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Antimicrobial activity of *Elettaria cardamomum*: Toxicity, biochemical and histological studies

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Abstract

Elettaria cardamomum is one of the most broadly used spices in Moroccan gastronomy. Its antimicrobial activity against both Gram-positive and Gram-negative bacterial species was demonstrated. Likewise, its toxicity was investigated on *Swiss albinos* mice. Daily, mice were treated orally with 0.003 and 0.3 mg during 7 days. Plasmatic markers and antioxidant defence systems were assessed and histological alterations were evaluated. A significant increase in creatine phosphokinase level was observed. The microscopic evaluation shows that *E. cardamomum* induce morphological perturbation in mice's heart. The results show also an inhibitory effect of glyceraldehyde 3-phosphate dehydrogenase and an important increase in the level of thiobarbituric acid reactive substances, succinate dehydrogenase and catalase activities. Results show that *E. cardamomum* induces toxicity at 0.3 mg/g mouse and affect energy metabolism and oxidative stress.

Keywords: *Elettaria cardamomum*; Antimicrobial activity; Natural antioxidant; Food; Clinical enzymes; Metabolic enzymes; Oxidative stress; Toxicity and histology

1. Introduction

Food-borne diseases are still a major problem in the World, even in well-developed countries (Mead et al., 1999). A variety of microorganisms also lead food spoilage that is encountered as one of the most important matter concerning the food industry. So far, many pathogenic microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Listeria monocytogenes* have been reported as the causal agents of food-borne diseases and/or food spoilage (Deak & Beuchat, 1996). Raw and/or processed foods are open to contamination during the production, sale and distribution of the foods (Deak & Beuchat, 1996). Due to the economical impacts and the consumer's concerns over safety, the reliability of synthetic chemicals used for treating foods has been in question. Thus, a lot of attention has been paid to naturally derived compounds or natural products (Alzoreky & Nakahara, 2003). Recently, there has been considerable interest in extracts and essential oils from aromatic plants with antimicrobial activities for controlling pathogens and/or toxin producing microorganisms in foods (Alzoreky & Nakahara, 2003).

The antimicrobial activity of some essential oil components against food-borne pathogens, including myco-

Abbreviations: DCIP, dichloroindophenol; CPK, creatine phosphokinase; EDTA, ethylenediaminetetraacetic acid; GOT, glutamic oxalic transaminase; GPT, glutamic pyruvic transaminase; KCN, potassium cyanide; MIC, minimal inhibitory concentration; NAD, nicotinamide adenine dinucleotide oxidized form.

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toxin-producing fungi, has also been tested (Kim, Wei, & Marshall, 1995; Ultee & Smid, 2001; Ultee, Slump, Steging, & Smid, 2000). Moreover, plant extracts have been developed and proposed for the same purpose in foods as natural antioxidants and/or antimicrobials (Del Campo, Amiot, & Nguyen-The, 2000; Ginesta-Peris, Garcia-Breijo, & Primo-Yùfera, 1994; Hsieh, Mau, & Huang, 2001).

L. monocytogenes is considered as one of the most dangerous microorganisms that spoil foods, it possesses several characteristics, which enable the pathogen to successfully contaminate, survive and grow in foods, thereby resulting in outbreaks. These traits include an ability to grow under refrigeration temperature, ability to survive in acidic conditions, e.g., pH 4.2, ability to tolerate up to 10% sodium chloride, and the ability to survive in biofilms on equipment in food processing plants and to resist superficial cleaning or disinfection treatments. Killing *L. monocytogenes* in foods would reduce the contamination of foods and limit the outbreaks of listeriosis, thus it would decrease economic loss to the food industry. A variety of chemicals and synthetic compounds have been developed for is inhibiting *L. monocytogenes* in foods. However, the instability of chemicals and synthetic compounds used as antimicrobials in foods, has lead on a significant increase in consumer's demand for naturally processed food, which has resulted in a huge increase in the use of naturally derived compounds such as plant extracts (Hao, Brackett, & Doyle, 1998).

Elettaria cardamomum as plant of Zingiberaceae family, has its seeds used commonly. Cardamom is a perennial shrub with thick, fleshy, lateral roots which can grow to a height of 8 feet (Kapoor, 2000). As a cooking spice, the darker seeds are removed from the seed pod and ground into a powder. Cardamom is primarily cultivated in southern India, Sri Lanka, Tanzania, and Guatemala. Morocco is also producer of cardamom which is used in cooking.

Historically known as the "Queen of all Spices" (Ravindran, 2002), cardamom has been used in India since ancient times. As a spice, cardamom is used in cuisine for curry, coffee, cakes, bread, and flavouring sweet dishes and drinks (Ravindran, 2002). The seed and the essential oil are used as a flavouring component in a variety of foods including alcoholic and non-alcoholic beverages, frozen desserts, candies, baked goods, puddings, condiments, relishes, gravies, meat, and meat products; it is also used a spice that makes Moroccan tajine, more tasty and generally used in cooking including that of meats.

