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Cytogenotoxicity of *Cymbopogon citratus* (DC) Stapf (lemon grass) aqueous extracts in vegetal test systems

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ABSTRACT

The lemon grass, *Cymbopogon citratus* (DC) Stapf, is an important species of Poaceae family commonly used in t folk medicine in many countries. The aim of this study was to investigate the cytotoxic and genotoxic effects of aqueo extracts from *C. citratus* leaves on *Lactuca sativa* (lettuce) root tip meristem cells by cytogenetic studies that have nev been done before for lemon grass extracts. For this, lettuce seeds were treated for 72h with different concentratio of lemon grass aqueous extracts (5; 10; 20 and 30 mg/mL). The percentage of germination, root development a cellular behavior were analyzed, and the results showed that the highest concentration of aqueous extracts reduced t mitotic index, the seed germination and the root development of lettuce. The extracts have also induced chromoson aberrations and cellular death in the roots cells of *L. sativa*.

Key words: aqueous extract, *Cymbopogon citratus*, cytotoxic effect, genotoxic effect, *Lactuca sativa*.

INTRODUCTION

The use of medicinal plants on phytotherapy is a result of empirical knowledge accumulated over the centuries about plant actions in several ethnic groups. Therefore, there are many questions about the standardization techniques for the production and exchange of phytotherapeutic agents (Negrelle and Gomes 2007).

The indiscriminate medicinal use of plants, usually toxic ones, may entail risks to health, because, similarly to the allopathic drugs, there is a threshold dosage for each phytotherapeutic agent. Thus, after an inadequate use, several disorders may occur, from intoxications to mutation events in somatic and germinative tissue, and it can lead to the development of somatic diseases, teratogenic effects and inherited genetic damages (Çelik et al. 2008). Most carcinogens, for example, their tumorigenic activity by the interaction of inductors of mutations with the DNA, leading manent genetic lesions, which are expressed as mutations or chromosomal aberrations involving cycle (Bronzetti et al. 1996, Gonzalgo and Jone Nunes and Carvalho 2003).

Among various species with medicinal pro *Cymbopogon citratus* (DC.) Stapf (Poaceae), a popularly known as "lemon grass", shows an number of applications and is popularly used b ple in many countries. In Brazil, for example, infusion and extracts of *C. citratus*, which are p with fresh or dry leaves, are often used in the medicine as a restorative, digestive, anti-tussis, e drug against colds, with an analgesic, anti-hermeti

lergic effect (Negrelle and Gomes 2007). Besides, the lemon grass extracts and their essential oils are also used in the food (flavoring), perfume and cosmetics industries, this use being of reasonable economical importance in various countries (Oliveira et al. 1997).

As a consequence of innumerous applications of *C. citratus*, several studies have been done aiming at enlarging the knowledge about the chemical composition of lemon grass leaves, which are the parts used for medicinal purposes. These studies have revealed that, although the chemical composition of the essential oil and aqueous extracts of *C. citratus* varies according to the geographical origin, the isolated and identified substances from the leaves are mainly alkaloids, saponin, â-sitosterol, terpenes, alcohols, ketone, flavonoids, chlorogenic acid, caffeic acid, p-coumaric acid and sugars (Matouschek and Stahl 1991, Chisowa et al. 1998, Negrelle and Gomes 2007).

Although plant extracts of lemon grass have been extensively used in the folk medicine, scientific research has found some potentially toxic substance in this species. Hepatotoxic and nephrotoxic effects in mice treated with fluid extracts of *C. citratus* (30% and 80%) were observed (Guerra et al. 2000), indicating the necessity of more detailed research on its cytotoxicity (Negrelle and Gomes 2007).

Considering different techniques used to investigate toxicity, cytogenetic bioassay is an important tool to identify the effects of substances at the chromosome level and also on cell cycle (Campos et al. 2008, Dragoeva et al. 2008). Among the various available methodologies, tests that use plant roots are extremely useful in biological assays, relatively inexpensive and can easily be handled. In addition, plant cytotoxic bioassays have a good correlation with mammalian test systems (Fiskesjö 1985, Jovtchev et al. 2002, Yi and Meng 2003, Çelik and Aslantürk 2006, 2007, Lubini et al. 2008).

