0163-4984/(Online) 1559-0720/07/11502–157 $30.00

Analysis of Cadmium and Lead in Mice Organs

Effect of Nigella sativa L. *(Black Cumin) on the Distribution and Immunosuppressive Effect of Cadmium–Lead Mixture in Mice*

Adnan M. Massadeh,*[,1] Saafan A. Al-Safi,[2] Idrees F. Momani,[3] Mohsen Al-Mahmoud,[1] and Ahmad S. Alkofahi[1]

[1]Departments of Medicinal Chemistry and Pharmacognosy and [2]Clinical Pharmacy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan; and [3]Department of Chemistry, Faculty of Science, Yarmouk University, Irbid, Jordan

Received April 12, 2006; Revised May 23, 2006; Accepted October 1, 2006

ABSTRACT

Analysis and distribution of Pb and Cd in different mice organs, including the liver, kidney, spleen, heart, and blood, were evaluated before and after treatment with different aqueous concentrations of *Nigella sativa* (1.25–10.0 mg/L). Atomic absorption spectrometry was used for analysis of Pb and Cd in these organs. Results indicated that the Pb in the unexposed group of mice without treatment with *N. sativa* (black cumin) was in the following order: liver > heart > spleen > kidney, and the distribution of Pb in various organs of the unexposed group was not affected significantly by *N. sativa.* Moreover, results of mice exposed for Pb show that the Pb concentrations in different organs were reduced significantly ($p < 0.05$) by 72.9%, 63.4%, 72.3%, 66.7%, and 39.5% at a dose of 10 mg/L of *N. sativa* for the liver, kidney, heart, spleen, and blood, respectively. Furthermore, the distribution of Cd in the unexposed Cd group of mice without treatment with *N. sativa* was in the following order: kidney > heart > spleen > liver. *Nigella sativa* at 10 mg/L reduced Cd levels in mice exposed to Cd by 75.5%, 83.3%, 47.0%, 95.3%, and 100% in the liver, kidney, heart, spleen, and blood, respectively, whereas blood Cd concentrations were lowered to below the detection limit of 0.05 μg/L.

*Author to whom all correspondence and reprint requests should be addressed.

A 28-d exposure of mice to a Cd–Pb mixture at a concentration of 1 ppm in drinking water induced a highly significant inhibition ($p < 0.0001$) of antibody response to human serum (80.5%). The suppressed immune responses in mice pretreated with the Cd–Pb mixture were reversed by 43.1% and 38.9% in the presence of 1.25 and 2.5 mg/mL of *N. sativa,* respectively, whereas higher concentrations (5–10 mg/mL) of *N. sativa* increased the immunosuppression significantly. *Nigella sativa* at 1.25–10 mg/mL did not induce any significant modulation of the antibody response in unexposed mice.

Index Entries: Cadmium; lead; analysis; atomic absorption spectrometry; *Nigella sativa;* immunosuppressive.

INTRODUCTION

Heavy metals have an extremely long biological half-life in humans and are accumulated in body tissues, particularly in the liver and kidney *(1,2).* Most of the heavy metals, especially Pb and Cd, are toxic pollutants and dangerous for humans. Many adverse health effects caused by Pb and Cd are the result of their accumulation in different body organs *(1,2).* Pb toxicity is induced in several organ systems, including nervous, hematopietic, renal, endocrine, and skeletal systems, reproductive system, and cardiovascular system. Exposure can cause impairment in intellectual functioning, kidney damage, infertility, miscarriage, and hypertension *(3–5).* Moreover, Pb is a special hazard for young children and had been shown to reduce the IQ of school-aged children *(4).*

Cadmium is an ubiquitous environmental pollutant *(6).* Acute high-dose exposures can cause severe respiratory irritation and prostate cancer, and lead to bone fractures (itai-itai disease) *(7,8).*