Another use of Cardamom is in traditional Chinese and Indian medicine as a digestive aid, and for the treatment of intestinal gas (Ravindran, 2002). It has also been added on massage oils and lotions as well as soaps, detergents, and perfumes for its soothing properties (Ravindran, 2002). Seeds from *E. cardamomum* have antibacterial gram-negative bacterium (Mahady, Stoia, Fabricant, & Chadwick, 2005), the content of essential oil in the seeds is strongly dependent on storage conditions, but may be as high as 8%.

Recent scientific research has shown that many plants used as food or in traditional medicine are potentially toxic, mutagenic and carcinogenic (De Sã Ferrira & Ferrão Vargas, 1999).

The aim of the present study was to determine the antimicrobial effect of *E. cardamomum* on pathogens, for potential application as an antimicrobial in foods, and the study of the impact of the spice extract on the metabolic markers, stress biomarkers, clinical parameters and histological injury on *Swiss albinos*' mice. In addition, to the best of our knowledge this is the first report showing the introduction of toxicity and perturbation of enzymatic system by *E. cardamomum*.

2. Materials and methods

2.1. Chemicals

NAD+ (free acid) was purchased from Boehringer (Mannheim, Germany) and all other chemicals were of analytical grade.

2.2. Antibacterial effect

2.2.1. Spice material

Seeds of *E. cardamomum* were collected in March 2006 from 1 km of Jbel Bou Hachem, near Chauen, Morocco; the climate is mild Mediterranean-type, with cool, humid winters and warm, dry summers. They were dried according to herbarium techniques, and stored one month before their use in the laboratory.

2.2.2. Preparation of spice extracts

One hundred grams of spice were finely ground and macerated at room temperature in 100% ethanol during 5 days. The extract was subsequently filtered and concentrated to dryness in vacuo. The residue was dissolved in distillate water to create a concentration of 150 mg/ml of stock solution.

2.2.3. Microorganisms

The isolated strains were obtained from different departments. Optimum incubation temperature is presented in Table 1. These bacteria were selected because they are frequently reported in human infection and are multiresistant to several antibiotics.

2.2.4. MIC agar dilution assay

Minimal inhibitory concentration (MIC) values of the isolates were studied based on the agar dilution method as described by Gulluce et al. (2003). The oil was added aseptically to sterile muller hinton (MH) agar (Difco) at the appropriate volume to produce the concentration range of 1–150 mg/ml. The resulting MH agar solutions were immediately poured into Petri plates after vortexing. The plates were spot inoculated with 5 μl of each bacteria isolate. The inoculated plates were incubated at corresponding

Table 1
Bacteria strains and growth conditions used for this study

Strains	Source	Growth temperature (°C)	Broth of growing
1. Gram positive bacteria			
Lactobacillus delbrueckii	LNM	30	MRS
Bacillus cereus 11778	ATCC	30	Muller Hinton
Listeria monocytogenes 4d	LMAB	37	Palcam agar
Staphylococcus aureus 25923	ATCC	37	Muller Hinton
2. Gram negative bacteria			
Shigella sonnei	LNM	37	Muller Hinton
Escherichia coli	IPM	37	Muller Hinton
Pseudomona aeruginosa	ATCC	37	Muller Hinton
Salmonella enteridis	LNM	37	Muller Hinton
Yersinia enterolitica	LNM	30	Muller Hinton
Proteus vulgarices	LNM	37	Muller Hinton
Proteus mettegeri	LNM	37	Muller Hinton
Proteus penneri	LNM	37	Muller Hinton
Klebsiella pneumoniae	LMBE	37	Muller Hinton
Enterobacter cloacea	LNM	37	Muller Hinton
Morganella morganii	LNM	37	Muller Hinton
Citrobacter freundii	LNM	37	Muller Hinton

LNM, Service d'Hygiène de Rabat (Maroc); ATCC, American Type Collection Culture; IPM, Institut Pasteur du Maroc (Casablanca); LMBE, Laboratoire Microbiologie Biotechnologie et Environnement (Faculté des sciences Aïn Chock, Casablanca, Maroc); LMAB, Laboratoire Microbiologie Appliquée et Biotechnologie (Faculté des sciences, El jadida, Maroc).

temperatures for each isolates for 24 h. At the end of incubation period, the plates were evaluated for the presence or the absence of growth. MIC values were determined as the lowest concentration of the essential oil where the absence of growth was recorded.