According to Campos et al. (2008), the great number of seeds, the great contact surface with the aqueous extracts, the high sensibility and the bigger chromosomes of *L. sativa* (lettuce) make this plant very useful for cytogenetic bioassay, this paper described the effects of aqueous extracts of *C. citratus* leaves on seed germination, root growing, chromosome structure and cellular cycle of *L. sativa*.

MATERIALS AND METHODS

Plant Material and Extracts Preparation

Fresh leaves of *C. citratus* were collected at the Botanical Experimental Area of the Universidade Federal de Juiz de Fora (UFJF), and four aqueous extracts were prepared (5; 10; 20 and 30 mg, respectively, in 1 mL of distilled water). For the extraction of extracts, pieces of leaves were macerated in distilled water and after 24h, at room temperature; the extracts were filtered in filter paper before their application in the seeds bioassays. A voucher specimen was deposited at the CESJ Herbarium of the UFJF.

Germination and Root Growth

The different extracts concentrations and control were arranged in a completely random design with four repetitions (each repetition corresponding to 60 seeds of lettuce placed in a Petri dish). For control, lettuce seeds were germinated in distilled water. The germination percentage and root growth were evaluated after 12, 24, 36, 48, 60 and 72h of exposure time.

Cytogenetics Studies

After 72h, 10 roots of control and different extracts concentrations were collected from each repetition (40 roots for treatment). These roots were fixed in cold methanol/glacial acetic acid 3:1 (v/v) during 24h. Following this step, the roots were submitted to an enzymatic maceration (Pectinex NOVO NORDISK^{TM}) at 34°C for 1:45h. Subsequently, the roots were hydrolyzed in 5N HCl for 11 minutes. The air dry technique (Carvalho and Saraiva 1993), five slides were prepared for repetition (20 slides for treatment, each one with 2 root-tips) and stained with Giemsa 10% for 3 minutes. The mitotic index was determined for each treatment and the presence of chromosomes abnormalities were also evaluated. Around

STATISTICAL ANALYSES

The percentage of germinated seeds, size of the roots, mitotic index and percentage of chromosome aberrations were obtained by the mean of each of the four repetitions of each treatment. The data were submitted to one-way analysis of variance (ANOVA) and comparison between the means of treatments with the means of control was performed using the Tukey test ($p < 0.05$).

RESULTS

In this study, the mean percentage of germinated seeds decreased as the concentrations of lemon grass extracts increased, and the treatments lem_2 (10 mg/mL) at 24, 36, 48 and 60h, and lem_3 (20 mg/mL) and lem_4 (30 mg/mL) at 36, 48, 60 and 72h showed significant difference from the control. The extract concentrations also showed a considerable influence in the time of seed germination. We observed that the seeds treated with higher concentrations of lemon grass (lem_3 and lem_4) started the germination only after 36h of exposure, and they exhibit lower index of germination when compared with the control, lem_1 and lem_2 (Table I).

In a general way, a similar behavior was observed on the root growth. lem_3 and lem_4 reduced significantly ($p < 0.05$) the root size when the average was compared with to control (Table II).

Regarding the cytotoxic evaluation, the higher concentrations of the extracts showed a cytotoxic effect on root cells of lettuce considering that mitotic index decreased significantly on lem_2, lem_3 and lem_4 when the average of mitotic cells were compared to the control (Table II).

Together with the mitotic index, the cytological evaluation also revealed the occurrence of chromosome aberrations. It was observed a great percentage of lagging migration of the chromosomes, chromosome bridges, chromosome breaks, chromosome stickiness, polar deviation and micronucleus (Table IV, Fig. 1). Nevertheless, only at the highest concentration the percentage of all the chromosome aberrations were significantly different when compared to the control (Table IV,

