

Effect of *Nigella sativa* seeds' extract Treatment on Neutrophils count

Batool A. Al-Haidary , Ph.D. in Clinical Immunology *

Summary:

Fac Med Baghdad
2008; Vol.50, No.2
Received May 2007
Accepted Dec.2007

Background: *Nigella sativa* seeds have been frequently used in folk medicine for treatment of various diseases such as inflammatory disease. The aim of this study is to show the effect of this herb on Neutrophils count as the main anti-inflammatory cells. **Materials & Methods:** Aqueous & oil extract of *Nigella sativa* seeds have been applied for treatment of 30 blood samples of apparently healthy individuals. Blood count has been performed after different incubation periods.

Results & Conclusions: This study revealed that treatment of blood samples with herbal extraction results in highly significant elevation of Neutrophils' count with time passing particularly the aqueous one and after 6 hrs. incubation period ($P < 0.001$). Our conclusion, that *Nigella sativa* aqueous extract can be used against bacterial infections which required recruitment of Neutrophils.

Introduction:

Nigella sativa is one of an annual herbaceous, plant growing in Western Asia and the Mediterranean region for its seeds. These seeds contain 40% fixed oil and 1.4% volatile oil [1]. *Nigella sativa* was discovered in Tutankhamen's tomb implying that it played an important role in an ancient Egyptian practices. The earliest written reference to black seed is found in the book of Isaiah in the Old Testament [2].

Seeds of *Nigella sativa* are frequently used in folk medicine in Middle East and some Asian countries for promotion of good health and treatment of various diseases [3]. The multiple use of *Nigella sativa* in folk medicine encouraged many investigators to isolate the possible active component and to conduct *in vivo* and *in vitro* studies on laboratory animals and human beings to understand its pharmacological actions [4-5]. These include immune stimulation, anticancer, anti-microbial and other studies [6-9]. *Nigella sativa* seed and its components are frequently used as a natural remedy for many ailments. A lot of work has been done to evaluate the pharmacological basis of these uses.

It was denoted that Black seed is effective at treating the body as a whole and fights the actual cause of the symptoms. Further more Black seed was observed to be a good regulator of too weak or too strong reactions of the immune system and is excellent for treating chronic, allergic and hormonal diseases.

In view of an investigation on the *Nigella sativa* effect on the immune system, it was reported that black seed enhanced immune functions [11].

Being highly motile, Neutrophils quickly concentrate at a focus of infection, attracted by cytokines expressed by activated endothelium, mast cells and macrophages. Neutrophils are much more numerous than the larger-lived Monocytes/ macrophages. The first phagocytes; that a pathogen is likely to encounter are Neutrophils. Some authorities feel that the short life time of Neutrophils is an evolutionary adaptation to minimize propagation of those pathogens that parasitize phagocytes [12].

The importance of this herb encourages us to study the effect of it on the some immunological parameters such as Neutrophils

*Assis. Prof. / College of Medical & Health technology

Materials & Methods:

An aqueous and oil extracts of Black seed were prepared [13]. These extracts has been applied for the treatment of 30 apparently healthy volunteers' blood samples after lymphocytes' isolation; in presence and absence of bacterial suspension. Lymphocytes have been estimated before and after each treatment [14]. Each treatment was followed after one, three and six hours of incubation periods.

Statistical Analysis:

All data have been statistically analyzed using t-Test and ANOVA (F-test) for analysis of comparison of differences before and after treatment and within different incubation periods [15].

Results:

I. Effect of Black seed's Oil extract on Neutrophils' count:

Thirty volunteers heparinized venous blood samples have been collected and tested Neutrophils' count before and after each treatment. These facts are clear in table and figure 1 which shows the highly elevation in Neutrophils count after treatment with black seeds oil extract particularly after six hours in comparison with its count before treatment. The variation in the mean of cells count after three hours may be related to generation time of the cells which exceeds three hours.

Table 1: Frequency of Neutrophils after treatment with oil extract for different durations

<i>Studied groups</i>	<i>Neutrophils frequency *</i>	<i>P value +</i>
<i>Before any Treatment</i>	53.7 ± 4.24	—
<i>After one hour</i>	61.6 ± 3.86	0.0 89 NS
<i>After three hours</i>	58.6 ± 9.11	0.285 NS
<i>After six hours</i>	67.3 ± 3.16	0.0 05 #

* = Mean of frequency ± SD,

+ = Comparison of significance by (t-test) co

NS = Non-significant.