2.2.5. Inhibitory effect by the agar-well diffusion method

Simple susceptibility screening test using agar-well diffusion method as adapted earlier was used (Bagamboula, Uyttendaele, & Debevere, 2004; Erdemog˘lu, Küpeli, & Yeşilada, 2003; Perez, Agnese, & Cabrera, 1999; Perez, Pauli, & Bazerque, 1990). Each microorganism was suspended in brain heart infusion (BHI) (Difco, Detroit, MI) broth and diluted with peptone water to provide initial cell counts of about 10^7–10^8 colony forming unit (CFU)/ml. Each strain of bacteria was inoculated on duplicate plates one agar. The plates were allowed to dry at 4 °C for 1 h. The wells were formed and filled with 30 µl from spice extract. The Discs of Antibiotics (Sigma) were then placed aseptically over the bacterial cultures on plates by the disc diffusion method (Collins, Lyne, & Grange, 1989), the plates were incubated for 24 h at temperature appropriate. The diameter of the inhibition zone was measured with calipers.

2.3. Animals and administration of E. cardamomum

Swiss albinos' mice were adapted to laboratory conditions at a temperature of 22 °C with food and water *ad libitum*. The light cycle during the entire experiment was set to 14 h light and 10 h dark.

Forty-five animals were randomized into five groups of nine mice each, and the *E. cardamomum* extract was administered daily by oral injection during 7 days. Mice were given *E. cardamomum* at 0.003 and 0.3 mg/g of mice's weight while corresponding groups were given sterile water serving as control. In each treatment

2.4. Blood analysis

The determination of the CPK, aminotransaminases glutamic oxalic transaminase (GOT) and glutamic pyruvic transaminase (GPT), creatinine and urea were carried out by "Laboratoire des analyses médicales du Centre National de la Sécurité Sociale (CNSS)", Casablanca.

2.5. Crude extract preparation

All procedures were carried out at 4 °C. Samples of liver were quickly weighed and then homogenized 1/3 (w/v) in 50 mM potassium phosphate buffer pH 7.4 containing 1 mM EDTA, 1 mM DTT. The homogenates were then filtered and stored at −20 °C until use.

2.6. Biochemical assays

All assays were conducted at 25 °C using Jenway 6405 UV/Visible spectrophotometer.

2.6.1. Catalase

The consumption of 7.5 mM H_2O_2 in 50 mM potassium phosphate buffer (pH 7) was monitored at 240 nm (Aebi, 1984).

2.6.2. Thiobarbituric acid reactive substances

The assessment of the extent of hepatic lipid peroxidation relied on the determinations of malondialdehyde (MDA) equivalent content in the crude liver homogenates. Duplicate determinations from each liver were made and the average of the two measurements was used in the subsequent statistical analysis of the data. Lipid peroxidation was estimated by the formation of thiobarbituric acid reactive substances (TBARS) and quantified in terms of malondialdehyde (MDA) equivalents according to the method described by Samokyszyn and Marnett (1990).

One milliliters of samples is added to 1 ml solution (0.375% thiobarbituric acid and 15% trichloracetic acid in 0.25 M hydrochloric acid). The tubes are heated at 100 °C during 15 min. Then, they are cooled in the ice to stop the reaction. A centrifugation was then carried out with 1000*g* during 10 min. The reading of supernatant is made to 535 nm, TBARS were calculated from a standard curve (8–50 nmol) of malondialdehyde (MDA), freshly prepared by acidification of TEP (1,1,3,3-tetraethoxypropane). Results are expressed as TBARS (nmol/mg protein) using $\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

2.6.3. Glyceraldehyde 3-phosphate dehydrogenase

GAPDH activity in the oxidative phosphorylation was determined by monitoring NADH generation at 340 nm (Serrano, Mateos, & Losada, 1991). The reaction mixture of 1 ml contained 50 mM Tricine–NaOH buffer pH 8.5, 10 mM sodium arsenate, 1 mM NAD+ and 2 mM D-G3P.

2.6.4. Succinate dehydrogenase

The enzyme was assayed according to King (1967) with assay conditions: 100 mM potassium phosphate buffer (pH 7.4), 0.3 mM EDTA, 0.053 mM DCIP and 100 μg of protein. The mixture was preincubated 10 min at 37°C before to added 50 μl of KCN-Succinate (containing 3.25 mg/ml of KCN in 0.5 M succinate). The measure of activity was done at 625 nm.

2.6.5. Protein quantification

Protein content in liver was measured according to the Bradford procedure, using bovine serum albumin (BSA) as standard (Bradford, 1976).

2.7. Histopathological analysis

After seven days of treatment, the liver, heart, brain, kidneys and bowel 4 μm sections were removed, fixed in Bouin and embedded in paraffin. The organ sections were stained with hematein-eosin, then examinated under light microscopy (Olympus-BH-2).

2.8. Statistical analysis

All experiments were replicated in four times and tests were duplicated, the experimental data represent the means ± standards deviations. Means were compared using the Student *t*-test, using SPSS statistical software Version 12.0. Differences were considered significant at the level $p < 0.05$ and very significant at the level $p < 0.01$.