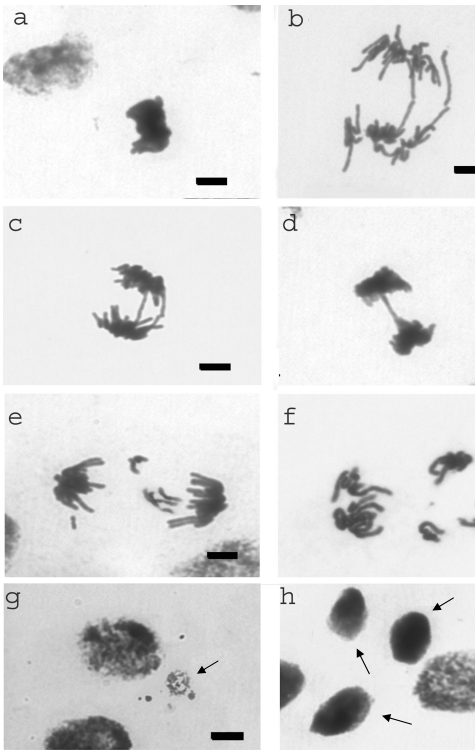

Fig. 1 – Chromosome aberrations observed in meristematic
lettuce exposed to aqueous extracts of lemon grass. Stickine
phase (a); Anaphase with chromosome lagging (b); Anapha
bridges (c and d); Chromosomes breakage due to bridge ter
(e); Polar deviation due to spindle alteration (f); Micronucle
tion (arrows) (g), Nuclear condensation due to cell death i
(arrows) (h). Bar = 5μm.

apoptotic bodies and some nucleus with comm
tions among them were observed. Again, lem_2, le
lem_4 treatments showed significant differences (P
when compared to the control (Table V, Fig. 2).

DISCUSSION

Cytogenetic assays have been widely used in ge
icity assessment to test compounds under both
and *in vivo* conditions. Changes on the mitotic
micronuclei formation and chromosome aberrati
important cytogenetic endpoints that are routine
in citotoxicity and genotoxicity evaluation (San

TABLE I
Percentage mean of germination of lettuce seeds after 12, 24, 36, 48, 60 and 72h of exposure to aqueous extracts of lemon grass.

Treatments	Exposure (h)					
	12	24	36	48	60	72
Control	—	87.81 (± 1.82)	90.54 (± 2.03)	94.27 (± 1.96)	96.77 (± 1.07)	97.81 (± 1.45)
lem_1	—	75.93 (± 3.12)	83.96 (± 3.88)	87.40 (± 2.65)	91.56 (± 1.80)	94.90 (± 2.64)
lem_2	—	30.83 (± 2.71)*	66.77 (± 3.18)*	77.69 (± 1.89)*	78.22 (± 1.75)*	85.62 (± 2.80)
lem_3	—	—	37.39 (± 2.17)*	72.28 (± 2.00)*	76.99 (± 2.85)*	79.16 (± 2.93)*
lem_4	—	—	25.00 (± 1.93)*	51.77 (± 1.48)*	55.31 (± 3.20)*	57.85 (± 4.00)*

lem_1: lemon grass aqueous extract (5 mg/mL), lem_2: lemon grass aqueous extract (10 mg/mL), lem_3: lemon grass aqueous extract (20 mg/mL), lem_4: lemon grass aqueous extract (30 mg/mL). *Significantly different from the control ($p < 0.05$) (Tukey test).

TABLE II
Mean size (cm) of lettuce roots during 72h of exposure in aqueous extracts of lemon grass.

Treatments	Exposure (h)					
	12	24	36	48	60	72
Control	—	0.75 (± 0.06)	1.09 (± 0.08)	1.59 (± 0.08)	2.84 (± 0.09)	3.80 (± 0.17)
lem_1	—	0.70 (± 0.04)	1.11 (± 0.05)	1.62 (± 0.01)	2.89(± 0.03)	3.45 (± 0.17)
lem_2	—	0.72 (± 0.02)	0.82 (± 0.04)*	1.45 (± 0.05)	2.19 (± 0.16)	3.55 (± 0.29)
lem_3	—	—	0.27 (± 0.03)*	0.70 (± 0.02)*	1.28 (± 0.03)*	1.52 (± 0.07)*
lem_4	—	—	0.18 (± 0.02)*	0.42 (± 0.07)*	0.91 (± 0.06)*	1.34 (± 0.06)*

lem_1: lemon grass aqueous extract (5 mg/mL), lem_2: lemon grass aqueous extract (10 mg/mL), lem_3: lemon grass aqueous extract (20 mg/mL), lem_4: lemon grass aqueous extract (30 mg/mL). *Significantly different from the control ($p < 0.05$) (Tukey test).

