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Research Paper

Antitumor and immunomodulating potential of *Coriandrum sativum*, *Piper nigrum* and *Cinnamomum zeylanicum*

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ABSTRACT

Phytochemicals are known to modulate immune function, and possess antitumor and antimicrobial properties. In the present study, *in vitro* antitumor and immunomodulating activities of aqueous and methanol extracts of *Coriandrum sativum* (leaf and seed), *Piper nigrum* and *Cinnamomum zeylanicum* seeds were evaluated. We observed that aqueous extract of *C. sativum* (leaf), *P. nigrum*, and *C. zeylanicum* caused significant (P<0.05) 24, 39 and 61 percent L5178Y-R lymphoma cells toxicity at 31.2, 31.2 and 7.8 µg/ml (MICs) respectively, whereas the methanol extract of *C. sativum* (seed and leaf), *P. nigrum*, and *C. zeylanicum* caused 40, 31, 26 and 39 percent cytotoxicity at 7.8, 62.5, 15.6 and 7.8 µg/ml (MICs), respectively. In addition, *C. sativum* leaf aqueous extract stimulated significant (P<0.01) 14 to 45 percent splenic cells lymphoproliferation at 7.8 to 125 µg/ml respectively, whereas *P. nigrum* caused significant (P<0.01) 79 percent proliferation at 125 µg/ml; similarly, *C. zeylanicum* aqueous extract significantly (P<0.01) induced 20 and 37 percent thymic cells lymphoproliferation at 62.5 and 125 µg/ml respectively, whereas the methanol extracts of *C. sativum* leaf extract caused significant (P<0.01) 43 to 59 percent lymphoproliferation at the concentrations tested. Furthermore, all spice aqueous extracts tested were observed to significantly (P<0.01) reduce up to 100% nitric oxide production by LPS-stimulated macrophages.

Keywords: Spices; Anticancer; Immunomodulation; Lymphoproliferation; Antiinflammatory.

INTRODUCTION

The use of preparations and infusions of plants to treat diseases and improve the immunological response against many pathologies has been practiced worldwide for centuries, but their effectiveness must be scientifically validated to increase the credibility of their use (Mainardi, et al., 2009; Gomez-Flores, et al., 2009, 2000; García-Alvarado, et al., 2001). The use of leaves, stems, flowers, seeds or roots is common among people, and

pharmaceutical industry is using this knowledge to synthesize and produce drugs after rigorous scientific protocols of biological activity validation (Grynkiewicz, et al., 2008).

Piper nigrum (black pepper, peppercorns) oil is used to treat pain, rheumatism, chills, colds, exhaustion, muscular aches, physical and emotional coldness, nerve tonic and fevers, and to increase blood flow. It also stimulates appetite, encourages peristalsis, tones the colon muscles and is a general digestive tonic; its oleoresin has bacteriostatic and fungistatic properties (Chaudhry and Tariq, 2006). Similarly, *Coriandrum sativum* (coriander, cilantro, Arab parsley, Chinese parsley) has been reported to possess antimicrobial properties (Chaudhry and Tariq, 2006). On the other hand, *Cinnamomum zeylanicum* (cinnamon bark, sweet wood, canela, dalchini, tamala patra) was added to food to prevent spoiling, and it was used in Egypt for embalming. Cinnamon has been prescribed for diarrhea, chills, influenza, diabetes, rheumatism and certain menstrual, dyspepsia with nausea, intestinal colic, and parasitic worms, among other uses. Cinnamon oil has been reported to possess antimicrobial (Wong, et al., 2008) and anti-inflammatory (Kanuri, et al., 2009) activities.