3. Results

3.1. Antibacterial activity

Table 2 shows that the average zone of inhibition of spices against microorganisms ranged from 7 ± 2 mm

Table 2
Mean clearance zone diameters for *Elettaria cardamomum* extract at (300 μg/μl) and for antibiotics

Microorganisms	Cardamom	Te	NA	VA	AM	DA	AMX	NV	C (10 μg)	C (30 μg)	ST	P	K	CB
Bacillus cereus ATCC 11778	16 ± 2.0	19 ± 1.0	12 ± 1.3	10 ± 2.4	–	–	–	13 ± 1.5	16 ± 1.2	14 ± 2.6	–	–	15 ± 1.4	–
Enterobacter clolacea	13 ± 1.5	18 ± 2.8	12 ± 1.5	16 ± 2.3	–	–	–	10 ± 1.4	–	13 ± 2.0	–	–	–	–
Citobacter freundii	8 ± 1.9	12 ± 1.5	–	–	–	–	10 ± 2.0	–	–	–	–	–	–	–
Lactobacillus delbrueckii	n.d.	–	–	–	–	–	–	–	10 ± 3.1	12 ± 2.4	–	–	–	–
Yersinia enterolitica	–	–	–	–	–	–	–	12 ± 1.5	9 ± 3.2	12 ± 1.6	–	–	6 ± 1.2	–
Proteus penneri	10 ± 2.3	–	10 ± 2.0	15 ± 2.8	–	–	–	12 ± 1.8	12 ± 1.4	16 ± 1.4	–	–	–	–
Proteus mettegeri	8 ± 1.8	17 ± 1.9	–	12 ± 1.1	–	–	–	15 ± 1.4	12 ± 1.2	11 ± 2.2	–	–	–	–
Staphylococcus aureus	10 ± 1.5	23 ± 2.0	–	–	–	–	–	14 ± 2.1	7 ± 2.2	20 ± 2.2	–	–	–	–
Escherichia coli	12 ± 2.4	–	–	–	–	–	–	–	–	–	–	–	14 ± 1.9	–
Shigella sonnei	8 ± 1.9	–	–	–	–	–	–	–	–	–	–	–	–	–
Listeria monocytogenes	13 ± 1.8	20 ± 1.9	–	16 ± 1.7	–	–	–	12 ± 2.2	15 ± 3.1	13 ± 1.9	–	–	–	–
Klebsiella pneumoniae	8 ± 1.4	12 ± 2.0	–	–	–	–	–	–	14 ± 2.2	15 ± 1.6	–	–	–	–
Salmonella enteridis	8 ± 2.6	–	–	–	–	–	–	–	–	–	–	–	–	–
Morganella morganii	8 ± 2.9	–	–	–	–	–	–	–	–	–	–	–	–	–
Proteus vulgaricus	8 ± 2.2	–	–	16 ± 1.6	–	–	–	12 ± 1.4	10 ± 1.8	13 ± 1.5	–	–	–	–
Pseudomonas aeruginosa ATCC 27853	7 ± 2.0	9 ± 1.5	–	–	–	–	–	12 ± 1.8	8 ± 1.4	11 ± 3.3		–	–	–

Values (mm) are expressed as mean ± standards deviations of four experiments; –, No inhibition zone; n.d., not determined. Te, Tetracycline 30 μg; NA, Acide nalidixic; VA, Vancomycine 30 μg; AM, Ampicillin 10 μg; DA, Clindamycin 2 mg; AMX, Amoxycillin 25 μg; NV, Novobiocine 5 μg; C, Chloramphenicol 10 μg/30 μg; ST, Sulfathiazol 0.25 mg; P, Penicilline G 10 μg; K, Kanamycine 30 UI; CB, Carbenicillin 100 μg.

(*Pseudomonas aeruginosa* ATCC 27853) to 16 ± 2 mm (*Bacillus cereus* ATCC 11778). Most discs of antibiotics indicate that they did not possess antibacterial effect on the pathogen. The antibacterial effect of spice extract on the different bacteria strains is shown in Table 3.

Although various strains of bacteria varied in their antimicrobial susceptibility, Cardamom extract was very effective in inhibiting the growth of all the tested strains of a majority of the pathogens, CMI ranged between 9.4 and 18.75 mg/ml with the exception of *E. coli*, *Bacillus cereus* ATCC11778 and *Enterobacter cloacae* which had a great sensitivity to the spice extract (CMI < 2.34 mg/ml).

3.2. Toxicity test

The general state and the mice mortality was followed during the 7 days of treatment. No sign of stress or difficulties on breathing in the gavages mice was observed.

No change was observed with the amounts of 0.003 mg/g and 0.03 mg/g during 7 days of treatment. On the other hand, a significant decrease in the weight was observed (32%) at 0.3 mg/g. Mortality was observed in the mice treated with 3 mg/g after 48 h of treatment (Fig. 1).