TABLE III
Total of cells evaluated and mean of mitotic index from lettuce roots cells after 72h of exposure to lemon grass aqueous extracts.

Concentration	Total of cells observed for treatment	Mean of Mitotic Index for treatment
Control	20,324	6.89 (± 0.71)
lem_1	18,432	6.63 (± 0.08)
lem_2	15,668	2.42 (± 0.49)*
lem_3	12,627	3.01 (± 0.13)*
lem_4	15,422	2.43 (± 0.37)*

lem_1: lemon grass aqueous extract (5 mg/mL), lem_2: lemon grass aqueous extract (10 mg/mL), lem_3: lemon grass aqueous extract (20 mg/mL), lem_4: lemon grass aqueous extract (30 mg/mL). *Significantly different from the control ($p < 0.05$) (Tukey test).

In the present study, aqueous extracts of lemon grass leaves showed cytotoxic and genotoxic effect, which can be observed by a significant decrease of mitotic index and the great number of chromosome aberrations. The different kind and number of chromosome aberrations from lettuce seeds treated with the highest

the action of other substances on living organisms by transforming promutagens (chemicals that are not mutagenic themselves, but that can be biologically transformed into a mutagen) into mutagens (Sarkar et al. 1996). Because of this, the property of activating promutagens in plants that may enter the food chain or are

TABLE IV

Mean percentage of chromosomes aberrations in meristematic cells of lettuce after 72h of exposure to lemon grass extract

Concentration	Chromosome aberrations					
	Lm	Brks	Stk	Sa	ABr	Mcn
Control	0.13 (± 0.01)	0.60 (±0.09)	3.89 (±0.54)	0.16 (±0.05)	0.42 (±0.07)	0.23 (±0
lem_1	0.17 (±0.01)	0.63 (±0.15)	4.17 (±0.62)	0.30 (±0.12)	0.58 (±0.09)	0.28 (±0
lem_2	0.14 (±0.01)	0.61 (±0.14)	6.20 (±0.31)*	0.22 (±0.04)	11.72 (±0,91)*	0.27 (±0
lem_3	15.31 (±2.02)*	0.66 (±0.08)	9.00 (±1.39)*	0.24 (±0.09)	23.68 (±2.28)*	0.30 (±0
lem_4	16.85 (±1.84)*	24.04 (±6.18)*	9.55 (±1.11)*	1.88 (±0.54)*	30.67 (±3.37)*	9.51 (±0,

lem_1: lemon grass aqueous extract (5 mg/mL), lem_2: lemon grass aqueous extract (10 mg/mL), lem_3: lemon grass aqueous extract (20 lem_4: lemon grass aqueous extract (30 mg/mL). Lm = lagging migration; Brks = breaks; Stk = stickiness; Sa = spindle alterations; ABr = a with bridge; Mcn = micronuclei. *Significantly different from the control ($p < 0.05$) (Tukey test).

TABLE V

Mean percentage of nuclear condensation, apoptotic bodies and nuclear communication in meristematic cells of lettuce after 72h of exposure to lemon grass extracts.

Concentration	Nuclear alterations		
	(%) Ncd	(%) Ab	(%) Ncm
Control	4.80 (± 0.61)	0.16 (± 0.03)	0.31 (± 0.15)
lem_1	5.42 (± 1.37)	0.18 (± 0.03)	0.30 (± 0.10)
lem_2	12.74 (± 1.24)*	0.18 (± 0.03)	0.31 (± 0.60)
lem_3	27.40 (± 3.62)*	4.37 (± 0.48)*	0.34 (± 0.08)
lem_4	37.22 (± 4.49)*	7.97 (± 0.85)*	5.65 (± 0.84)*

lem_1: lemon grass aqueous extract (5 mg/mL), lem_2: lemon grass aqueous extract (10 mg/mL), lem_3: lemon grass aqueous extract (20 mg/mL), lem_4: lemon grass aqueous extract (30 mg/mL). Ncd = nuclear condensation; Ab = apopitotic bodies; Ncm = nuclear communications. *Significantly different from the control ($p < 0.05$) (Tukey test).