Cancer is one of the leading causes of death worldwide. It is well known that chemotherapy and radiotherapy are toxic not only to tumor cells, but also to healthy cells; the use of natural sources, particularly plant-derived products, in cancer treatment, may reduce adverse side effects of conventional therapy (Kaefer and Milner, 2008). There are many reports related to the use of medicinal plants in cancer, mainly from Asia (Plaeger, 2003); taxol, vincristine, vinblastine, tepocan, irinotecan, and etoposide-teniposide are plant products that are widely used in tumor therapy (Kong, et al., 2003). In addition, plants have been shown to modulate the immune system and the inflammatory response (Salem, 2005); compounds present in plants such as alcaloids, quinones, terpenoids, phenolcarboxylic acids, polysaccharides, and glycoproteins have been linked to immunostimulating and antitumor potential (Wagner, 1990).

In the present study, we evaluated the *in vitro* antitumor (toxicity to murine L5178Y-R lymphoma cell line) and immunomodulating (lymphoproliferation and nitric oxide production by macrophages) activities of *Coriandrum sativum*, *Piper nigrum* and *Cinnamomun zeylanicum*.

MATERIALS AND METHODS

***Reagents and culture media**:* Penicillin-streptomycin solution, L-glutamine, and RPMI 1640 and AIM-V media were obtained from Life Technologies (Grand Island, NY). Fetal bovine serum (FBS), lypopolysacharide (LPS) from *Escherichia coli* 0111:B4 strain, sodium dodecyl sulfate (SDS), N, N-dimethylformamide (DMF), and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO). The tumor cell line L5178Y-R (mouse DBA/2 lymphoma) was purchased from The American Type Culture Collection (Rockville, MD), and was maintained in culture flasks with RPMI 1640 medium supplemented with 10% FBS, 1% L-glutamine, and 0.5% penicillin-streptomycin solution (referred as complete RPMI 1640 medium) at 37ºC, in a humidified atmosphere of 5% CO_2 in air; cellular density was kept between 10^5 and 10^6 cells/ml. Extraction buffer was prepared by dissolving 20% (wt/vol) SDS at 37^{o}C in a solution of 50% each DMF and demineralized water, and the pH was adjusted to 4.7.

***Animals**:* Six- to eight-week old Balb/c female mice were purchased from Harlan Mexico S.A. de C.V. (Mexico, D.F.). They were kept in a pathogen- and stress-free environment at 24^0 C, under a light-dark cycle (light phase, 06:00-18:00 h) in a One Cage 2100™ System (Lab Products, Inc., Seaford, DE), and given water and food *ad libitum*. Animals were euthanized by asphyxiation in a 100% CO_2 chamber. Experiments involving the use of animals were reviewed and approved by our institutional animal care and use committee before being initiated, and were performed in accordance with the Guiding Principles in the Use of Animals in Toxicology, adopted by the Society of Toxicology in March 1999.

***Preparation of extracts**:* *Coriandrum sativum* (Apiaceae family) leaves and seeds, *Piper nigrum* (Piperaceae family) and *Cinnamomun zeylanicum* (Lauraceae family) used in this study were identified by M.Sci. María del Consuelo González de la Rosa, Chief of the

Herbarium of the Biological Sciences College at Autonomous University of Nuevo Leon. Plant material was obtained at a local market in San Nicolás de los Gaza N. L., México with a voucher specimen #1361 for *Coriandrum sativum*, #12789 for *Piper nigrum*, and #023494 for *Cinnamomun zeylanicum*. They were rinsed with tap water to eliminate dust and other contaminating material, dried at 37°C for 36 h, and pulverized. The aqueous extract was prepared by placing 5g of the powder in 80 ml boiling distilled water for 10 min. The resulting extracts were then centrifuged at 2800 rpm for 15 min at room temperature, and supernatants were placed in 1 ml glass tubes, previously weighted. Then this material was dried by lyophilizing, and dissolved in distilled water. The methanol extract was prepared by placing 5g of the powder in 80 ml absolute methanol under shaking for 24 h at room temperature. The resulting extracts were centrifuged at 2800 rpm for 15 min at room temperature, and supernatants were placed in 1 ml Eppendorf tubes, previously weighted. Then this material was dried under vacuum using a speed-vac concentrator (Savant Instruments Inc., Hicksville, NY), and dissolved in distilled water. Stock solutions of both aqueous and methanol extracts were then prepared at 1 mg/ml in complete RPMI 1640 medium (for L5178Y-R cells) or in AIM-V medium (for macrophage and lymphocyte cultures), and sterilized by filtering through a 0.22-microns membrane (Millipore, Bedford, MA).