3.3. Effect of E. cardamomum extract on plasmatic parameters

The blood analysis indicated an important increase in CPK level (×1.21) at 0.3 mg/g. No changes were observed in GOT, GPT, urea and creatinine (Table 4).

3.4. In vivo effect of E. cardamomum extract on stress and metabolic biomarkers

For stress biomarkers, the treatment of the mice showed that *E. cardamomum* induced a significant increase in the level of TBARS (×2.02) at 0.3 mg/g and on catalase activity (×2.57 and ×2.60) at 0.003 and 0.3 mg/g, respectively, compared with the control (Table 5).

For the metabolic markers, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was very sensitive to *E. cardamomum* extract at both concentrations; it strongly decreased (76% and 56%) at 0.003 and 0.3 mg/g, respectively. In contrast, the succinate dehydrogenase activity increased significantly at both concentrations (×2.32 and ×3.49, respectively).

Table 3
Minimum inhibitory concentrations (MIC) of spice extracts of cardamom

Bacteria	MIC (mg/ml)
Shigella sonnei	18.75 ± 0.11
Listeria momocytogens	18.75 ± 0.08
Lactobacillus delbrueckii	18.75 ± 0.09
Staphylococcus aureus	9.4 ± 0.05
Pseudomonas aeruginosa ATCC27853	9.4 ± 0.07
Escherichia coli	<2.34
Morganella morganii	18.75 ± 0.01
Salmonella enteridis	9.4 ± 0.02
Citrobacter freundii	18.75 ± 0.02
Yersinia enterolitica	18.75 ± 0.06
Proteus vulgaricus	18.75 ± 0.04
Klebsiella pneumoniae	9.4 ± 0.07
Proteus mettegeri	9.4 ± 0.17
Enterobacter cloacea	< 2.34
Proteus penneri	9.4 ± 0.05
Bacillus cereus ATCC11778	< 2.34

Values (mm) are expressed as means ± standards deviations of four experiments.

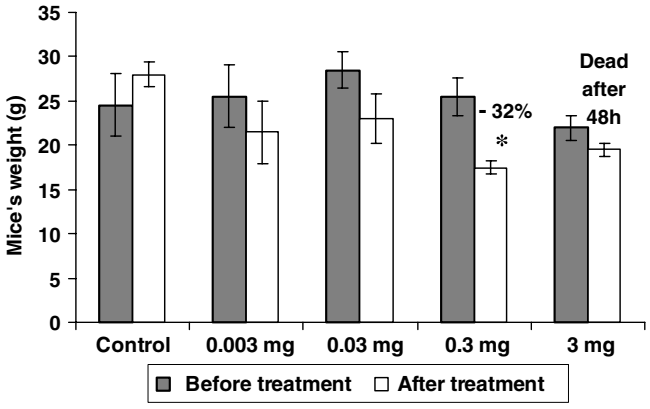

Fig. 1. Weight variation of the *Swiss albinos* mice before and after gavages (7 days) with 0.003 mg/g mouse, 0.03 mg/g mouse, 0.3 mg/g mouse, 3 mg/g mouse of Cardamom extract. Values are means ± standards deviations. $^{*}p < 0.05$ (Student *t*-test), $n = 9$. %, indicate the percentage of decrease or increase of the values comparing with the control.

Table 4
Plasmatic parameters from control and cardamom treated mouse at 0.003 and 0.3 mg

	Control	Cardamom 0.003 mg	Cardamom 0.3 mg
GOT (U/l)	285 ± 56	307 ± 10	270.5 ± 91.2
GPT (U/l)	45.6 ± 7	40 ± 2.8	44.5 ± 2.1
CPK (U/l)	2915 ± 134	3475 ± 35[a] (×1.19)	3532 ± 53[a] (×1.21)
Urea	0.32 ± 0.10	0.38 ± 0.02	0.39 ± 0.03
Creatinine	8.66 ± 1.52	10 ± 1.41	9.5 ± 2.12

Values (mm) are expressed as mean ± standards deviations ($n = 9$).
[a] $p < 0.05$ (Student *t*-test). Numbers in brackets indicate how many times the values have increased comparing with the control values.

3.5. Immunological study

In order to explore the impact of *E. cardamomum* extract on immunological cells, the number of lymphocytes, monocytes and polynuclears were calculated. Fig. 2 shows an important increase in monocytes level (155%). No changes were observed for lymphocytes and polynuclears.

3.6. Morphological and histopathological study

The macroscopic analysis of the removed organs during the dissection does not reveal any changes compared to the control.