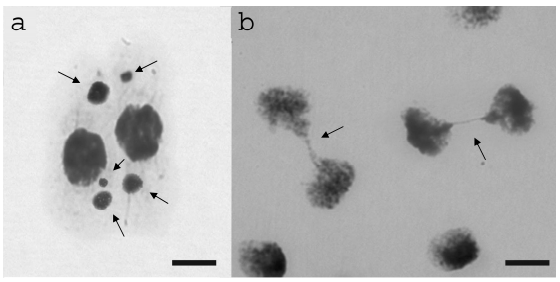

Fig. 2 – Nuclear modifications in roots cells of lettuce treated with aqueous extracts of lemon grass. Apoptotic bodies (arrows) and two condensed nucleus (a); Nuclear communications (arrows) (b). Bar = 5μm.

Regarding different types of mutagenic alterations

fects on spindle) effects, while chromosomes
tions are thought to arise from chromosome br
and exchange (Pugliesi et al. 2007). We obser
these effects in our study, mainly when 30 mg/m
applied (lem_4). As we observed more prominent
with chromosome aberrations at the highest con
tion, it is possible that the types of alterations a
specific. For example, only lem_4 induced the for
of apoptotic bodies, chromosomes breakage, ab
spindle behavior, micronuclei and nuclear comm
tion. Thus, our results suggested caution with
of lemon grass extracts, since some chromosome
tions can be produced when elevated doses are ab

The reduction of the mitotic index can be ex

In agreement with the second hypothesis, we observed various cells with cytoplasm shrinkage, nuclear condensation and apoptotic bodies, which are morphological aspects very common in the programmed cell death in plants (Solomon et al. 1999). These aspects were observed mainly in the lem4 treatment, where the mitotic index showed a significant decrease in relation to the control. Similar results were observed in previous studies and cell death was the major depressor of the mitotic index (Çelik and Aslantürk 2006, 2007, Campos et al. 2008, Lubini et al. 2008).

In addition to the alterations discussed above, we also observed a great percentage of stickiness, mainly on metaphases observed at the highest concentration (lem_3 and lem_4). This alteration in chromosome morphology reinforces the toxicity potentiality of some doses of *C. citrates* extracts, once these alteration evidences the toxic effect on the chromatin allowed by cell death (Campos et al. 2008, Lubini et al. 2008).

We also observed that the extracts affect root development and seed germination of lettuce. The reduction of the germination can be explained by the toxicity observed at the highest concentration of the lemon grass extracts. The decrease of mitotic index can also explain the reduction of root sizes at the highest concentration considering that the cell division is directly associated with root growth (Campos et al. 2008).

Valarini et al. (1996) also showed inhibitory effects of the essential oil suspension of *C. citratus*. The authors related total inhibition of seeds from *Digitaria horizontalis*, *Sorghum halepense*, *Bidens pilosa*, *Euphorbia heterophylla* and *Raphanus raphanistrum*. They suggested that lemon grass has a promissory application as a natural herbicide.

Finally, plant species represent a great source of biologically active compounds whose effects on heritable material are mostly unknown. The results obtained in the present study showed that, although *C. citratus* has a beneficial effect as a medicinal plant, serious problems and damages on cells by incorrectly usage, can be observed.

In order to reach more information and certain con-

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RESUMO

O capim-limão, *Cymbopogon citratus* (DC) Stapf, é uma importante espécie da família Poaceae com uma comum utilização na medicina popular em vários países. O objetivo deste estudo foi investigar os efeitos citotóxicos e genotóxicos do extrato aquoso das folhas de *C. citratus* em células meristemáticas de *Lactuca sativa* (alface) por meio de estudos citogenéticos, uma vez que estudos desta natureza não existem para extratos aquosos de capim-limão. Para isso, sementes de alface foram tratadas por 72h com diferentes concentrações de extratos aquosos feitos das folhas de capim-limão (5, 10, 20 e 30 mg/mL). O percentual de germinação, desenvolvimento radicular e o comportamento celular foram avaliados e os resultados mostraram que as concentrações mais elevadas dos extratos aquosos reduziram o índice mitótico, o percentual de germinação das sementes e desenvolvimento radicular da alface. Os extratos também induziram aberrações cromossômicas e morte celular nas células das raízes de *L. sativa*.

Palavras-chave: extrato aquoso, *Cymbopogon citratus*, efeito citotóxico, efeito genotóxico, *Lactuca sativa*.

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