= Highly significant differences.

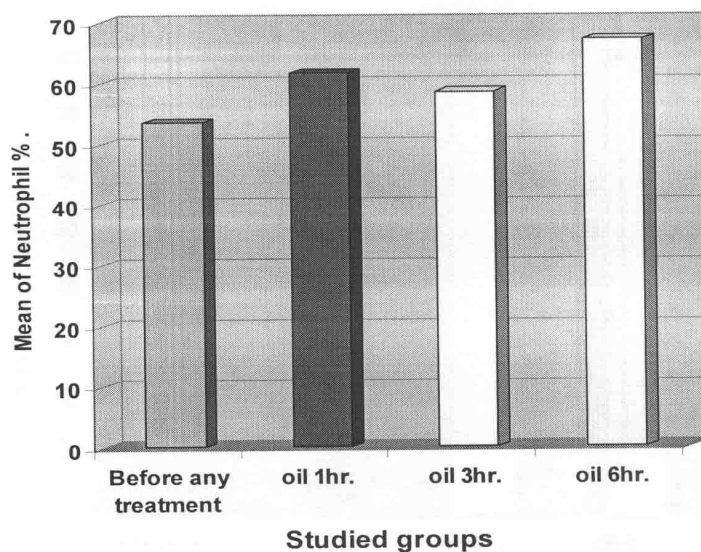


Figure 1: Frequency of Neutrophils after treatment with *Nigella sativa* oil extract.

II. Effect of Black seed's Aqueous extract on Neutrophils count:

This investigation includes the study of aqueous extract effect on Neutrophils count. The result was listed in table 2. This table shows that the effect of aqueous extract of *N. sativa* is higher than that for oil extract. Moreover, there is a highly significant difference before and after treatment which increases with time passing to reach it's maximum effect after 6 hours of incubation. Figure 2 confirms these facts too.

Table 2: Neutrophil's frequency according to aqueous extract treatment in different incubation periods.

<i>Studied groups @</i>	<i>Neutrophils frequency (%)*</i>	<i>P value +</i>
<i>Before any Treatment</i>	53.70 ± 4.24	—
<i>After one hour</i>	73.90 ± 7.13	< 0.00 #
<i>After three hours</i>	75.20 ± 5.51	< 0.001
<i>After six hours</i>	79.40 ± 4.62	< 0.001

*= Mean of frequency ± SD, + = Comparison of significance by (t-test)
= Highly significant differences. @ = No. of samples is 10 with 40 treatments.

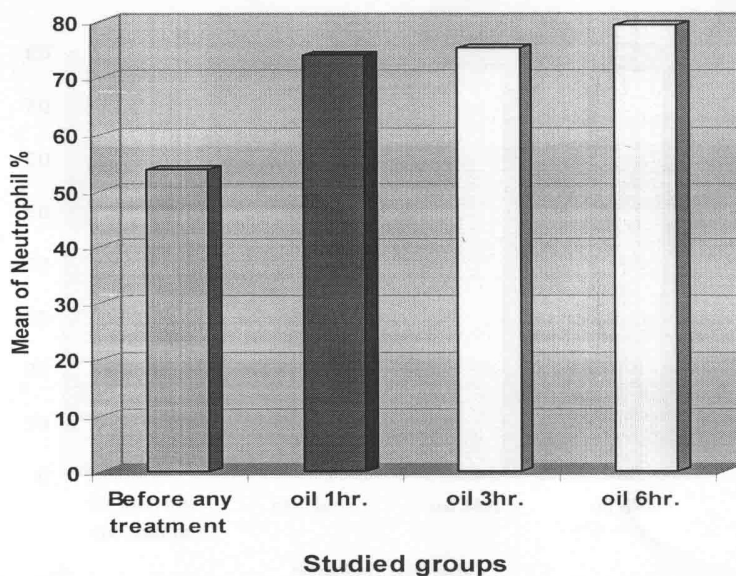


Figure 2: Frequency of Neutrophils according to liquid extract treatment in different incubation periods.