Table 5
Effect in vivo of Cardamom at 0.003 mg and 0.3 mg on response of oxidative stress and metabolic biomarkers

	Control	Cardamom 0.003 mg	Cardamom 0.3 mg
Catalase (μmol/min/mg of protein)	3.51 ± 0.58	$9.05 \pm 0.54^{*}$ (×2.57)[a]	$9.16 \pm 1.15^{*}$ (×2.60)[a]
Thiobarbituric acid reactive substances (nmol/mg of protein)	0.42 ± 0.16	0.55 ± 0.09	$0.85 \pm 0.13^{*}$ (×2.02)[a]
Glyceraldehyde-3-phosphate dehydrogenase (μmol/min/mg of protein)	0.50 ± 0.15	$0.12 \pm 0.06^{*}$ (−76%)[b]	$0.22 \pm 0.05^{*}$ (−56%)[b]
Succinate dehydrogenase (Absorbance/min/mg of protein)	1.48 ± 0.76	$3.44 \pm 0.89^{*}$ (×2.32)[a]	$5.17 \pm 1.23^{*}$ (×3.49)[a]

Values (mm) are expressed as mean ± standards deviations ($n = 9$).
[a] Indicate how many times the values have increased comparing with the control values.
[b] Indicate the percentage of decrease of the values comparing with the control values.
* $p < 0.05$ (Student t-test).

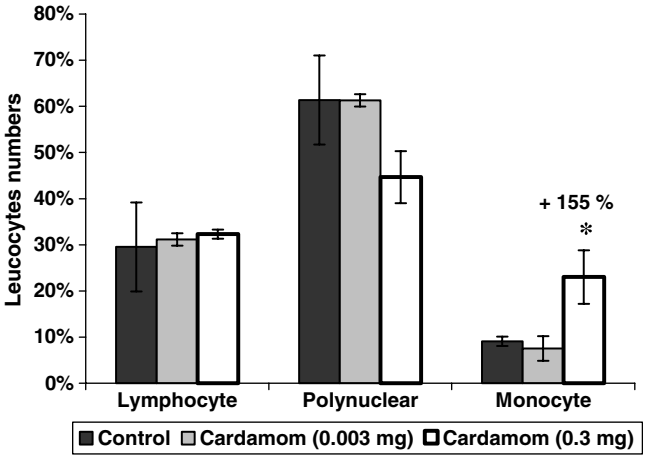

Fig. 2. Leucocytes numbers after 7 days of gavages in comparison with control. Values are means ± standards deviations. $^{*}p < 0.05$ (Student t-test), $n = 9$. %, indicate the percentage of decrease or increase of the values comparing with the control.

Optical microscopy of the heart of mice treated with 0.3 mg/g showed a necrosis and dissolution of the nucleus and nuclear membrane (karyolysis) (Fig. 3).

In brain of the mice treated with 0.3 mg/g, an increase of the number of the nucleus as well as a hypercondensation of the chromatin was observed. Also, we observed the presence of some multinucleated giant cells that indicate the inflammation in brain (Fig. 4).

4. Discussion

Natural products and naturally derived compounds from plants may have applications in controlling pathogens in foods (Bowles & Juneja, 1998; Davidson, 1997). The challenge is to isolate, stabilize and incorporate natural antimicrobials into foods without adversely affecting sensory, nutritional and safety characteristics (Beuchat & Montville, 1989). This has to be achieved without significantly increasing production, processing and marketing costs.

Some compounds of essential oil have been shown by authors to inhibit peptidoglycan synthesis (Ogunlana, Hoeglund, Onawunmi, & Skoeld, 1987), damage microbial membrane structures (Cox, Mann, Bell, & Warmington, 2000), modify bacterial membrane surface hydrophobicity (Turi, Turi, Koljalg, & Mikelsaar, 1997), and modulate quorum sensing (Gao, Teplitski, & Bauer, 2003).

Ethanolic extracts have been investigated for their antibacterial activity against bacteria. These observations are likely due to the existing differences in cell wall structure between Gram-positive and Gram-negative bacteria, with the Gram-negative outer membrane acting as a barrier to many environmental substances, including antibiotics (Tortora, Funke, & Case, 2001).

It is difficult to perform a detailed comparison with the results obtained by other authors in this type of study. Among the problems, it is worth mentioning those that arise from the material used, in terms of its nature (spice, extract or essential oil), origin (country of origin, altitude at which it grows, harvest season), production process, level of purity and preservation, all of which help to determine the presence of variable concentrations of antimicrobials in the final product. On the other hand, many tests have been carried out on synthetic growth media, with dilution or diffusion in a solid medium, using discs impregnated with antimicrobial agents (Ginesta-Peris et al., 1994; Hsieh et al., 2001; Kim et al., 1995).

Seeds of *E. cardamomum* have an antibacterial activity for gram-negative bacterium (Mahady et al., 2005). Gram-positive bacteria are common pathogens responsible for disease conditions. These include *S. aureus* for skin infections (Noble, 1998); *L. monocytogenes* has emerged into a highly problematic and fatal food-borne pathogen. A wide spectrum of foods, including milk, cheese, beef, pork, chicken, seafood, fruits, and vegetables, have been identified as vehicles of *L. monocytogenes* in causing listeriosis (Hao et al., 1998). *Bacillus cereus* is a spore-forming pathogen often associated with food-borne diseases caused by toxins. It is present in ready-to-eat vegetable-based foods (Carlin et al., 2000; Del Torre, Della Corte, & Stecchini, 2001; Valero, Hernàndez-Herrero, Fernàndez, & Salmeron, 2002a).