L5178Y-R cell preparation and culture*:* In order to determine the direct *in vitro* effect of the extracts on tumor cell growth, L5178Y-R cell cultures were collected and the cellular suspensions obtained were washed three times in RPMI 1640, and re-suspended and adjusted to 5 x 10^4 cells/ml with complete RPMI medium. One hundred microliters of the cell suspensions were then added to flat-bottomed 96-well plates (Becton Dickinson, Cockeysville, MD), containing 100 µl triplicate cultures of complete RPMI (unstimulated control) or the extracts at various concentrations (extracts were dissolved in complete RPMI 1640 medium). After incubation for 44 h at 37°C with 5% CO_2, MTT (0.5 mg/ml, final concentration) was added, and cultures were additionally incubated for 4 h. Next, cell cultures were incubated for 16 h with extraction buffer (100 µl/well) and optical densities, resulting from dissolved formazan crystals, were then read in a microplate reader (Bio-Tek Instruments Inc., Winooski, VT) at 540 nm (Gomez-Flores, et al., 2009). The percentage of cytotoxicity was calculated as follows:

$$\% \text{ Cytotoxicity} = 100-[(A_{540} \text{ in extract treated cells}/ A_{540} \text{ in un-treated cells}) \times 100]$$

Macrophages and lymphocytes preparation and culture*:* Spleens and thymuses were immediately removed after rat death. Single-cell suspensions were independently prepared by disrupting the organs in RPMI 1640 medium. Cell suspensions were washed three times in this medium, and suspended and adjusted at appropriate densities with AIM-V medium (containing 2.5 mM L-glutamine, streptomycin (50 µg/ml), and gentamycin (10 µg/ml)). The culture medium was changed at this step to the serum-free medium AIM-V which supports cell culture (Gomez-Flores, et al., 2009, 2000).

Lymphocyte proliferation assay*:* Lymphoproliferation was determined by a colorimetric technique using MTT (Gomez-Flores, et al., 2009, 2000). Thymus or spleen cell suspensions (100 µl of 1 X 10^7 cells/ml) were added to flat-bottomed 96-well plates (Becton Dickinson) containing triplicate cultures (100 µl/well) of AIM-V medium (unstimulated control), or plant extracts at various concentrations for 48 h at 37°C in 95% air-5% CO_2 atmosphere. After incubation for 44 h, MTT (0.5 mg/ml, final concentration) was added, and cultures were additionally incubated for 4 h. Cell cultures were then incubated for 16 h with extraction buffer (100 µl/well), and optical densities, resulting from dissolved formazan crystals, were then read in a microplate reader (Bio-Tek Instruments Inc.) at 540 nm (Gomez-Flores, et al., 2009, 2000). The lymphocyte proliferation index (LPI) was calculated as follows:

$$\text{LPI} = A_{540} \text{ in extract treated cells}/ A_{540} \text{ in un-treated cells}$$

Macrophage cultures*:* Peritoneal macrophages were prepared by washing the peritoneal cavity with cold RPMI 1640 medium, and washing the resulting cell suspension twice in this medium. One hundred-microliter cell suspensions at 1.7 x 10^6 cells/ml in AIM-V medium were then plated in flat-bottomed 96-well plates (Becton Dickinson) for 2 h at 37°C. Non-

adherent cells were removed, and adherent cells (about 70% of the input cells or about 1 x 10^6 cells/ml) were then utilized for determining nitric oxide production. The final adherent cell monolayer consisted of 95-99% macrophages as judged by Giemsa's stain procedures.

