58)	491.75 (± 135,55)	9733.75 (± 2286.38)
Lia_1	5.11 (± 0.07)	387.00 (± 44.11)	7571.75 (± 833.61)
Lia_2	4.62 (± 0.90)	420.50 (± 79.12)	9159.00 (± 1266.02)
Lia_3	1.92 (± 0.04)*	210.50 (± 19.14)	10985.00 (± 1119.24)
Lia_4	—	—	—

MI: Mitotic Index; MCD: average number of division cells; MC: average number of observed cells. Lac_1: *L. camara* aqueous extract (5g/L), Lac_2: *L. camara* aqueous extract (10g/L), Lac_3: *L. camara* aqueous extract (20g/L), Lac_4: *L. camara* aqueous extract (30g/L). Lia_1: *L. alba* aqueous extract (5g/L), Lia_2: *L. alba* aqueous extract (10g/L), Lia_3: *L. alba* aqueous extract (20g/L), Lia_4: *L. alba* aqueous extract (30g/L). *Significantly different from the control ($p < 0.05$) (Tukey test).

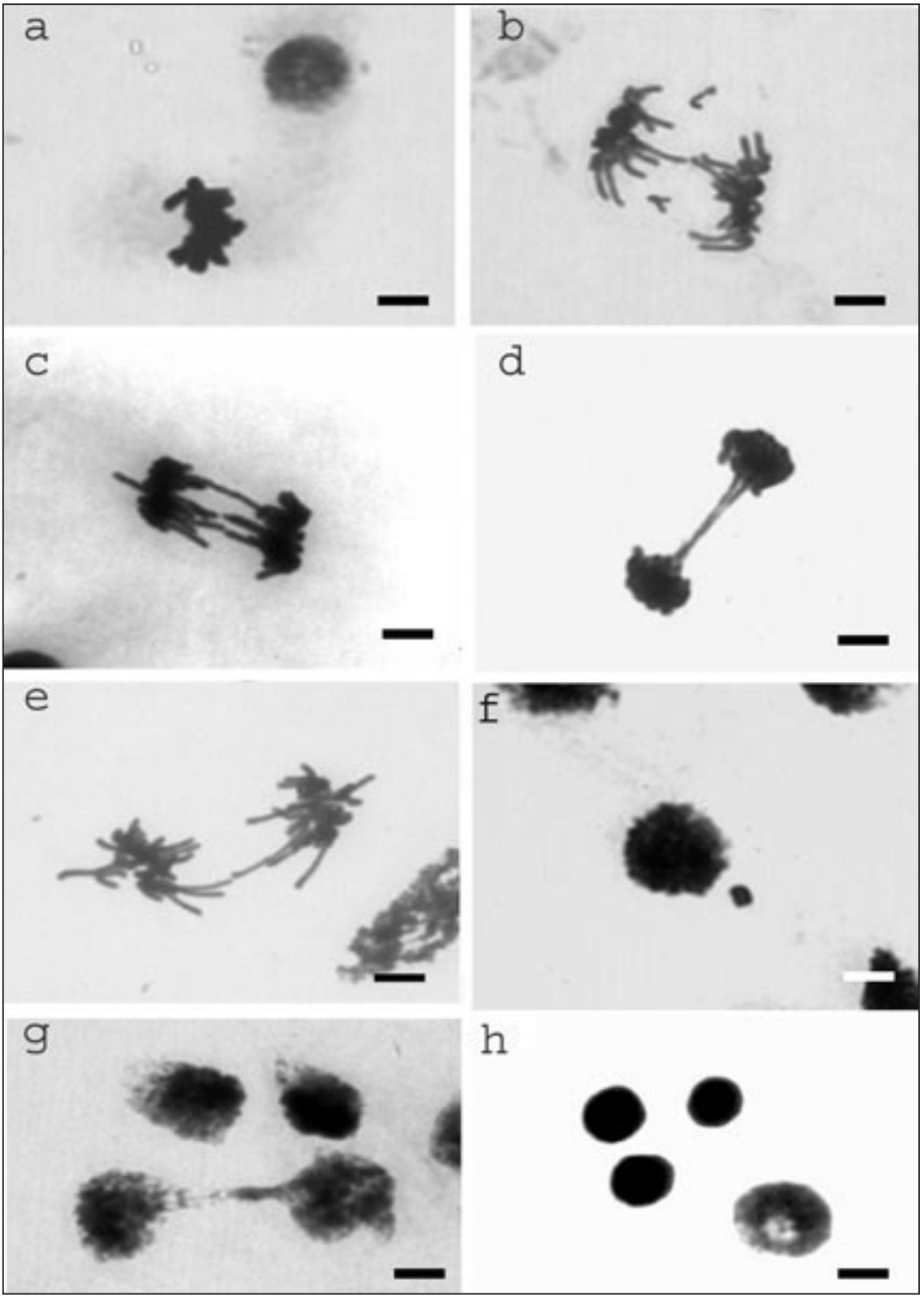

Fig. 1 — Chromosome and cellular aberrations observed in lettuce root meristem exposed to *Lantana camara* and *Lippia alba* aqueous extracts. (a) metaphases stickiness; (b) anaphase with laggard chromosome; (c) anaphase with bridges; (d) telophases with bridges; (e) anaphase with chromosome bridge; (f) micronuclei formation; (g) nuclear communication; (h) condensed nuclei. Bar = 5µm.

Table 4 — Percentage of chromosomes aberrations from root tips of lettuce after 72h of exposition to *Lantana camara* and *Lippia. alba* extracts.

Concentration	Lm	Brks	Stk	Nc	ABr	Mcn
Lantana camara						
Control	0.18 (± 0.04)	0.88 (± 0.19)	2.87 (± 0.97)	0.28 (± 0.08)	0.76 (± 0.21)	0.31 (± 0.11)