Researchers studied these elements in soils *(9),* road dust *(10,11),* and cigarets *(12,13).* Others studied their effects on the immune system *(14–16),* the effect of heavy metals on *Drosophila (17),* and removal of heavy metals by halophilic bacteria *(18).* Good indicators of exposure to heavy metals are measurements of their concentrations in different mice organs such as blood, liver, kidneys, amd spleen *(14).*

Nigella sativa seeds have been used for thousands of years as a spice and food preservative, as well as a protective and treatment for various diseases *(19).* The English name for *N. sativa* is black cumin. Its crude oil produces many actions, including antihistamine *(20),* antihypertensive *(21),* hypoglycemic *(22),* antimicrobial *(23),* and immunopotentiating *(24)* effects. The main constituents of *N. sativa* are thymoquinone and dithymoquinone *(25).* Other constituents include alkaloids such as nigellicine, nigellimine, and nigellidine *(26).*

In this study, the effect of *N. sativa* on Pb and Cd distribution in mice organs and the immunosuppressive effect of Cd and Pb were evaluated in mice.

MATERIALS AND METHODS

Materials

Concentrations of 1 ppm of Cd and Pb were prepared from two stock solutions (1000 ppm) of analytical grade $Cd(NO_3)_2 \cdot 4H_2O$ and $Pb(NO_3)_2$ obtained from Scharlau (Spain). Ten groups of male and female Balb/c mice (11 mice/group) weighing 15.8–30.4 g (mean: 26.9 g) were obtained from the Biological Research Center of Jordan University of Science and Technology. All mice were fed a normal diet. During d 1–28, mice of groups 1–5 were allowed to drink distilled water while mice of groups 6–10 were exposed to a Pb–Cd mixture (1 ppm each). At the end of d 28, Pb–Cd exposure was terminated. During d 29–50, mice of group 1 were left on distilled water while groups 2–5 were allowed to drink distilled water containing *Nigella* extract at 10, 5, 2.5, and 1.25 mg/mL, respectively. At the end of d 50, exposure to *Nigella* extract was terminated. Moreover, during d 29–50, mice of group 7 were left on distilled water while mice of groups 7–10 were exposed to distilled water containing *Nigella* extract at 10, 5, 2.5, and 1.25 mg/mL, respectively. At the end of d 50, exposure to *Nigella* extract was terminated.

On d 51, 52, and 53, each mouse of groups 1–3, 4–6, and 7–10, respectively, was immunized intraperitoneally with 0.25 mL of human serum emulsified in Freund's complete adjuvant (FCA) according to the standard immunization procedures *(27).* Secondary immunization was repeated on d 61, 62, and 63 for groups 1–3, 4–6, and 7–10, respectively. Furthermore, tertiary immunization was performed on d 71, 72, and 73 for groups 1–3, 4–6, and 7–10, respectively.

On d 81, 82, and 83, blood samples were obtained from mice of groups 1–3, 4–6, and 7–10, respectively, by cardiac puncture while the mice were under general ether anesthesia.

The titer of the antibody response was estimated using the Rheumatoid Factor Kit (Labkit, Spain). The approximate titer, in international units (IUs), of the serum was calculated by multiplying the highest dilution giving a positive agglutination by the kit's sensitivity (8 IU/mL).

Methods

Organ Samples Pretreatment

Organ tissue samples were dried in an oven at 105°C. A weight of about 200 mg of dried sample of each organ was placed in a PTFE vessel and allowed to digest with a mixture of 4 mL of HNO_3 and $HClO_4$ at a ratio of (3 : 1, v/v) by heating the PTFE vessel in a water bath–shaker at 80°C for 18 h. After cooling, a volume of 6 mL deionzed H_2O for each sample was added and shaken for 6 h and then each sample was filtered via filter paper (Whatman no. 41). Each extract was completed to 10 mL with deionized H_2O and kept in a refrigerator at 4°C until analysis.