Nitrite determination: Accumulation of nitrite in the supernatants of macrophage cultures was used as an indicator of nitric oxide production by resident or activated cells. Peritoneal macrophages were incubated for 72 h in 200 µl AIM-V medium, in the presence or absence of various concentrations of the extracts and LPS (3 µg/ml) in triplicates, in a total volume of 200 µl AIM-V medium. After incubation, supernatants were obtained and nitrite levels were determined with the Griess reagent (Gomez-Flores, et al., 2000), using $NaNO_2$ as standard. Optical densities at 540 nm were then determined in a microplate reader (Bio-Tek Instruments Inc.). Macrophage viability was determined by the MTT reduction assay as previously described (Gomez-Flores, et al., 2000).

The percentage of viability was calculated as follows:

$$\% \text{ Viability} = A_{540} \text{ in extract treated cells/ } A_{540} \text{ in un-treated cells x } 100$$

Statistical analysis: The results were expressed as means ± SEM of triplicate determinations from a representative experiment. All experiments were repeated at least three times with similar results. Statistical significance was assessed by one-way analysis of variance and by Student's't' test.

RESULTS

In vitro cytotoxic activity of extracts: Aqueous and methanol extracts were tested at concentrations ranging from 7.8 to 125µg/ml. Table-1 shows that aqueous seed extract of *C. sativum* did not alter L5178Y-R cell growth, whereas its leaf caused significant ($P<0.05$) 24 (MIC), 27 and 49 percent cytotoxicity at concentrations of 31.2, 62.5 and 125 µg/ml respectively. In addition, *P. nigrum* induced significant ($P< 0.01$) 39 (MIC), 64 and 71 percent cytotoxicity at concentrations of 31.2, 62.5 and 125 µg/ml respectively, and *C. zeylanicum* caused significant ($P<0.01$) 60 to 72 percent cytotoxicity at the concentrations tested (MIC = 7.8 µg/ml) (Table-1). The methanol extract of *C. sativum* seed was observed to cause significant ($P< 0.01$) 40 to 78 percent cytotoxicity at the concentrations tested (MIC = 7.8 µg/ml), and its leaf induced significant ($P< 0.05$) 31 and 51 percent cytotoxicity at 62.5 (MIC) and 125 µg/ml respectively (Table-2). In addition, *P. nigrum* induced significant ($P< 0.05$) 26 to 75 percent cytotoxicity at concentrations of 15.6 (MIC) to 125 µg/ml respectively, and *C. zeylanicum* caused significant ($P< 0.01$) 39 to 72 percent cytotoxicity at the concentrations tested (MIC = 7.8 µg/ml) (Table-2).

Effect of extracts on spleen and thymus lymphocyte proliferation: *C. sativum* seed and *C. zylanicum* aqueous and all methanol extracts did not alter spleen lymphoproliferation (data not shown), whereas *C. sativum* leaf aqueous extract stimulated significant ($P<0.01$) 14 to 45 percent spleen lymphocyte proliferation at concentrations ranging from 7.8 to 125 µg/ml, respectively, and *P. nigrum* caused significant ($P<0.01$) 79 percent proliferation at 125 µg/ml (Table-3). In addition, *C. sativum* (seed and leaf) and *P. nigrum* aqueous extracts did not alter thymus lymphoproliferation (data not shown), whereas *C. zylanicum* extract significantly ($P<0.01$) induced 20 and 40 percent proliferation at 62.5 and 125 µg/ml respectively (Table-4). The methanol extracts of *C. sativum* seed, *P. nigrum* and *C. zeylanicum* did not alter thymus proliferation (data not shown), whereas *C. sativum* leaf extract caused significant ($P<0.01$) 44 to 59 percent proliferation at the concentrations tested (Table-4).