For stress biomarkers, a significant increase in lipid peroxidation (TBARS) was observed at 0.3 mg/g, this increase in activities shows the beginning of oxidative stress in liver. Effectively, oxidative stress occurs as a consequence of imbalance between the formation of oxygen free radicals and inactivation of these species by antioxidant defence system. Lipid peroxidation has been implicated in the

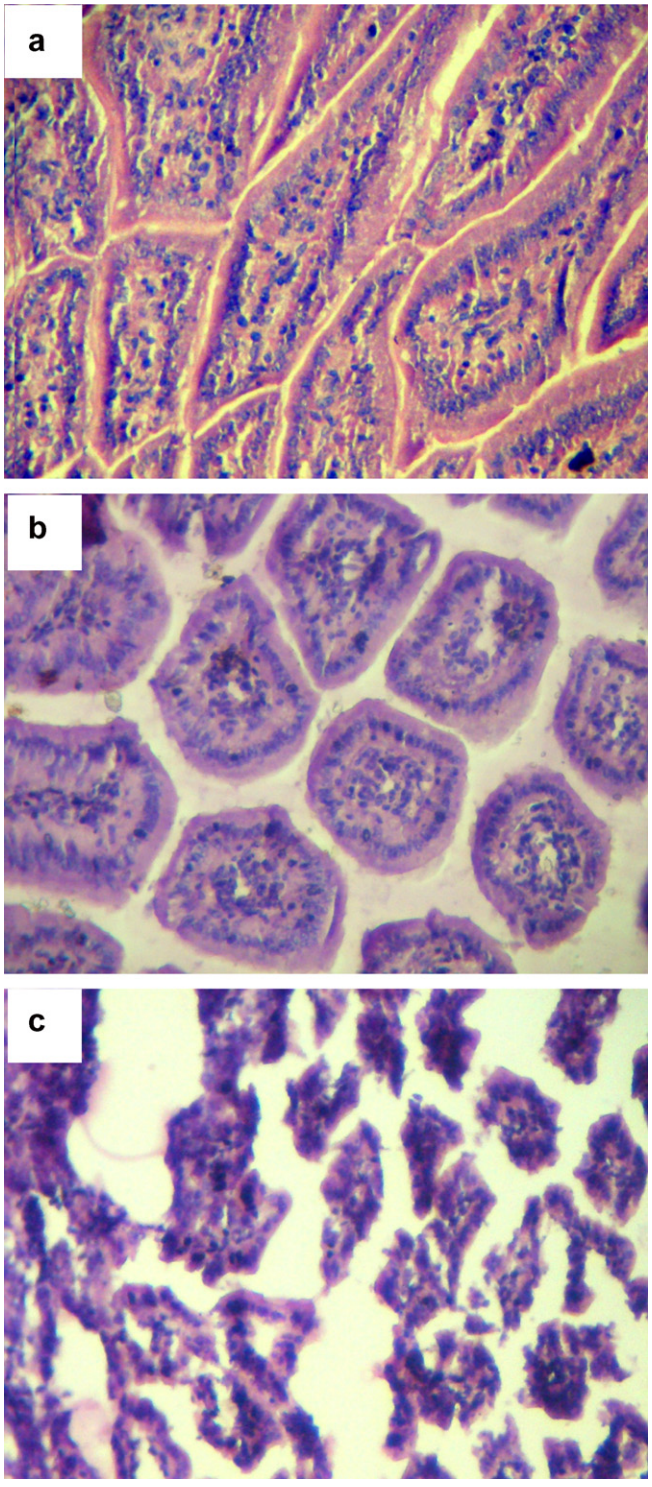

Fig. 3. Histological sections of heart from control (a) and gavage with cardamom 0.003 mg (b) and 0.3 mg (c) after 7 days of treatments. Sections are stained with hematein-eosin. Magnification: ×400.

