Effect of Nigella Sativa on Immunity in young healthy Saudi volunteers

Literature review:

For many centuries, seeds of Nigella sativa (NS) (black cumin), a dicotyledon of the Ranunculaceae family, have been used as a seasoning spice and food additive in the Middle East and Mediterranean areas [(1)]. It was used for a long time as an herbal medicine and was showing very good effects in many aspects of health. Researchers started from a long time to search for the benefits of this amazing seed, and until now they are discovering new benefits. Black seed has two types of oil; volatile and essential oil. The volatile oil which does not exceed 1% of the seed weight has more than a hundred ingredients with therapeutic potential. Thymoquinone (TQ) is the most important known ingredient present in black seed volatile oil. Black seeds had shown to have anticancer, antioxidant, gastroprotective, hepatoprotective, analgesic, anti-inflammatory, antihypertensive, antidiabetic, antihistaminic, anthelmintic, and antimicrobial impacts [(1)].

Anti-Microbial Effects:

Numerous studies have reported antimicrobial effect of NS on several Gram-negative and Gram-positive bacteria, viruses, parasites, Schistosoma and fungi. NS showed an excellent anti-bacterial effect on MRSA. The anti-microbial properties are mainly due to TQ and melanin. Bacterial death happens due to components of NS inhibiting part of the bacteria which lead to it death [(1)]. In an in vitro study, all stains of Helicobacter pylori that were tested were inhibited, NS extract produced, within 60 minutes, a 100% inhibition of their growth [(2)]. In a clinical trial, NS seeds in a dose of 2g/d with omeprazole 40mg/d showed a potent anti-Helicobacter pylori, however the doses of 1g/d and 3g/d were less effective. The cause of the less effectiveness if the dose was increased to 3 grams was not clear, the authors proposed a possible explanation that NS contains numerous ingredients which could have countered the anti-H. Pylori effect with a higher dose [(3)].

Anti-Inflammatory Effects:

Black seeds showed huge improvement in many inflammatory conditions and diseases. It has a direct effect on inflammatory cells which are responsible for creating an inflammation. NS has been shown to strongly prevent leukotrienes production in human blood cells through a decrease in 5-lipoxygenase and leukotriene B4[(4)]. TQ also showed evidence in decreasing

inflammatory cytokines secretion and inhibiting inflammatory alterations in liver and many other organs. In another study, Black seeds extract had a direct effect on asthmatic patients in reducing airway inflammation and improving all pulmonary function tests. [5]

Immunomodulatory Effects:

The immune system consists of multiple linked network of cells, proteins, and lymphoid organs to provide protection against millions of microbes and infections. The immune system includes the innate immunity and the adaptive immunity, the innate immunity provides an immediate protection to the body, and its effect is merely the same in different individuals, while the adaptive immunity, takes more time to develop, but it is more specific and fatal to invasive pathogens[6]. NS have shown so many immunomodulatory effects in both in-vivo and in-vitro studies.

In-Vitro Studies:

A study tested the in vitro effects of NS seeds and soluble fractions on lymphocyte response to different mitogens and on polymorphonuclear leukocyte phagocytic activity. NS increased the production of interleukin-3 (IL-3) by human lymphocytes it did show an effect on macrophages by increasing IL-1β [7]. Another in-vitro study on the effect of TQ on immunity showed that TQ injected directly to cells in low concentrations (10, 2.5 or 0,62 μg/mL), increased the survival of activated T-cells, CD8+ T-cells ability to generate IFN-γ, which indicates that TQ may be beneficial against infectious diseases and enhancing immunity[8]. A study was designed to investigate the direct effect of purified extract containing: nigellone, thymohydroquinone, and thymol at total concentration of 2 mg/ml on several immune responses in sheep cells. Fifty Balady sheeps around one-year-old of both sex weighing 45–80 kg were used as blood donor in this study for peripheral blood mononuclear cell separation and autologous sera, blood was collected from at least eight sheep in each experiment which were selected randomly. Each experiment was repeated at least 3 times for reproducibility. NS extract enhanced sheep macrophage function and produced morphological changes which started 2 hours post culturing and reached maximum after 18 hours. NS extract-treated monocytes-derived-macrophages (MDM) cells size increased to 2–3 time that of the control untreated cells. Also, MDM phagocytic capacity to engulf labeled yeast was significantly higher in the NS extract treated MDM than in the untreated cells. Overall the results

in this study showed better host defense, preventing infections, enhancing innate immunity and regulating adaptive immunity[9]. A study on mouse dendritic cells (DC) which are important in the regulation of innate and adaptive immunity, DC maturation and cytokine release is triggered by bacterial components such as lipopolysaccharides (LPS). The present study explored whether TQ modifies LPS-induced DC maturation, survival and cytokine release. Mouse bone marrow derived DC's were treated with LPS and different concentrations of TQ and the surface expression of CD11c, CD86, MHCII, CD54 and CD40 was determined by FACS analysis. The formation of the interleukins 10 (IL-10) and 12 (IL-12p70) as well as tumor necrosis factor (TNF)-alpha was evaluated by ELISA. LPS elevated the percentage of CD11c(+)CD86(+), CD11c(+)MHCII(+), CD11c(+)CD40(+) and CD11c(+)CD54(+) cells and stimulated the release of IL-10, IL-12p70 and TNF-alpha. These effects were reduced by TQ in a concentration dependent manner (1-20 microM). In conclusion, TQ compromised the maturation, cytokine release and survival of DC's[10].

