



IMMUNOMODULATORY EFFECT OF VOLATILE OIL FROM *NIGELLA SATIVA* ON THE ANIMALS LABORATORY (IN VIVO)

Essam F. Al-Jumaily* and Anwar Jalil Shihab Al-Jumaily

Biotechnology Dept. Genetic Engineering and Biotechnology Institute for Post Graduate Studies. Baghdad University, Baghdad/Iraq.

*Author for Correspondence: Dr. Essam F. Al-Jumaily

Biotechnology Dept. Genetic Engineering and Biotechnology Institute for Post Graduate Studies. Baghdad University, Baghdad/Iraq.

Article Received on 16/12/2015

Article Revised on 06/01/2016

Article Accepted on 26/01/2016

ABSTRACT

The study has employed an *in vivo* evaluation of the immune system effect of volatile and fixed oil in male albino mice at single dose concentrations (0.5 mg / kg) given injection for 7 days including measuring the IgM level and histopathological changes. At day 14, the animal was killed and the spleen was kept in 10% formalin for the preparation of histopathological sections, the serum was isolated from the blood for the biochemical tests. The statistical results showed the presence of significant changes on IgM between the groups of mice. Examination of the spleen tissue confirmed potential histopathological effects for volatile oil as immunomodulatory in treated mice as widening of white pulp with a reduction of red pulp, so hyperplasia of lymphoid follicles with germinal center and platelet cytokines.

KEYWORDS: *Nigella sativa*; volatile oil; immunomodulatory effect; IgM

INTRODUCTION

Medicinal plants have been used for curing diseases for many centuries in different indigenous systems of medicine as well as folk medicines. Moreover, medicinal plants are used in the preparation of herbal medicines as they are considered to be safe as compared to modern allopathic medicines. Many researchers are focusing on medicinal plants since only a few plant species have been thoroughly investigated for their medicinal properties, potential, mechanism of action, safety evaluation and toxicological studies. Among various medicinal plants, *Nigella Sativa* (Family Ranunculaceae) is emerging as a miracle herb with a rich historical and religious background since many researches revealed its wide spectrum of pharmacological potential. *N. Sativa* is commonly known "black seed". *N. Sativa* is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world like Middle Eastern Mediterranean region, South Europe, India, Pakistan, Syria, Turkey and Saudi Arabia.^[1]

Most of the therapeutic properties of this plant are due to the presence of thymoquinone (TQ) which is a major active chemical component of the essential oil. Black seeds are also used in food like flavoring additive in the breads and pickles because it has a very low level of toxicity.^[2]

Many studies have shown that *N. Sativa* and TQ have an effect on the immune system; they are able to inhibit

many inflammatory mediators and could ameliorate inflammatory and autoimmune conditions.^[3,4] Based on the results obtained from the *in vitro* experiments *N. Sativa* constituents have shown to down-regulate B cell-mediated immunity.^[5]

Mohammed^[6] reviewed that certain constituents of *N. Sativa* oil possess potent potentiating effects on the cellular immunity, while other constituents possess suppressor effects on B cell-mediated immunity. These findings suggest also that the stimulatory effects of *N. Sativa* on the cellular immunity are dependent on the nature of the immune response. However, Hannan et al.,^[7] found that *N. Sativa* oil caused suppressions in both cellular and humoral immunity.

The study by Ghonime et al.,^[8] on two groups of mice were immunosuppressed with cyclophosphamide, the one which pretreated with the black seed extracts significantly ($P < 0.01$) restored their resistance against lethal infection with the predominately granulocyte-dependent *Candida albicans*. These results confirmed the immunomodulatory activity of black seed, and may have therapeutical implications in prophylactic treatment of opportunistic infections and as supportive treatment in oncogenic cases. The present study aims to the effect of the volatile oil (thymoquinone) of *Nigella Sativa* on the immune response in lab mice.

MATERIALS AND METHODS

The dried seeds of *Nigella Sativa* were collected from the local market in Baghdad during November/2014 and identified by the department of biology in the college of sciences/Baghdad University. The seeds have been grinded by a coffee grinder to a fine powder to be used directly after grinding.