Blood Sample Pretreatment

A volume of 1 mL of each blood sample was digested in a 3 mL mixture of HNO_3 and $HClO_4$ at a ratio of (1 : 2, v/v) and heated in a water bath–shaker at 70°C for 12 h. For each extract sample, a volume of 6 mL deionized H_2O was added and shaken for 12 h. Each extract was filtered via Whatman filter paper no. 41, completed up to 10 mL with deionized H_2O, and kept at –20°C until analysis.

Instrumentation for Heavy Metal Analysis

An atomic absorption spectrometer (Spectra AA-50/55; Varian, Australia) with a computer system, at the Department of Chemistry, Yarmouk University, Jordan, was used. Hollow-cathode lamps for Pb and Cd were operated at wavelengths of 283.3 nm and 228.8 nm, respectively, with a spectral bandpass of 0.7 nm.

Validation of Methods for Heavy Metal Analysis

To confirm the reliability of the method used for the analysis of Cd and Pb in the mice organ tissues. For accuracy and reproducibility of results, every run was started with a control blank and testing several quality control (QC) solutions. This procedure was repeated after every tenth sample. Results were within 5% of the QC values. For every sample, three replicates were taken and the average value was calculated. The levels of Cd and Pb were estimated in the heart, spleen, kidney, and liver. Calibration checks and blanks were analyzed for five replicate determinations.

Statistical Analysis of Results

The Minitab statistical program was used for analysis of data in this study. The results were analyzed utilizing a computerized program of the unpaired *t*-test. Results were considered statistically significant when $p<0.05$. Comparison was made between each test group and the control group.

RESULTS AND DISCUSSION

Lead Distribution and Analysis

Lead levels in different organs of unexposed and exposed mice and the effect of *N. sativa* on its distribution in these organs are shown in Tables 1 and 2, respectively.

The distribution of Pb in the unexposed group of mice without treatment with *N. sativa* was in the following order: liver > heart > spleen > kidney. Results show a reduction in Pb concentrations in all organs after treatment with *N. sativa* in same order (Table 1). The distribution for the Pb-exposed group of mice in the absence of *N. sativa* was in the following

Table 1
Effect of *N. sativa* on Distribution of Pb
in Various Organs in Pb-Unexposed Mice

Group	Nigella Mg/mL	N	Mean ± SEM (mg/kg)				
			Liver	Kidney	Heart	Spleen	Blood
I	0	11	0.149±0.007	0.116±0.009	0.137±0.006	0.130±0.003	0.052±0.0025
II	10	3	0.068±0.015 (0.020<P)	ND	0.035±0.0001 (0.015<P)	0.011±0.0008 (0.000<P)	0.048±0.0031 (0.47>P)
III	5	10	0.054±0.009 (0.056<P)	0.055±0.007 (0.00<P)	0.057±0.0001 (0.00<P)	0.010±0.0005 (0.0001<P)	0.045±0.0018 (0.047<P)
IV	2.5	3	0.047±0.006 (0.00>P)	0.054±0.001 (0.00<P)	0.047±0.0003 (0.00<P)	0.049±0.0003 (0.000<P)	0.032±0.0007 (0.3>P)
V	1.25	10	0.050±0.004 (0.00<P)	0.044±0.003 (0.00<P)	0.042±0.002 (0.000<P)	0.047±0.0016 (0.000<P)	0.020±0.0025 (0.000<P)

order: liver > kidney > spleen > heart > blood. There was a significant reduction in Pb concentrations in all organs after treatment with *N. sativa* (Table 2). Moreover, there was a significant difference before and after treatment with *N. sativa.* The percentage removal of Pb concentrations in liver ranged from 70.05% to 76.92% at 10, 5, 2.5, and 1.25 mg/mL *N. sativa.* The percentage removal of Pb concentrations in the kidney, heart, spleen, and blood ranged from 63.43% to 77.77%, from 31.73% to 72.28%, from 5.93% to 66.66%, and from 32.54% to 39.55%, respectively, at the same doses of *N. sativa.* In the heart, the percentage removal of Pb ranged from 31.73% to 72.29 at 10, 5, 2.5, and 1.25 mg/mL *N. sativa.*

The distribution of Pb in various organs of the unexposed group was not affected significantly by *N. sativa* (Table 1), whereas the opposite is true for the exposed group (Table 2).