Effect of spice extracts on nitric oxide production: Nitrite levels in supernatants of LPS-stimulated murine macrophage cultures treated with all the extracts were significantly lower than the untreated controls. At concentrations ranging from 31.2 to 500 µg/ml, *C. sativum* leaf and seed, *P. nigrum* and *C. zeylanicum* aqueous extracts respectively caused significant ($P< 0.01$) 50% to 100%, 22% to 100%, 33% to 100%, and 53% to 100% decreases in nitrite levels, as compared with untreated control (Table-5). In addition, *C. sativum* leaf and seed, *P. nigrum* and *C. zeylanicum* methanol extracts respectively caused significant ($P < 0.01$) 70% to 100% (at 31.2 to 500 µg/ml respectively), 67% to 100% (at 15.6 to 500 µg/ml respectively), 85% to 100% (at 125 to 500 µg/ml respectively), and 100% (from 62.5 µg/ml) decreases in

nitrite levels, as compared with untreated control (Table-6). Macrophage viability was not significantly altered by the aqueous and methanol extracts (Tables-5 and 6); however, the methanol vehicle was observed to significantly ($P<0.01$) reduce nitrite levels in the supernatants of LPS-stimulated macrophages (data not shown), thus indicating that the observed effect for the methanol extracts was due to the vehicle.

DISCUSSION

C. zeylanicum aqueous extract caused the highest toxicity (Table-1), whereas *C. sativum* seed, *P. nigrum* and *C. zeylanicum* methanol extracts showed high tumor cell toxicity (Table-2), compared with untreated control. *C. sativum, P. nigrum* and *C. zeylanicum* have been also reported to affect a variety of tumor cells or possess anti-cancer properties different from our findings related to murine L5178Y-R lymphoma cells *in vitro* toxicity; in this regard, *P. nigrum* was reported to suppress colon carcinogensis (Nalini, et al., 2006) and inhibit lung metastasis induced by B16F-10 melanoma cells (Pradeep and Kuttan, 2002), and one of its components, piperine (also present in cinnamon), was shown to inhibit human colon cancer cell growth (Duessel, et al., 2008), possess anti-mutagenic potential (Wongpa, et al., 2007) and prevent lung carcinogenesis in the rat (Selvendiran, et al., 2006). On the other hand, *C. sativum* was reported to protect against experimental colon cancer in rats (Chithra and Leelamma, 2000), and its methanol extract was cytotoxic against the tumor cell lines MK-1, HeLa and B16F10 (Nakano, et al., 1998). In addition, *C. zeylanicum* was shown to be chemopreventive against colon carcinogenesis (Bhattacharjee, et al., 2007), suppress melanoma progression and stimulate cytotoxic T cells (Kwon, et al., 2009), and inhibit proliferation of the myeloid cell lines Jurkat and U937 (Schoene, et al., 2005).

In addition to the antitumor activity observed, only *C. sativum* leaf and *P. nigrum* aqueous extracts were observed for the first time to stimulate spleen lymphocyte proliferation (Table-3), whereas *C. zeylanicum* aqueous and *C. sativum* leaf methanol extracts stimulated thymic lymphocyte proliferation (Table-4). Furthermore, all spice extracts were observed to inhibit nitric oxide production by macrophages (Tables-5 and 6). Potentiating lymphocyte functions and suppressing those of macrophages may be advantageous for these spices. Generation of nitric oxide is known to depend on TNF-alpha induction (Frankova and Zidek, 1998); nitric oxide and TNF-alpha are produced during inflammation and can be both beneficial and detrimental for the organism (MacMicking, et al., 1997; Muñoz-Fernandez and Fresno, 1998). Although these molecules are usually associated with antimicrobial and antitumor activities (Muñoz-Fernandez and Fresno, 1998), they also induce immunosuppression by affecting macrophage functions through direct action on these cells (Albina, et al., 1993). In addition, suppression of macrophage functions by the spices may be mediated by an autocrine mechanism involving the induction of IL-10 or TGF-β (Clarke, et al., 1998). Therefore, spices may potentiate lymphoproliferative responses, and suppress macrophage functions and potential pathological states.