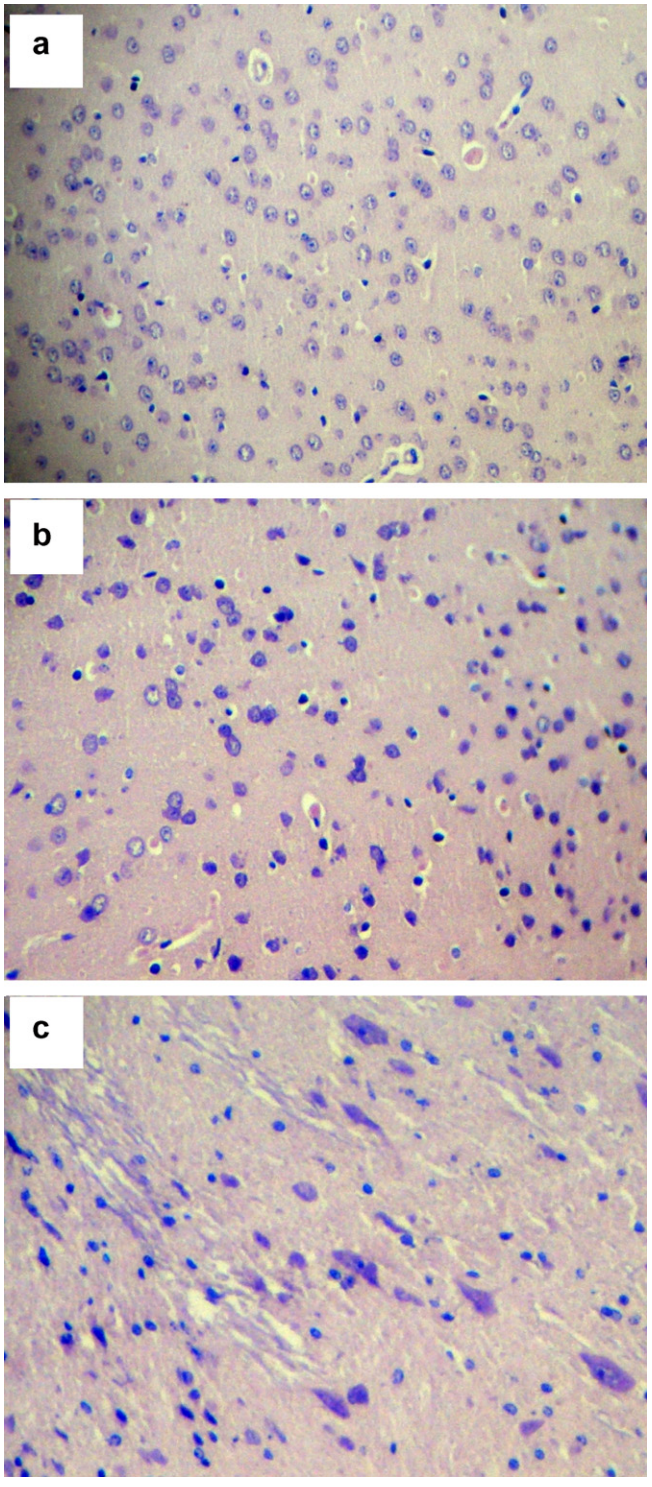

Fig. 4. Histological sections of brain from control (a) and gavage with cardamom 0.003 mg (b) and 0.3 mg (c) after 7 days of treatments. Sections are stained with hematein-eosin. Magnification: ×400.

pathogenesis of increased membrane rigidity, osmotic fragility, reduced erythrocyte survival and perturbations in lipid fluidity. We found that increase in lipid peroxidation (TBARS) correlated positively with increase administrative dose and disease activity score of the heart.

Free radical-induced oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficiency of natural antioxidant defences. Potential antioxidant therapy should therefore include either natural free radical scav-

enging antioxidant enzymes or agents which are capable of augmenting the activity of these enzymes, which include superoxide dismutase, catalase and glutathione peroxidase.

For the metabolic biomarkers, GAPDH was strongly suppressed, SDH increased significantly at 0.3 mg/g. This increased SDH activity, suggests that recovery process increased the production of energy in liver, and that metabolism energetic in heart and liver are perturbed which caused for long time serious problems, such as cirrhoses of liver and myocardial infarcts.

Other plants, like *Emblica officinalis* have the same effects as Cardamom against free radical damage induced during stress (Rege & Dahanukar, 1999); among other effects, we found inhibition of lipid peroxidation (Kumar, 1999), and antibacterial effect (Ahmad & Mohammad, 1998).

The body weight of the mice was altered by the extract *E. cardamomum* at 0.3 mg/g. Furthermore, mice treated with 3 mg/g were dead after 2 days of the gavages. These results agree with those obtained with the enzymatic markers and also suggest that *E. cardamomum* ethanolic extract was toxic at doses *ges* 0.3 mg/g.

There appeared to be no renal toxicity in the mice because we found no change in creatinine and urea.

Lymphocyte proliferation is a very sensitive test and is used as a potential biomarker for toxic exposures. In the present study, Fig. 2 showed an important increase in monocytes level. Further, the brain and heart of the mice treated with 0.3 mg/g, show cells necrosis and inflammation. The necrosis of the cells causes the importation of the immunizing cells and the development of the inflammatory reactions.

5. Conclusions

The present study confirmed the antibacterial effect of *E. cardamomum* extract, and that the toxicity of cardamom at 0.3 mg/g, which induced inflammation in brain, oxidative stress and cells necrosis in heart. The use of *E. cardamomum as* spice should not exceed the 0.003 mg/g since at this amount no negative effects were observed.

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