Cadmium Distribution and Analysis

Cadmium levels in different organs in unexposed and exposed Pb mice taking in our consideration the effect of *N. sativa* on its distribution in these organs are shown in Tables 3 and 4, respectively.

The distribution of Cd in the Cd-unexposed group of mice without treatment with *N. sativa* was in the following order: liver > spleen > kidney > blood. Results show a reduction in Cd concentrations in all organs after treatment with *N. sativa* in the following order: blood > kidney > heart > spleen > liver (Table 3). The distribution for the Cd-exposed group

Table 2
Effect of *N. sativa* on the Distribution of Pb
in Various Organs in Exposed Mice

Group	Nigella mg/mL	N	Mean ± SEM (mg/kg)				
			Liver	Kidney	Heart	Spleen	Blood
VI	0	9	4.763±0.275	4.242±0.143	1.541±0.071	1.584±0.091	0.488±0.132
VII	10	4	1.290±0.164 (0.00<P)	1.551±0.116 (0.0001>P)	0.427±0.035 (0.000<P)	0.528±0.030 (0.00<P)	0.295±0.037 (0.007<P)
VIII	5	4	1.113±0.262 (0.00<P)	1.329±0.056 (0.00<P)	1.036±0.038 (0.000<P)	1.265±0.114 (0.014<P)	0.297±0.021 (0.003<P)
IX	2.5	7	1.331±0.074 (0.00<P)	1.218±0.229 (0.00<P)	0.553±0.020 (0.000<P)	1.490±0.177 (0.78>P)	0.329±0.025 (0.016<P)
X	1.25	6	1.405±0.068 (0.00<P)	0.943±0.071 (0.00<P)	1.052±0.039 (0.000<P)	1.190±0.097 (0.003<P)	0.329±0.013 (0.007<P)

of mice without treatment of *N. sativa* was in the following order: kidney > spleen > liver > heart > blood. Results show a significant reduction in Cd concentrations in all organs after treatment with *N. sativa* in the following order: kidney > liver > spleen > heart > blood (Table 4). The percentage removal of Cd concentrations in the liver, kidney, heart, spleen, and blood was 55.74–76.17%, 75.63–88.91%, 47–84.47%, 85.16–95.34%, and 100% respectively, at 10, 5, 2.5, and 1.25 mg/mL of *N. sativa.* The percentage removal of Cd concentrations in the kidney ranged from 75.63% to 88.91%. In the heart, the percentage removal of Cd ranged from 47.00% to 84.47%.

A previous study by Massadeh and Al-Safi *(15)* revealed that Cd and Pb were distributed in various body tissues and organs. The highest levels of Pb were in the liver and kidney and the lowest level was in the heart. Moreover, when mice were exposed to each metal separately or in combination, the liver, kidney or liver, spleen, and kidney remained the main targets *(14).* Pb is distributed in various body tissues and organs of mice, including red blood cells, liver, kidney, spleen, heart, bone, and nervous and reproductive systems *(28).* For Cd, it was reported that about 60% of total body burden is concentrated in the liver and kidney where the majority is bound to a low-molecular-weight metal-binding protein known as metallothionein *(29).* In addition, Cd is distributed to other organs, including the stomach, intestines, pancreas, and reproductive organs *(30).* In this study, *N. sativa* decreases both Pb and Cd concentrations in different mice organs in high percentages compared with their concentrations before treatment with *N. sativa.*

Table 3
Effect of *N. sativa* on Distribution of Cd in Various Organs in Cd-Unexposed Mice