Sixty male albino mice (25-28 g) of about (6-8) weeks old were obtained from the Department of Drug Control and the animals were used in this study. They were selected randomly and kept in 15 groups of 4 mice in each the groups. Each group was kept in a separate cage. All animals were fed with mice developed commercially and feed ad libitum tap water supplied by the center. They were cleaning their cages daily and were changed food and water daily. The animals were allowed to acclimatize for 10 days.

Experimental protocols

Mice were divided into two main groups, the first group preventiveness experiment and the second group Therapeutic Experiment then the first Group (preventiveness experiment) was divided into two groups (*Candida* and *Salmonella*). The (*Candida*) group was infected with *Candida albicans* and *Salmonella* group was infected with *Salmonella paratyphi*. Then the (*Candida*) group was divided into five groups; 4 animals in each group. *Salmonella* group was divided into five groups 4 animals each group. Additionally, there was another control group consisting from 5 animals which was considered the negative control.

The first group (preventiveness Experiment) was divided in to groups (*Candida* and *Salmonella*)

The groups (*Candida*): Group A

Four mice infected with *Candida* after subcutaneously injection dose of 25 micron for 24h are considered a positive control.

Group B

Four mice subcutaneously injected with a single dose of 0.5 ml/kg from thymoquinone and after 7days wounds were made in the mice contaminated by *C.albicans* as in feeding after taking a dose of 25 micron from prednisolone. Then after five days animals, blood was tacked then the IgM protein was determined.

Group C

Four mice subcutaneously injected with a single dose of 0.5 ml/kg from *Nigella Sativa* oil was extracted by chloroform and after 7days infected with *Candida albicans* after taking dose of 25 micron from prednisolone then after five days, the animals blood was tacked then the IgM protein was determined.

Group D

Four mice were subcutaneously injected with a single dose of 0.5 ml/kg from *Nigella Sativa* oil which was

extracted by methanol and after 7days, they were infected with *Candida albicans* after taking a dose of 25 micron from prednisolone. After five days animals blood was tacked then the IgM protein was determined.

Group E

Four mice were subcutaneously injected with a single dose of 0.5 ml/kg from *Nigella Sativa* oil which was Extract aqueous. After 7days they were infected with *Candida albicans* after taking a dose of 25 micron from prednisolone. After five days animals blood was tacked then the IgM protein was determined.

The groups (*Salmonella*)

Group A

Four mice were subcutaneously infected with *Salmonella paratyphi* and they were considered a positive control.

Group B

Four mice were subcutaneously injected 0with a single dose of 0.5 ml/kg from thymoquinone and after 7days infected with *Salmonella paratyphi*, Then after five days, the animals blood was tacked and the IgM protein was determined.

Group C

Four mice were subcutaneously injected with a single dose of 0.5 ml/kg from *Nigella Sativa* oil which was extracted by chloroform and after 7days infected with *Salmonella paratyphi*, then after five days animals blood was tacked then the IgM protein was determined.

Group D

Four mice were subcutaneously injected with a single dose of 0.5 ml/kg from *Nigella Sativa* oil which was extracted by **methanol** and after 7days they were infected with *Salmonella paratyphi*, then after five days animals blood was tacked then the IgM protein was determined.

Group E

Four mice were subcutaneously injected with a single dose of 0.5 ml/kg from *Nigella Sativa* oil which was **Extract aqueous** and after 7days they were infected with *Salmonella paratyphi*, then after five days the animals blood was tacked then the IgM protein was determined.

The second group (Therapeutic Experiment) was divided in to groups (*Candida* and *Salmonella*)

The groups (*Candida*)

Group A

Four mice were subcutaneously infected *Candida albicans* after injected with 25 micron from prednisolone. Then after two weeks they were injected with a single dose of 0.5 ml/kg from *Nigella Sativa* oil extracted by **chloroform** and after five days the animals blood was tacked then the IgM protein was determined.

Group B

Four mice were subcutaneously infected *Candida Albicans* after injection with 25 micron from prednisolone, then after two weeksthey were injected a single dose of 0.5 ml/kg from thymoquinone. After five

days the animals blood was tacked then the IgM protein was determined.

The groups (Salmonella)

Group A

Four mice were subcutaneously infected Salmonella paratyphoid. Two weeks later injected with a single dose of 0.5 ml/kg from *Nigella Sativa* oil was extracted by chloroform. After five days the animals blood was tacked then the IgM protein was determined.