Lac_1	0.15 (± 0.03)	0.73 (± 0.25)	2.89 (± 0.12)	0.19 (± 0.22)	3.82 (± 0.73)*	0.40 (± 0.21)
Lac_2	0.13 (± 0.01)	0.91 (± 0.18)	3.76 (± 0.22)	0.25 (± 0.14)	8.21 (± 1.02)*	0.17 (± 0.03)
Lac_3	1.98 (± 0.12)*	0.76 (± 0.23)	4.90 (±2.01)	0.34 (± 0.09)	9.68 (± 1.08)*	0.22 (± 0.06)
Lac_4	3.04 (± 0.84)*	5.94 (± 1.18)*	7.08 (±2.31)*	2.13 (± 0.81)*	11.23 (± 2.83)*	4.12 (± 0.81)*
Lippia alba						
Control	0.22 (± 0.07)	0.76 (± 0.10)	1.92 (± 0.12)	0.32 (± 0.04)	0.87 (± 0.36)	0.26 (± 0.13)
Lia_1	0.19 (± 0.06)	1.21 (± 0.23)*	3.21 (± 0.23)*	0.47 (± 0.02)	0.92 (± 0.26)	0.32 (± 0.15)
Lia_2	0.31 (± 0.02)	2.74 (± 0.34)*	5.64 (± 0.31)*	1.78 (± (0.43)	9.79 (± 1.01)*	0.21 (± 0. 11)
Lia_3	0.84 (± 0.11)*	6.31 (± 0.28)*	5. 21(± 0.44)*	4.21 (± 0.34)*	14. 95 (± 1.43)*	0. 36 (± 0.10)
Lia_4	—	—	—	—	—	—

Lac_1: *L. camara* aqueous extract (5g/L), Lac_2: *L. camara* aqueous extract (10g/L), Lac_3: *L. camara* aqueous extract (20g/L), Lac_4: *L. camara* aqueous extract (30g/L). Lia_1: *L. alba* aqueous extract (5g/L), Lia_2: *L. alba* aqueous extract (10g/L), Lia_3: *L. alba* aqueous extract (20g/L), Lia_4: *L. alba* aqueous extract (30g/L). Lm = lagging migration; Brks = breaks; Stk = stickiness; Nc = nuclear communications; ABr = anaphases with bridge; Mcn = micronuclei. *Significantly different from the control ($p < 0.05$) (Tukey test).

Table 5 — Cellular dead in lettuce meristem after 72h of exposition to *Lantana camara* and *Lippia alba* extracts.