III- Effect of black seeds oil extract and bacteria on Neutrophils' count:

Highly significant elevation of Neutrophils has been observed after treatment with bacteria in addition to the oil extract as shown in table 3 particularly after three hours duration. The mean of cells percentage elevated from 53.7% to 75.3%, 77.0%, 76.6% after one, three and six hours respectively with highly significant differences before and after each treatment ($P < 0.0001$ for each).

Table 3: Neutrophil's frequency according to oil and bacteria treatment in different incubation periods.

<i>Studied groups #</i>	<i>Neutrophils frequency (%)*</i>	<i>P value +</i>
<i>Before any Treatment</i>	53.70 ± 4.24	—
<i>After one hour</i>	75.30 ± 4.69	< 0.00 @
<i>After three hours</i>	77.00 ± 3.30	< 0.00 @
<i>After six hours</i>	76.60 ± 3.24	< 0.00 @

* = Mean of frequency ± SD, + = Comparison of significance by (t-test)
@ = Highly significant differences. # = No. of samples is 10 with 40 treatment.

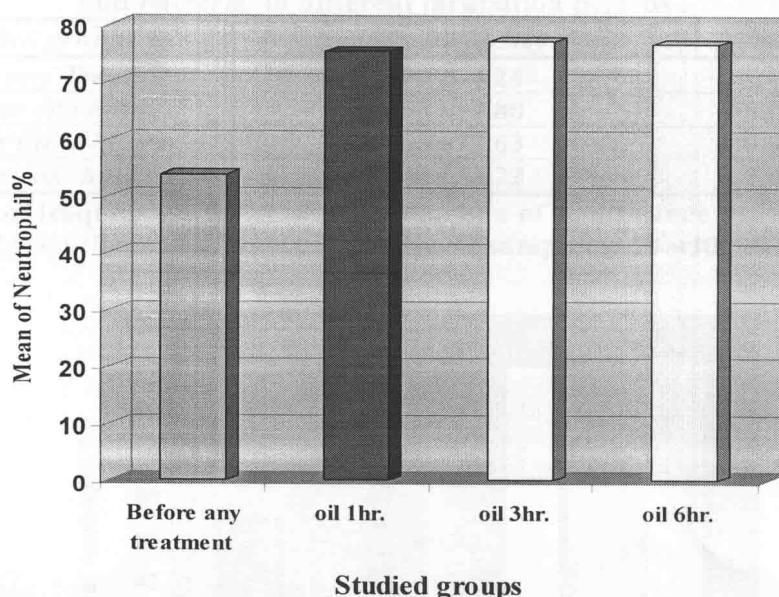


Figure 3: Neutrophil's % according to oil & bacteria treatment in different incubation periods.

Figure 3 reveals that the addition of bacteria to the Neutrophils in addition to the black seeds' oil extract induce Neutrophils' proliferation in highly significant manner, particularly after passing 3 hours duration.

IV- Effects of Black seeds aqueous extract and bacteria on Neutrophils' count:

Aqueous extract of *Nigella sativa* has been tested in addition to bacterial suspension on the Neutrophils¹ count. This extract induces an arising in Neutrophils¹ count more than that observed in the previous treatment as shown in the table and figure below.

Table 4: Neutrophil's frequency according to aqueous extract treatment and bacteria in different incubation periods .

Studied groups #	Neutrophils frequency (%)*	P value +
Before any Treatment	53.70 ± 4.24	—
After one hour	86.50 ± 2.80	< 0.01 @
After three hours	90.30 ± 2.63	< 0.01 @
After six hours	92.40 ± 2.22	< 0.01 @

* = Mean of frequency ± SD, + = Comparison of significance by (t-test)
@ = Highly significant differences. # = No. of samples is 10 with 40 treatment.