Group B

Four mice were subcutaneously infected Salmonella paratyphoid .Two weeks later injected with a single dose of 0.5 ml/kg from thymoquinone. After five days the animals blood was tacked then the IgM protein was determined.

Determination of the IgM protein by radial immunodiffusion plate

Single radial immunodiffusion tests have evolved from the work of (Fahey and McKelvey,^[9] and Mancini et al.,^[10] They are specific for the various proteins in serum or other fluids and depend on the reaction of reagent.

Histopathological Examination

Spleen tissues have been prepared for histopathological examination according to the method of Junqueira et al.,^[11] using paraffin sections technique.

The Statistical Analysis System- SAS^[12] program was used to effect of difference factors in study parameters . Least significant difference –LSD test was used to significant compare between means in this study.

RESULTS AND DISCUSSION

The effect of *Nigella Sativa* oil as stimulated for the immune system in male mice immune response such as spleen function, as well as immunoglobuline M (IgM) profiles showed a low effect of Nagilla sativa oil on serum IgM.

Table (1) and Figure (1) shows that the serum (IgM) concentrations were significantly increased (P<0.01) in the *Salmonella paratyphi* for one week immunodulatory experiment groups (group2) of mice injection with Thymoquinone (120.63±15.58bc) compared to the control negative (group1) (24.40±1.38c) indicating the immunostimulant.^[13] *Nigella Sativa* oil has been shown to be effective against a wide spectrum of organisms-bacteria like *Salmonella paratyphi* and pathogenic yeast like *Candida albicans*^[14;15], and the essential oil antimicrobial activity against fifteen pathogenic microbial strains including Gram+ve, Gram-ve bacteria and Yeast *Candida albicans*[16]. Also, the results on Table (1) show that the serum (IgM) concentrations significantly increased (p<0.01) in the *Salmonella paratyphi* for one week immunodalatory experiment groups (group 3) of mice injection with chloroform (167.90±48.52b) compared to the control group. But when injected with a methanol extracted (group 4) it gave (81.33±30.28bc) for one week and that may be part

of the act by inhibiting the synthesis of the cell wall by inducing changes in the structure of the membranes by inhibiting bacterial protein synthesis.^[17] The highest immunodulatory effect the study get when injecting the mice with aqueous extracted (group5) (368.30±49.48a) for one week showed a significant increase (p<0.01) compared to the control group.

Table:1 Compare between difference groups of Salmonella in IgM (immunodulatory exp.)

No	Salmonella group	IgM ± SE
1	Control	24.40 ± 1.38 c
2	Thymoquinone	120.63 ± 15.58 bc
3	Chloroform Extract	167.90 ± 48.52 b
4	Methanol Extract	81.33 ± 30.28 bc
5	Aqueous Extract	368.30 ± 49.48 a
LSD value	---	106.20 **
P-value	---	0.0002

** (P<0.01).

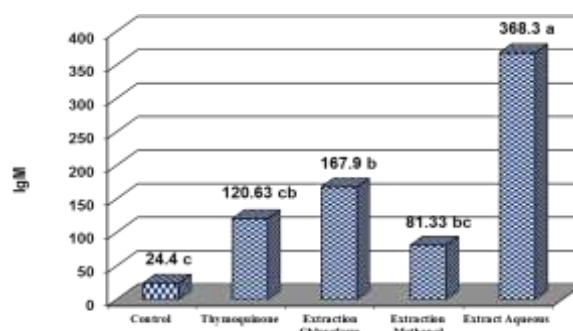


Figure (1): The compare between difference extracted groups of Salmonella in IgM (immunodulatory exp.)

Table (2): Compare between difference group of Candida in IgM (immunodulatory exp)

No	Candida group	IgM ± SE
1	Control	93.48 ± 31.95 c
2	Thymoquinone	264.17 ± 23.62 ab
3	Extraction Chloroform	360.38 ± 42.12 a
4	Extraction Methanol	236.25 ± 52.63 ab
5	Extract Aqueous	150.73 ± 46.30 bc
LSD value	---	129.95 **
P-value	---	0.0044

** (P<0.01).

Table:3 Compare between difference group of Salmonella in IgM (treatment exp.)