Treatments	Cellular death
Lantana camara	
Control	1.23 (± 0.31)
Lac_1	1.34 (± 0.45)
Lac_2	12.32 (± 4.32)*
Lac_3	18.76 (± 5.87)*
Lac_4	33.76 (± 4.92)*
Lippia alba	
Control	1.46 (± 0.42)
Lia_1	2.57 (± 0.65)
Lia_2	21.34 (± 5.76)*
Lia_3	39.21 (± 7.87)*
Lia_4	—

*Significantly different from the control ($p < 0.05$) (Tukey test).

obtained from *L. camara* and *L. alba* induces the inhibition of seed germination. In the higher concentration (30g/L) of *L. alba* extract a biocide activity was detected, once no germination was observed. Similar results involving the same species were previously reported. In *L. camara* SHAUKAT and SIDDIQUI (2002), for example, showed an inhibitory effect of the germination of mungbean seeds after the application of extracts obtained from roots and leaves of this species. As observed in our study, they observed a reduction of the germination and the root development as the concen-

tration extracts increased. For *L. alba*, MAIRESSE *et al.* (2007) also observed similar results in lettuce, indicating a drastic effect in the survival of lettuce seedlings as the extracts concentration increased. In both studies, the authors suggested the utilization of these two extracts for the natural control of weeds in crop plants.

Allelochemicals can act by innumerous manner to affect the plant germination and growth. The principals mechanisms involves the alteration of the mitotic index and the suppression of hormones synthesis (RICE 1984). The inhibition of cellular cycle by the decline of the mitotic index indicates the occurrence of a cytotoxic effect. The cytotoxic potential of *L. alba* was investigated by Costa et al. (2004) using Hep-2 and NCI-H292 cells. According to these authors, the cytotoxic potential is dependent to the solvent utilized and the concentrations administered. On the other hand, about the cytotoxic effect of *L. camara* extracts, no study was carried out. Our results indicate that some concentrations of the aqueous extracts of *L. camara* and *L. alba* reduced the mitotic index of lettuce root meristem, and revealed a possible cytotoxicic effect. Comparing to *L. alba*, the *L. camara* extracts showed more cytotoxic effect once significant difference was observed also in low concentrations (10g/L). On the other hand *L. alba* extracts exhibit the same effect only in the highest concentration (20g/L). These data can also explain the reduction of the root development as the concentration of the extract increased.

The reduction of the mitotic index can be explained by the arrest of the division of the interphasic nucleus, as well as by death of intherfasics nucleus, hindering the onset of the prophase and, thus, the division of the cells. In agreement of the second hypothesis, we observed many cells with nuclear condensation, which constitutes common morphological aspects of the programmed cell death in plants (SOLOMON *et al.* 1999). These aspects were observed mainly with the increment of the concentrations of the extracts, where the mitotic index showed a significantly decrease in relation to the control. Similar results were observed in previous studies about other medicinal plant extracts and the cell death was considered the major depressor of the mitotic index (ÇELIK and ASLANTÜRK 2006, 2007; CAMPOS *et al.* 2008; LUBINI *et al.* 2008).

In addition to the citotoxicity, both species can be also considered as genotoxic once innumerous chromosome alterations were observed. According to SARKAR *et al.* (1996), natural plant products can, apart from inducing mutations, modify the action of other known mutagens on the living organisms by activating the existing mutagens within the cell, inhibiting the production of mutagens in the cell, synergizing the activity of existing mutagens, or activating the promutagens within the cell into mutagens. So, the property of plants to activate promutagens that may enter in the food chain or in the phytotherapy is of a great significance in a view of a large number and types of chemicals produced by plants. As we observed, the more prominent results with chromosome aberrations were on the highest concentrations of the extracts for the both species studied, suggesting that the effect can be considered dose dependent. Thus, our results suggest caution in the use of *L. camara* and *L. alba* in folk medicine.

Finally, although *L. camara* and *L. alba* can be utilized in folk medicine, serious problems and damages on cells by incorrectly usage can be observed. In order to obtain more information and precise conclusions about this subject, further researches should be performed with different models and systems. In spite of that, our results also demonstrated the potential of these two plant extracts as a source of active biological substances that can be use in agriculture to weed control.

Acknowledgements — The authors thank FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for the financial support.

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Received June 1st 2009; accepted September 4th 2009