It was also shown that spice extracts activated lymphocytes proliferation, but they were cytotoxic to L5178Y-R tumor cells *in-vitro*. To our knowledge, this is the first report showing that spice extracts can not only potentiate lymphoproliferation, but also directly kill tumor cells. However, limited information on the immunomodulatory properties and tumor cell toxicity of spice extracts or their bioactive compounds is available. On this regard, a pure compound from *P. nigrum*, piperine, was shown to significantly reduce nitric oxide production by LPS-stimulated macrophages (Pradeep and Kuttan, 2003). On the other hand, conflicting results on *C. zeylanicum* nitric oxide and/or TNF-alpha production have been reported. This spice was shown to both suppress iNOS and TNF-alpha expression (Kanuri, et al., 2009) and increase TNF-alpha mRNA levels (Cao, et al., 2008) in RAW macrophages, whereas significant inhibition of nitric oxide production by LPS-treated murine peritoneal macrophages was observed in the present study (Tables-5 and 6). Such differences might be related to several factors including the target cell source and the final product evaluated (gene expression versus compound production and secretion). Activation of lymphoproliferation is relevant since lymphocytes produce cytokines capable to stimulate lymphocyte and macrophage functions. In our study, we observed that spice extracts stimulated

lymphoproliferation in the absence of co-stimulatory signals like concanavalin A or LPS, without altering lymphocyte viability. Spice extracts not only stimulated lymphocyte proliferation, but also were cytotoxic to tumor cells. In order to elucidate this differential activity of spices on lymphocytes and tumor cells, it will be necessary to isolate and test the spices´ bioactive compounds.

CONCLUSION

We have shown for the first time and in a single study the antitumor potential of *C. sativum*, *P. nigrum* and *C. zeylanicum* aqueous and methanol extracts, and their effect on stimulating lymphoproliferation and suppressing nitric oxide production by macrophages without altering their viability.

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Table-1: Effect of spice aqueous extracts on L5178Y-R lymphoma cell toxicity[a].

Concentration (µg/ml)	*C. sativum* (seed)	*C. sativum* (leaf)	*P. nigrum*	*C. zeylanicum*
7.81	9.3±0.3[b]	15.6±0.4	10.1±0.2	61.2±16.7**
15.65	13.4±1.1	17.9±0.54	18.8±0.5	71.8±6.9**
31.25	9.0±0.7	23.9±1.0*	38.8±5.7**	70.7±5.1**
62.5	2.5±0.3	27.2±2.2*	63.7±3.7**	67.6±3.5**
125	0±1.6	48.5±15.9**	70.9±1.6**	60.4±5.3**

- [a] Optical density value for proliferation of L5178Y-R cells untreated control was 0.381 ± 0.035.
- [b]Values represent percent cytotoxicity.
- **P<0.01; *P<0.05, compared with untreated control.

Table-2: Effect of spice methanol extracts on L5178Y-R lymphoma cell toxicity[a].

Concentration (µg/ml)	*C. sativum* (seed)	*C. sativum* (leaf)	*P. nigrum*	*C. zeylanicum*
7.81	40.0±9.1**[b]	4.0±0.3	15.1±1.3	38.8±5.1**
15.65	69.4±19.0**	7.8±0.9	26.0±4.6*	58.2±13.1**
31.25	78.4±9.3**	15.0±2.0	51.7±21.1**	70.0±13.3**
62.5	78.1±4.9**	30.6±6.6*	73.7±10.5**	72.2±5.6**
125	76.2±2.1**	50.8±21.8**	74.9±7.1**	70.8±6.6**

- [a]Optical density value for proliferation of L5178Y-R cells untreated control was 0.381 ± 0.035.
- [b]Values represent percent cytotoxicity. **P<0.01; *P<0.05, compared with untreated control.

Table-3: Effect of spice aqueous extracts on spleen lymphocyte proliferation[a].