No	Salmonella	IgM ± SE
1	Control (Before treatment)	50.10 ± 5.41 b
2	Extraction Chloroform	168.65 ± 24.87 a
3	Thymoquinone	133.00 ± 35.56 a
LSD value	---	106.20 **
P-value	---	0.00442

** (P<0.01).

Table:4 Compare between difference group of *Candida* in IgM (treatment exp.)

No	<i>Candida</i>	IgM \pm SE
1	Control (Before treatment)	50.10 \pm 5.41 c
2	Extraction Chloroform	340.67 \pm 50.47 a
3	Thymoquinone	151.63 \pm 15.73 b
LSD value	---	89.37 **
P-value	---	0.0117

** (P<0.01).

In the current study, the mice were exposed two weeks (spleen) of examination. The light microscopic examination by specific staining of spleen in control tissues showed a normal parenchymal tissue which consisted of lymphocyte cells, Figure (2).

The spleen was examined mainly. Spleen section of mice treated with *Nigella sativa* oil extraction with chloroform (0.5 mg/kg) then infected with *Salmonella paratyphi* showed a widening of white pulp with a reduction of red pulp, Figure (3). When infected with *Candida albicans*, showed a hyper plasia of lymphoid follicles with a germinal center, Figure (4).

The spleen section of mice treated with *Nigella Sativa* oil extraction with aqueous extracted (0.5mg/kg) then infected with *Salmonella paratyphi* showed widening white pulp and red pulp, Figure (5). When it was infected with *Candida albicans*, showed a germinal center, Figure (6).

The spleen section of mice treated with *Nigella Sativa* oil extraction with methanol extracted (0.5mg/kg) then infected with *Salmonella paratyphi* showed a wide necrosis, Figure (7), and when infected with *Candida albicans* it showed platelet cytokines, Figure (8).

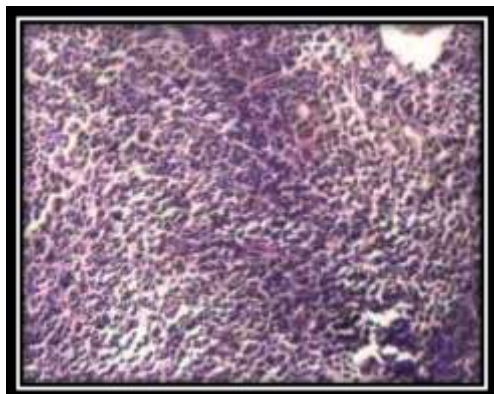


Figure (2): section of spleen showing normal parenchymal tissue of the spleen which consist of lymphocyte cell (H & E 40X).

Although, previous studies showed that lipid - soluble component such as thymoquinone is mainly responsible for the anti-inflammatory effect of the plant.^[18,19] Therefore, the hydroethanolic extract of the plant was used to extract the both lipid and water soluble constituents of *N. Sativa*. However, more investigation should be done to evaluate the safety of long-term administration of herbal medicine and their constituents.^[18]

A combination of fatty acids, volatile oils and trace elements are believed to contribute to its effectiveness. The *N. Sativa* seed active ingredients, in particular thymoquinone (TQ), have shown the potential immunomodulatory and immunotherapeutic potentials. The immunotherapeutic efficacy of TQ is linked to its antitoxic, anti-histaminic and anti-inflammatory properties. These effects with its immunomodulatory properties can explain the anti-microbial and anticancer properties of *N. Sativa* oil or TQ. The immunotherapeutic efficacy of ingestion or administration of the whole seeds, oil or its purified constituents should be measured by the nature of the disease.^[19]

Most of the therapeutic properties of this plant are due to the presence of thymoquinone (TQ) which is a major active chemical component of the essential oil.^[18]

Ghonime et al.^[8] found that spleen weight of the black seed treated groups was significantly increased (P<0.01). Two groups of mice were immunosuppressed with cyclophosphamide, the one which pretreated with the black seed extracts significantly (P<0.01) restored their resistance against lethal infection with the predominately granulocyte-dependent *Candida albicans*. The results confirmed the immunomodulatory activity of black seed, and may have therapeutically implications in prophylactic treatment of opportunistic infections and as supportive treatment in oncogenic cases.