Group	Nigella mg/mL	N	Mean ± SEM (µg/kg)				
			Liver	Kidney	Heart	Spleen	Blood
I	0	11	1.427±0.329	0.298±0.084	0.297±0.235	0.347±0.054	0.244±0.004
II	10	3	0.760±0.387 (0.33>P)	ND	0.015±0.004 (0.62>P)	0.051±0.009 (0.00<P)	0.131±0.007 (0.028<P)
III	5	10	1.025±0.294 (0.47>P)	0.040±0.012 (0.00<P)	0.145±0.107 (0.0037<P)	0.940±0.11 (0.041<P)	ND
IV	2.5	3	0.887±0.192 (0.27>P)	0.0280±0.008 (0.00<P)	0.025±0.001 (0.00<P)	1.116±0.046 (0.004<P)	ND
V	1.25	10	1.355±0.677 (0.90>P)	0.031±0.103 (0.00<P)	0.063±0.015 (0.00<P)	0.757±0.059 (0.0068<P)	ND

ND, not detected.

Table 4
Effect of *N. sativa* on Distribution of Cd in Various Organs in Cd-Exposed Mice

Group	Nigella mg/mL	N	Mean ± SEM (µg/kg)				
			Liver	Kidney	Heart	Spleen	Blood
VI	0	9	14.100±100	60.58±8.73	8.85±1.16	36.73±3.37	0.422±0.197
VII	10	4	3.453±0.827 (0.024<P)	10.01±0.752 (0.0045<P)	4.69±0.718 (0.012<P)	1.710±0.347 (0.00<P)	ND
VIII	5	4	6.24±1.890 (0.090>P)	14.76±1.00 (0.0017<P)	3.17±0.306 (0.0011<P)	5.45±0.408 (0.00<P)	ND
IX	2.5	7	3.077±0.323 (0.019<P)	7.089±0.976 (0.0012P)	1.65±0.085 (0.0002<P)	4.180±0.196 (0.00<P)	ND
X	1.25	6	3.800±1.100 (0.027<P)	6.72±1.27 (0.0012<P)	4.56±0.362 (0.0065<P)	2.447±0.368 (0.00<P)	ND

ND, not detected.

Table 5
Effect of *N. sativa* on Antibody Response in Mice

Nigella sativa (mg/ml)	N	Antibody titer Mean ± SEM	P
0 (control)	11	687 ± 104	---
1.25	11	465.5 ± 67.5	NS
2.50	11	488.7 ± 64.2	NS
5.00	11	535.3 ± 54.1	NS
10.00	11	535.3 ± 80.6	NS

NS, not significant.

Table 6
Effect of *N. sativa* on Antibody Response in Mice Pretreated with Cd–Pb Mixture (1 ppm Each)

Nigella sativa (mg/ml)	N	Antibody titer Mean ± SEM	P
0 (No Cd-Pb) -Control	11	687 ± 104	---
0	11	133.8 ± 20.2	0.0004
1.25	11	372.4 ± 50.2	0.017
2.50	11	349.1 ± 49.11	0.011
5.00	11	87.27 ± 9.74	0.0002
10.00	11	87.27 ± 9.74	0.0002

Immune System Results

Table 5 shows that *N. sativa* at 1.25–10 mg/mL did not induce any significant modulation of the antibody response.

The suppressed immune responses in mice pretreated with the Cd–Pb mixture were reversed by 43.1% and 38.9% in the presence of 1.25 and 2.5 mg/mL of *N. sativa,* respectively (Table 6). However, higher concentrations (5–10 mg/mL) of *N. sativa* did not cause any improvement of the depressed immune response (Table 6).