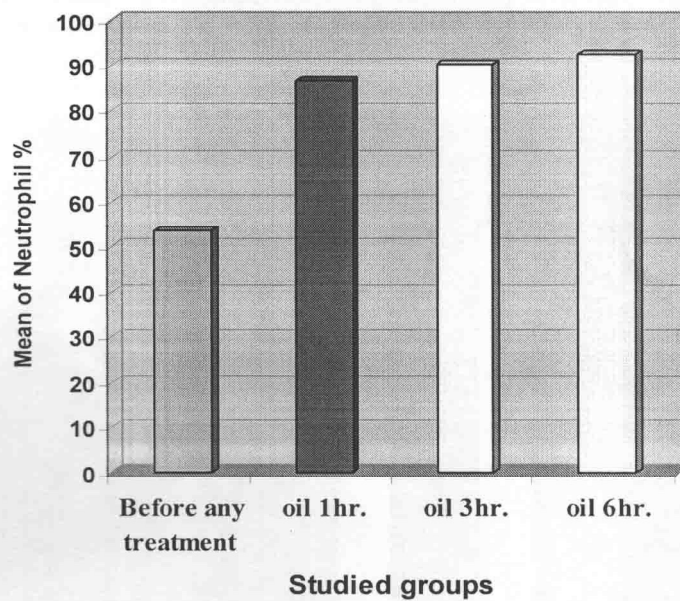


Figure 4: Distribution of Neutrophil's % according to extract & bacterial treatment in different incubation periods.

V- Effect of Incubation period with different treatment on Neutrophil's Count:

1. One hour Incubation:

Comparisons of the different treatments on Neutrophil's count after one hour are listed in the table below which shows highly significant differences before and after each treatment particularly for liquid extract. Figure 5 confirms these data.

Table 5: Neutrophil's % according to studied groups in incubation periods 1hr.

Studied groups #	Neutrophil's frequency (%)*	P value +
Before any Treatment	53.70 ± 4.24	0.0001 @
With oil extract	61.60 ± 3.86	
With oil extract & bacteria	75.30 ± 4.69	
With aqueous extract	73.90 ± 7.13	
With aqueous extract & bacteria	86.50 ± 2.80	
Comparison of significance by LSD (F-test)		< 0.001

* =Mean of frequency ± SD, += Comparison of significance by ANOVA (F-test)
@ = Highly significant differences. # = No. of samples is 10 with 50 treatment.

2. Three Hours incubation:

After three hours of incubation with different treatment highly significant difference is observed as shown in table 6. These facts are clearly appeared in figure 6 too.

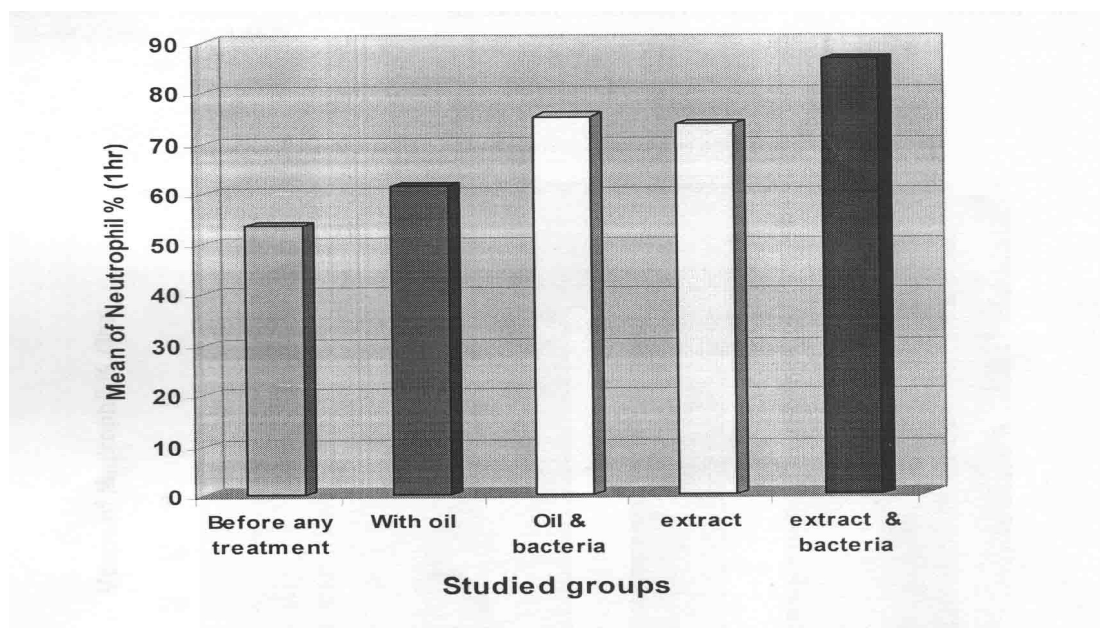


Figure 5: Neutrophil's % according to studied groups in incubation periods 1hr.