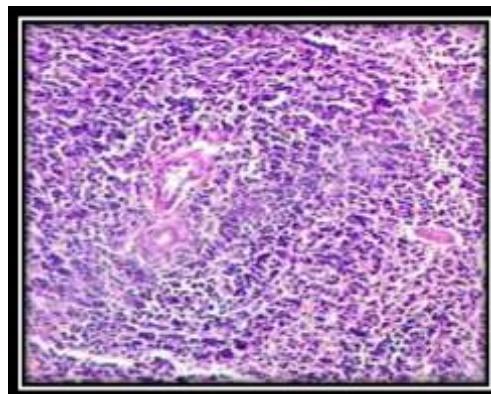


Figure (3): Histopathological section in the spleen of mice at 2 weeks does *Nigella Sativa* oil extraction with chloroform (0.5 mg/kg) then infected *Salmonella paratyphi* showing widening of white pulp with a reduction of red pulp (H&E stain 20X).

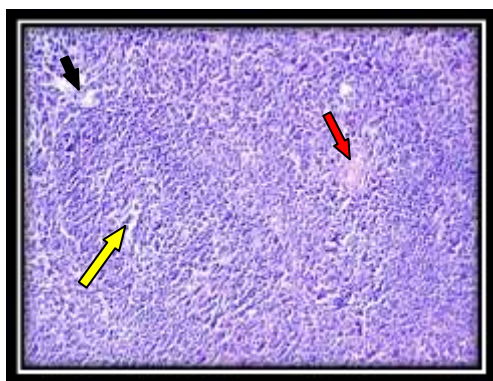


Figure (4): Histopathological section in the spleen of mice at 2 weeks does *Nigella Sativa* oil extraction with chloroform (0.5 mg/kg) then infected with *Candida Albicans* showing hyper plasia of lymphoid follicles with germinal center (H&E stain 20X).

→ Germinal Center

→ Red pulp

White Pulp →

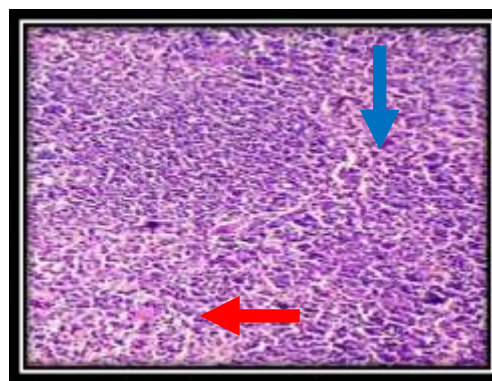


Figure (5): Histopathological section in the spleen of mice at 2 weeks does *Nigella Sativa* oil extraction - aqueous(0.5 mg/kg) - then infected with *Salmonella paratyphi* showing. (H&E stain 20X).

→ Widening white pulp

→ Red pulp

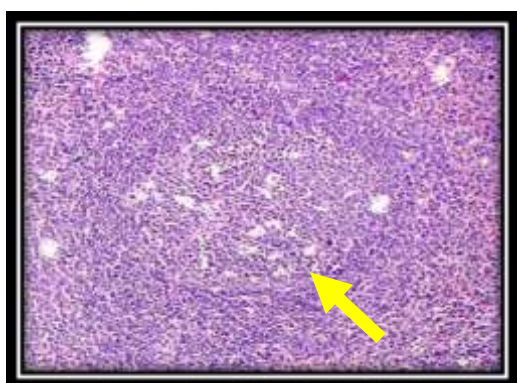


Figure (6): Histopathological section in the spleen of mice at 2 weeks does *Nigella Sativa* oil extraction aqueous (0.5 mg/kg) then infected with *Candida Albicans* showing germinal center.

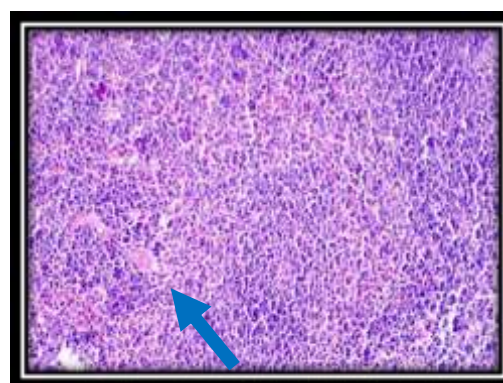


Figure (7): Histopathological section in the spleen of mice at 2 weeks does *Nigella Sativa* oil extraction with methanol (0.5 mg/kg) then infected with *Salmonella paratyphi* showing wide necrosis (H&E stain 20X).