Nigella sativa seeds have been shown to induce immunopotentiating effects on human lymphocytes *(31).* Moreover, *N. sativa* seeds stimulate T-

lymphocytes to secrete interleukin-3 (IL-3) and to enhance IL-1β production *(32).* However, *N. sativa* extract has no immunostimulatory activity on mouse splenocytes unless activated by optimal doses of mitogen *(25). N. sativa* seeds contain immunostimulatory and immunosuppressive proteins *(33).* The reversal of Cd–Pb-induced immunosuppression by low concentrations (1.25–2.5 mg/mL) of *N. sativa* extract might be the result of the predominant effect of the immunostimulatory proteins $CD4^+T_H$ cells. On the other hand, the enhanced immunosuppression by higher concentrations (5–10 mg/mL) might be the result of either the predominant effect of the immunosuppressive proteins on $CD4^+T_H$ cells or the immunostimulant effect on suppressor lymphocytes ($CD8^+T_s$). Our results are in agreement with other investigators *(31).*

ACKNOWLEDGMENTS

We acknowledge the support from the Faculty of Scientific Research at Jordan University of Science and Technology. Thanks are due to Mr. M. Damrah (Animal House, Faculty of Medicine, JUST) and Asma Ayoub for their technical assistance in the preparation of the samples.

REFERENCES

1. World Health Organization, *Evaluation of Criteria Food Additives and Contaminants,* Technical Report Series No. 837, WHO, Geneva (1993).
2. World Health Organization, Environmental Health Criteria 165, International Programme on Chemical Safety, WHO, Geneva (1995).
3. E. Silbergeld, *The Elimination of Lead from Gasoline: Impacts of Lead in Gasoline on Human Health, and the Costs and Benefits of Eliminating Lead Additives,* The World bank, Washington, DC (1996).
4. R. A. Goyer, Results of lead research: prenatal exposure and neurological consequences, *Environ. Health Perspect.,* **104(10),** 1050–1054 (1996).
5. National Research Council, *Measuring Lead Exposure in Infants, Children and Other Sensitive Populations,* National Academy Press, Washington, DC (1993).
6. M. G. Cherian and R. A. Goyer. Cadmium toxicity, *Commun. Toxicol.* **3,** 191–206 (1989).
7. S. Benoff, A. Jacob, and I. R. Hurley, Male infertility and environmental exposure to lead and cadmium, *Hum. Rep. Update* **6,** 107–121 (2000).
8. J. Ye, S. Wang, M. Barger, V. Castranova, and X. Shi, Activation of androgen response element by cadmium: a potential mechanism for a carcinogenic effect of cadmium in the prostate, *J. Environ. Pathol. Toxicol. Oncol.* **19,** 275–280 (2000).
9. A. M. Massadeh, M. Tahat, Q. Jaradat, and I. F. Al-Momani, Lead and cadmium contamination in roadside soil in Irbid City, Jordan, *J. Soil Sediment Contam.: Int. J.* **13(4),** 347–359 (2004).
10. A. M. Massadeh, Distribution of copper and zinc in different fractions of particle sizes in road dust samples in Irbid City, Jordan using atomic absorption spectrometry, *Res. J. Chem. Environ.* **7(4),** 49–54 (2003).
11. A. M. Massadeh and R. D. Snook, Determination of Pb and Cd in road dusts over the period in which Pb was removed from petrol in the UK, *J. Environ. Monit.* **4,** 567–572 (2002).