Table 6: Neutrophil's % according to studied groups in incubation periods 3hr

<i>Studied groups #</i>	<i>Neutrophil's frequency (%)*</i>	<i>P value+</i>
<i>Before any Treatment</i>	53.70 ± 4.24	0.0001 @
<i>With oil extract</i>	58.60 ± 19.11	
<i>With oil extract & bacteria</i>	77.00 ± 3.30	
<i>With aqueous extract</i>	75.20 ± 5.51	
<i>With aqueous extract & bacteria</i>	90.30 ± 2.63	
Comparison of significance by LSD (F-test)		<0.001 \$

* = Mean of frequency ± SD, + = Comparison of significance by ANOVA (F-test)

@ = Highly significant differences, # = No. of samples is 10 with 50 treatment.

\$ = All the treatment are show highly significant differences except with oil in comparison with liquid extract (P = 0.667)

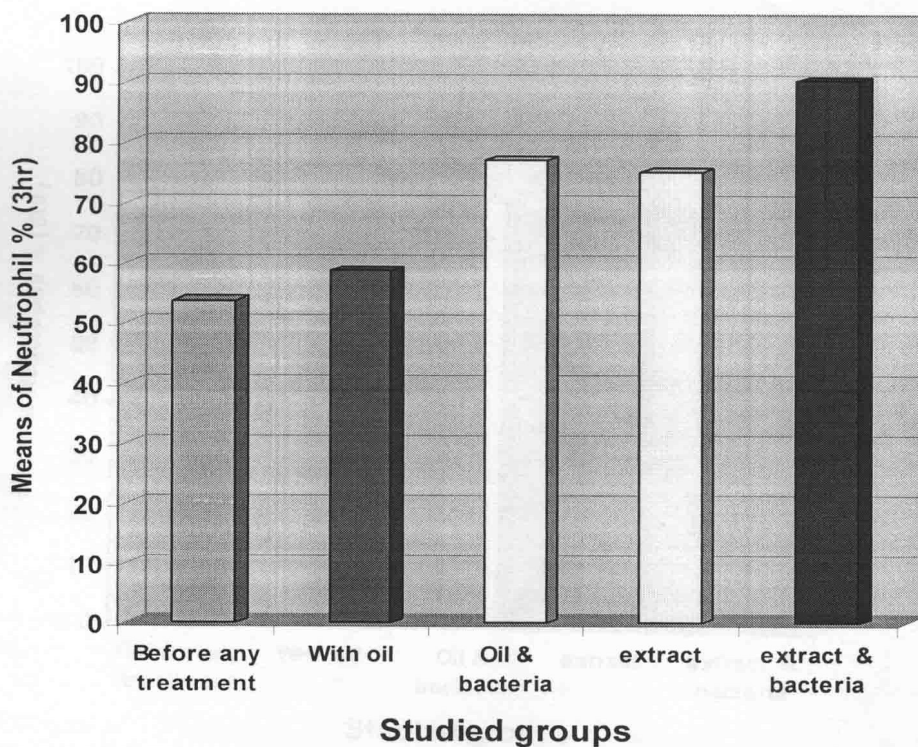


Figure 6: Neutrophil's % according to studied groups in incubation periods 3hr.

3. Six Hours incubation:

The table and figure below show the effect of 6 hours incubation with different treatment.

Table 7: Neutrophil's % according to studied groups in incubation periods 6 hr

<i>Studied groups #</i>	<i>Neutrophil's frequency (%)*</i>	<i>P value +</i>
<i>Before any Treatment</i>	53.70 ± 4.24	0.0001 @
<i>With oil extract</i>	67.30 ± 3.16	
<i>With oil extract & bacteria</i>	76.60 ± 3.24	
<i>With aqueous extract</i>	79.40 ± 4.62	
<i>With aqueous extract & bacteria</i>	92.40 ± 2.22	
Comparison of significance by LSD (F-test)		<0.001 \$

* = Mean of frequency ± SD, += Comparison of significance by ANOVA (F-test)

@ = Highly significant differences, # = No. of samples is 10 with 50 treatment.

\$ = All the treatment are show highly significant differences except with oil and bacteria in comparison with extract (P =0.089).