REFERENCES

1. Khare, C.P. (2004) Encyclopedia of Indian medicinal plants. NewYork: Springes-Verlag Berlin Heidelberg.
2. Al-Ali, A.; Alkhawajah, A.A.; Randhawa, M.A. & Shaikh, N.A. Oral and intraperitoneal LD50 of thymoquinone, an active principle of *Nigella Sativa*, in mice and rats. J. Ayub Med. Coll. Abbottabad, 2008; 20: 25-27.
3. Yarnell E, Abascal K; *Nigella Sativa*: holy herb of the middle East. Altern Compl Therap, 2011; 17(2): 99–105.
4. Padhye, S.; Banerjee, S.; Ahmad, A.; Mohammad, R. and Sarkar, F.H. From here to eternity-the secret of Pharaohs: Therapeutic potential of black cumin seeds and beyond. Cancer Ther, 2008; 6: 495–510.
5. Bakathir H.A. and Abbas N.A. Detection of the antibacterial effect of *Nigella Sativa* ground seeds with water. Afr J Tradit Compl Altern Med., 2011; 8(2): 159–164.
6. Mohamed, A.M.; Metwally, N.M. and Mahmoud, S.S. *Nigella Sativa* seeds against *Schistosoma mansoni* different stages. Mem Inst Oswaldo Cruz, 2005; 100(2): 205–211.
7. Hannan, A.; Saleem S, Chaudhary S, Barka M, Arshad. Anti-bacterial activity of *Nigella Sativa* against clinical isolates of methicillin resistant *Staphylococcus aureus*. J Ayub Med Coll Abbottabad, 2008; 20(3): 72–74.
8. Ghonime M, Eldomany R, Abdelaziz A, Soliman H. Evaluation of immunomodulatory effect of three herbal plants growing in Egypt. Immunopharmacol Immunotoxicol, 2011; 33(1): 141–145.
9. Fahey, J.L. and McKelvey, E.M, Quantitative determination of serum immunoglobulins in antibody agar plates. J. Immunol, 1965; 94: 84.
10. Mancini, G., Carbonara, AQ and Heremans, J.F. Immunochemical, quantitation of antigens by single radial immunodiffusion. Immunochemistry, 1965; 2: 235.
11. Junqueira, L. C., Carneiro, and Kelley, R. Basic Histology. (8th eds.), Lange Medical Book, 1995; 1-2: 306-314.

12. SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
13. Swamy S.M.K. and Tan B.K.H. Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella Sativa* L. seeds. *J. Ethnopharmacol*, 2000; 70: 1-7.
14. Damjanova, S.; Encheve, R.; Stojanova, A.; Perofanova, N. and Miziiski, F. *Hranitelno Vkusova Promishlenost*, 2002; 5: 10-11.
15. Gurdip S., M. Sumitra, M.P. de Lampasona and C. Catalan, Chemical constituents, antimicrobial investigations and antioxidative potential of volatile oil and acetone extract of star anise fruits. *The Science of Food and Agriculture*, 2005; 86: 111–121.
16. Ara, N.; Choudhary, S.A.R. and Amin, R. In vitro Antimicrobial Activity of the Volatile Oil of *Nigella Sativa* Linn Seeds. *TAJ*, 2005; 18(2): 109-112.
17. Mohammad, H.I.; Iffat, Z.A. and Mohammad, T.S. (2013). Antibacterial activity of *Nigella Sativa* seed in various germination phases on clinical bacterial strains isolated from human patients. *J. Biotech. Pharma. Res*, 4(1): 8-13.
18. Ahmad, A.; Husain, A.; Mujeeb, M.; Khan, S.A.; Najmi, A.K.; Siddique, N.A.; Damanhour, Z.A. and Anwar, F. A review on therapeutic potential of *Nigella Sativa*: A miracle herb. *Asian Pac J Trop Biomed*, 2013; 3(5): 337–352.
19. Salem, M.L. Immunomodulatory and therapeutic properties of the *Nigella Sativa* L. seed. *International Immunopharmacology*, 2005; 5: 1749–1770.