Concentration (µg/ml)	*C. sativum* (leaf)	*P. nigrum*
7.8	1.14±0.12**[b]	1.09±0.12
15.6	1.17±0.12**	1.08±0.12
31.2	1.27±0.15**	1.08±0.13
62.5	1.34±0.15**	1.13±0.12
125	1.45±0.17**	1.79±0.12**

- [a]Optical density values for spleen lymphocyte proliferation in medium alone(untreated control) was 0.904 ± 0.00.
- [b]Values represent lymphocyte proliferation indexes. **P<0.01, compared with untreated control.

Table-4: Effect of spice aqueous and methanol extracts on thymus lymphocyte proliferation[a].

Concentration (µg/ml)	Aqueous extract *C. zeylanicum*	Methanol extract *C. sativum* (leaf)
7.8	0.98±0.02[b]	1.58±0.00**
15.6	1.03±0.03	1.58±0.00**
31.2	1.09±0.03	1.50±0.02**
62.5	1.20±0.02**	1.53±0.01**
125	1.37±0.04**	1.43±0.00**

- [a] Optical density values for spleen lymphocyte proliferation in medium alone (untreated control) was 0.684 ± 0.044.
- [b]Values represent lymphocyte proliferation indexes. **P<0.01, compared with untreated control.

Table-5: Effect of spice aqueous extracts on viability and nitric oxide production by LPS-treated peritoneal macrophages[a]

		Aqueous extract concentration (µg/ml)						
		0	15.6	31.2	62.5	125	250	500
C. sativum (leaf)	%V	100	97	98	99	100	100	98
	$[NO_2]$	0.17±0.03	0.14±0.04	0.02±0.05 **b	0.08±0.04 **	0 ±0.05**	0 ±0.03**	0 ±0.04**
C. sativum (seed)	%V	100	98	97	96	96	96	100
	$[NO_2]$	0.60±0.07	0.52±0.09	0.54±0.03	0.46±0.04 *	0.12±0.06 **	0 ±0.05**	0 ±0.05**
P. nigrum	%V	100	97	100	100	100	100	97
	$[NO_2]$	0.79±0.15	0.83±0.05	0.39±0.05 **	0.52±0.09 **	0.05±0.17 **	0.08±0.11 **	0 ±0.13**
C. zeylanicum	%V	100	100	100	100	95	94	98
	$[NO_2]$	0.54±0.06	0.40±0.14	0.25±0.07 **	0 ±0.11**	0±0.05**	0±0.05**	0 ±0.06**

[a]Values represent percent macrophage v iability (%V) and nitrites concentration ($[NO_2]$) in nanomoles/well.

[b]**,P<0.01; *, P<0.05, compared with medium alone (untreated control), which did not induce nitrite release nor cytotoxicity.

Table-6: Effect of spice methanol extracts on viability and nitric oxide production by LPS-treated peritoneal macrophages[a]

		Methanol extract concentration (μg/ml)						
		0	15.6	31.2	62.5	125	250	500
C. sativum (leaf)	%V	100	98	100	98	98	97	98
	$[NO_2]$	0.96±0.07	0.80±0.16	0.29±0.12 **[b]	0.14±0.15 **	0 ±0.13 **	0 ±0.05 **	0 ±0.02 **
C. sativum (seed)	%V	100	86	88	87	90	92	85
	$[NO_2]$	1.17±0.12	0.39±0.16 **	0.48±0.15 **	0±0.08 **	0±0.09 **	0 ±0.07 **	0 ±0.15 **
P. nigrum	%V	100	98	97	100	100	99	97
	$[NO_2]$	1.01±0.10	1.07±0.08	1.11±0.15	0.99±0.16	0.15±0.07 **	0±0.04 **	0 ±0.04 **
C. zeylanicum	%V	100	100	99	100	100	100	100
	$[NO_2]$	1.17±0.09	1.06±0.16	0.88±0.30	0 ±0.06 **	0.±0.06 **	0±0.053 **	0 ±0.03 **

[a]Values represent percent macrophage viability (%V) and nitrites concentration ($[NO_2]$) in nanomoles/well.

[b] **, $P<0.01$, compared with medium alone (untreated control), which did not induce nitrite release nor cytotoxicity.