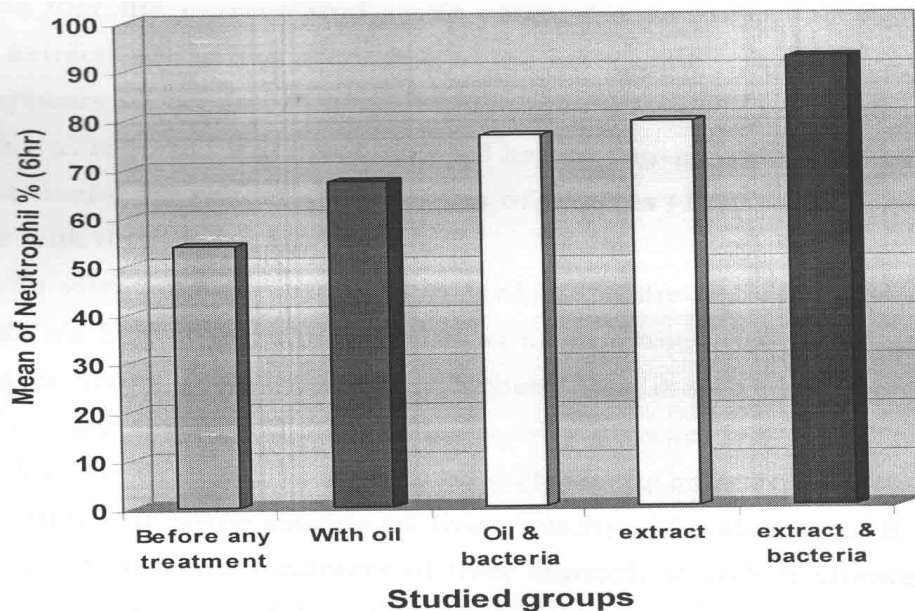


Figure 7: Neutrophil's % according to studied groups in incubation periods 6hr.

Discussion:

It is of interest that *Nigella sativa* seed, used for centuries for medicinal and culinary purposes and reported to possess a number of pharmacological properties, including antimicrobial activity. In 1959, the active ingredient in black seed, (*Nigella sativa*) crystalline Nigellone, was first isolated and identified as providing many health benefits. Clinical trials have validated the efficacy of black seed in promoting health and wellness.

In light of the above research findings, Black cumin stimulates Neutrophils' activity. These are the short-lived immune cells normally found in bone marrow but mobilized into action when there is a bacterial infection. Extracts of black cumin have also been shown to modulate production of interleukins such as GM-CSF which increased the cellular proliferative activity. These facts explain the results that the current study was conducted to [16]. The results of using different extracts are varied according to the other studies. However, antibacterial activity appears to be due to Thymol, Thymoquinone, Thymohydroquinone and tannins. It was reported that black seed oil has an anti-inflammatory effect and that it could be useful for relieving the effects of arthritis [17]. These criteria are quite consistent with the present study.

In vitro studies performed in Jordan and the USA have determined that the volatile oil is anti-leukemic as well as an immune system stimulant. Studies performed in Spain as well as England found that the fixed oil is useful in the treatment of rheumatism and other inflammatory diseases [18-19].

More over, it has been reported that *Nigella sativa* oil possesses Hepato-protective effects in some models of liver toxicity [20]. However, it is *N. sativa* seeds that are used in the treatment of liver ailments in folk medicine rather than its oil. Therefore, the aim of this study was to investigate the effect of the aqueous suspension of Black cumin in comparison with the oil extract. The data of current study were assumed in agreement with the others that the aqueous extract has best effect in some aspects such as those studies here [21-22].

References:

1. Chevallier A. "**The encyclopedia of medicinal plants**". Dorling Kindersley publishers, London. P: 237. (1996)
2. Brutis M & Bucar F. "Antioxident activity of *Nigella sativa* essential oil." *Phytother. Res. JA* (5):323-28. (2000)
3. Gilani AH, Jabeen Q & Khan MAU. "A review of medicinal uses and pharmacological activities of *Nigella sativa*." *Pak. J. Biol. Sci.* 7:441-51. (2004)
4. Khan MAU, Ashfaq MK, Zuberi HS, Mohamood MS & Gilani AH. "The *in vivo* antifungal activity of the aqueous extract from *Nigella sativa* seeds." *Phytother. Res.* 17(2): 183-86. (2003)
5. El-Abhar HS, Abdallah DM, & Saleh S. "Gastro-protective activity of *Nigella sativa* oil and its constituent, thymoquinone, against gastric mucosal injury induced by ischaemia / reperfusion in rats." *Ethanolpharmacol.* 85: 251-8-58. (2003)
6. Worthen DR, Ghosheh OA & Crooks PA. "The *in vivo* anti-tumor activity of some crude and purified components of black seed, *Nigella sativa* L." *Anticancer Res.* 18: 1527-32. (1998)
7. Hajhashemi V, Ghannadi A, & Jafarabadi H. "Black cumin seed essential oil, as a potent analgesic and anti-inflammatory drug." *Phytother. Res.* 18(3): 195-99. (2004)
8. Fararh KM, Atoji y, Shimizu Y & takewaki T. "Isulinotropic properties of *Nigella sativa* oil in Streptozotocin plus Nicotinamide diabetic hamster." *Res. Vet. Sci.* 73:279-82. (2002)
9. El-Dakhkhny M, Madi NJ, Lember N, & Ammon HP. "Nigella sativa oil, nigellone and derived oil and Thymoquinone inhibit synthesis of 5-lipoxygenase products in polymorphonuclear leukocytes from rats." *Ethanolpharmacol.* 8k 161-64. (2002)
10. El-Kadi A & Kandil O. "Effect of *Nigella sativa* on immunity." *Proceeding of the 4* International Conference on Islamic Medicine, Kuwait. Bull. Islamic Med.* 4: 344-48. (1986)
11. Haq A, Abdullatif M, Lobo PI, et al, "Black seed: effect on human lymphocytes and polymorphonuclear leukocytes phagocytic

activity." *Immunopharmacol.* 30_(2): 147-55 (1995).

12. Haq A, Lobo PL, Al-Tufail M, Rama MR, & Al-Sedairy ST. " Immunomodulatory effect of *Nigella sativa* protein fractionated by ion exchange chromatography." *Int. J. Immunopharmacol.* 21: 283-95 (1999).

13. Mashhadian NV & Rakhshandeh H." Antibacterial and anti-fungal effects of *Nigella sativa* extracts against *S. aureus*, *P. aerogenosa* and *C. albicans* " *Pak. J. Med. Sci.* 21(1): 47-52. (2005)

14. Davidsohn I & Nelson DA. "The Blood." In: *Todd-Sanford Clinical Diagnosis by Laboratory methods* by Davidsohn I & Henry JB. (eds.) 15th (Ed.) Saunders WB Co. Philadelphia, London, Toronto P: 100-126. (1979)

15. Sorlie, DE "Medical biostatistics and Epidemiology: Examination and Broad Review." 1st Ed; Norwalk Connecticut Appleton and Lange Com: 74-88 (1995)

16. Morsi, NM. "Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria." *Acta Microbiologica Polonica.* 49(1): 63-74. {a} Botany Department, Faculty of Science, Cairo University, Cairo, Egypt, (2000)

17. Al, O. S. Y., Ammar NM., et al., "Studies of some biochemical, nutritional and anti-inflammatory effects of *Nigella sativa* seeds." *Egyptian Journal of Pharmaceutical Sciences,* 38(4-6): 451-469. {a} Food Sciences and Nutrition Department, National Research Center, Dokki, Cairo, Egypt, (1997).

18. Khan N, Sultana S. "Inhibition of two stage renal carcinogenesis, oxidative damage and hyperproliferative response by *Nigella sativa*." *European Journal of Cancer Prevention.* 14(2):159-168, 2005 IFT Annual Meeting, July 15-20 - New Orleans, Louisiana (2005).

19. Corder C, Benghuzzi H, Tucci M, Cason Z. "Delayed apoptosis upon the treatment of Hep-2 cells with black seed." University of Mississippi Medical Center, Jackson, MS 39216, USA (1996).

20. Al-Ghamdi MS. "Protective effect of *Nigella sativa* seeds against carbon tetrachloride-induced liver damage." Department of Pharmacology, College of Medicine, King Faisal University, Dammam, Saudi Arabia.
<http://www.mghamdi@4ammam.kfu.edu.sa/>

21. Daba MH, Abdel-Rahman MS. Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. *Toxicol Lett;* 95(1):23-9 (1998).

22. Nagi MN, Alam K, Badary OA, et al. Thymoquinone protects against carbon tetrachloride hepatotoxicity in mice via an antioxidant mechanism. *Biochem Mol Biol Int;* 47(1): 153-9 (1999).