12. A. M. Massadeh, F. Q. Alali, and Q. Jaradat, Determination of copper and zinc in different brands of cigarettes in Jordan, *Acta Chim. Slov.* **50,** 375–381 (2003).
13. A. M. Massadeh, F. Q. Alali, and Q. Jaradat, Determination of cadmium and lead in different brands of cigarettes in Jordan, *Environ. Monit. Assess.* **104,** 163–170 (2005).
14. A. M. Massadeh and Saafan Al-Safi, Analysis of cadmium and lead: their immunosuppressive effects and distribution in various organs of mice, *Biol. Trace Element Res.* **108(1–3),** 278–286 (2005).
15. P. E. Bigazzi, Metal and kidney autoimmunity, *Environ. Health Perspect.,* **107,** 753–765 (1999).
16. A. Karrkaya, B. Yccesoy, and O. S. Sardas, An immunological study on workers occupationally exposed to cadmium, *Hum. Exp. Toxicol.* **13,** 73–75 (1994).
17. F. Momani and A. M. Massadeh. The effect of different heavy metal concentrations on *Drosophila melanogaster* larval growth and development, *Biol. Trace Element Res.* **108(1–3),** 271–278 (2005).
18. A. M. Massadeh, F. Momani, and H. Haddad. Removal of lead and cadmium by hahophilic bacteria isolated from the Dead Sea shore, Jordan, *Biol. Trace Element Res.* **108(1–3),** 259–270 (2005).
19. A. Abdel-Fattah, K. Matsumoto, and H. Watanabe. Antinociceptive effects of *Nigella sativa* oil and its major component, thymoquinone, in mice, *Eur. J. Pharmacol.* **400,** 89–97 (2000).
20. M. El-Dakhakhny, Studies on the Egyptian *Nigella sativa* L part IV: some pharmacological properties of the seed's active principle in comparison to its dihydro compound and its polymer, *Arzneim-Forsch.* **15,** 1227–1229 (1965).
21. K. Al-Tahir, and M. Ashour, M. Al-Harbi, The cardiovascular actions of the volatile of the black seed *(Nigella sativa)* in rats, elucidation of the mechanism of action, *Gen. Pharmacol.* **24,** 1123–1131 (1993).
22. A. A. Al-Hader, M. B. Aqel, and Z. A. Hasan, Hypoglycemic effects of the volatile oil of *Nigella sativa* seeds, *Int. J. Pharmacogn,* **31,** 96–100 (1993).
23. M. S. M. Hanafy and M. E. Hatem. Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin), *J. Ethnopharmacol.* **34,** 275–278 (1991).
24. D. R. Worthen, A. O. Ghosheh, and P. A. Crooks, The in vitro antitumor activity of some crude and purified components of blackseed, *Nigella sativa* L., *Anticancer Res.* **18,** 1527–1532 (1998).
25. S. M. K. Swamy and B. K. H. Tan, Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sativa* L. seeds, *J Ethnopharmacol.* **70,** 1–7 (2000).
26. M. S. Atta-ur-Rahman and M. K. Zaman, Nigellimine: a new isoquinoline alkaloid from the seeds of *Nigella sativa, J. Nat. Prod.* **55,** 676–678 (1992).
27. F. C. Hay, Immunological manipulations in vivo, in *Practical Immunology,* O. Westwood, ed., Blackwell Science, Oxford (2002).
28. C. Taupeau, J. Poupon, F. Nome, and B. Lefevre, Lead accumulation in the mouse ovary after treatment-induced follicular atresia, *Report Toxicol.* **15,** 385–391 (2001).
29. Y. Liu, J. Liu, and C. D. Klaassen, Metallothionein-null and wild-type mice show similar cadmium absorption and tissue distribution following oral cadmium, Administration, *Toxicol. Appl. Pharmacol.* **175,** 253–259 (2001).
30. M. Radike, D. Warshawsky, J. Caruso, et al., Distribution and accumulation of a mixture of arsenic, cadmium, chromium, nickel, and vanadium in mouse small ntestine, kidneys, pancreas, and femur following oral administration in water or feed, *J. Toxicol. Environ. Health* **65,** 2029–2052 (2002).
31. A. El-Kadi and O. Kandil, Effect of *Nigella sativa* (the black seeds) on immunity. Proceedings of the Fourth International Conference on Islamic Medicine, *Bull Islamic Med.* **4,** 344–348 (1986).

32. A. Haq, M. Abdullatif, P. I. Lobo, K. S. A. Khabar, K. V. Sheth, and S. T. Al-Sedairy, *Nigella sativa:* effect on human lymphocytes and polymorphonuclear leukocyte phagocytic activity, *Immunopharmacology* **30,** 147–155 (1995).
33. A. Haq, P. Lobo, M. Al-Tufail, N. Rama, and S. T. Al-Sedairy, Immunomodulatory effect of *Nigella sativa* proteins fractionated by ion exchange chromatography, *Int. J Immunopharmacol.* **21,** 283–295 (1999).