Evidence showed that NS and its major active compound TQ have an immunomodulatory activities. These activities are reflected on the cellular immunity, humoral immunity, Th1/Th2 paradigm and Natural Killer cytotoxicity. The effect of NS on cellular immunity showed that in vitro increasing in the proliferative capacity of T lymphocytes and splenocytes and response to a different mitogens of the human peripheral blood mononuclear cells (PBMC), increased in the IL-3 of PBM which shows that NS extract has stimulatory effect on cellular immunity[7]. NS has a suppressing effect on the humoral responses, that effect was documented on a splenic mixed lymphocyte culture and in an in-vivo study which reported a decrease of serum antibody titer in Long-Evans rats that were challenged with typhoid antigen, the experimental animal antibody titer was found 1280 compared to the control group 2560[7]. Another in vitro study, NS aqueous extraction (10–500 μg/ml) significantly enhanced the activity of NK cells against tumor cells. The enhancement of the NK cells activity after the use of NS aqueous extraction was due to its effect in the increase expression of IFNγ and TNFα which are cytokines having a tumoricidal effects, and granzyme A "GZMA", and N-acetyl-β-D-glucosaminidase "NAGase" which are enzymes involved in the cytotoxic effect of the NK against tumor cells[11].

In-Vivo Studies:

Several in-vivo studies showed significant effects of NS on immunity, autoimmune diseases and toxicity. A study demonstrated that NS aqueous extract of NS of 5 mg, showed a significant increase in the Natural Killer "NK" cytotoxic activity against YAC-1 cells after 1 week of oral administration in a 10 week old BALB/c female mice, increased the cytotoxic activity of NK cells by 62.3% ± 6.4%[12]. A group of researchers investigated the possible immunopotentiating effect of NSO on peritoneal macrophages in streptozotocin-induced diabetic hamsters. Phagocytic activity was evaluated by injection of fluorescent latex (2 microm diameter) intraperitoneally, followed by collection of preotoneal macrophages 24 hrs later[13]. The phagocytic index (number of macrophages that phagocytosed fluorescent particles) and phagocytic rate (number of fluorescent particles in each macrophage) were significantly higher in STZ-diabetic hamsters treated with NSO (400 mg/kg) for 4 weeks compared with untreated diabetic animals, as demonstrated by fluorescence microscopy. This was represented by significant decrease in the number of macrophages that contain 1, 2 and 3 fluorescent particles and significant increase in the number of macrophages that contain 4–20 fluorescent particles in diabetic animals treated with NSO. Flow cytometric analyses also showed a significant increase in macrophage phagocytic activity as indicated by the increase in fluorescence intensity after treatment with NSO[13]. Another study on gestational diabetes rats showed improvement in their offspring immune status, after giving the mothers 20mg/kg of oral TQ during pregnancy, they showed reversing in the decreased levels of IL-2, T-cell reproduction, and improvement in both circulating and thymus homing T-cells proliferation[14].

NS anti-toxic effect have been investigated in a study which concluded that intraperitoneal administration of TQ 1mg/kg in imidacloprid (IC) intoxicated rats showed improvement in immunity, which was achieved by enhancing chemokinesis, chemotaxis, phagocytic activity[15]. NSO was shown to possess a protective role against vitamin A hypervitaminosis (HVA). Rats treated with 800 mg/kg NSO orally showed higher serum levels of IgG and IgM than either the control or those with high doses of vitamin A.[16]

In a study on Newcastle virus vaccinated broilers, NS supplementation was given in 3 doses (5, 10 and 20 g kg^{-1}) for 42 days, anti-bodies against Newcastle virus significantly increased on day 35[17]. Another study revealed that the use of NS at 40 g kg^{-1} diet improved anti-body production against both Newcastle virus and infectious bursal disease.[18] This immunomodulatory effect is

done by stimulating T-cell mediated immune responses and suppression of B cell-mediated immune responses[19, 20].

Another study investigated the prophylactic effect of NS and its ability to stimulate immunity in broilers. Crushed NS in different doses (1.4%, 2.8%, 4.2%, and 5.6%) were supplemented to 5 groups of 1-day-old chicks, and then challenged with Escherichia coli. The study revealed highly significant increase ($p<0.01$) in IgG and IgA in NS 4.2% and NS 5.6% fed groups, on the other hand, IgM showed non-significant difference in all groups. A highly significant ($p<0.01$) decline in total bacterial count and total Enterobacteriaceae count was also noticed in the NS 5.6% fed broilers[21].

In vivo study was done to evaluate the immunoregulatory effect of NSO (90 mg/kg/day) after inhalation of different doses of Formaldehyde in rats for 30–60 days. The inhalation of formaldehyde increased the serum level of IgA, IgM and Complement protein "C3" and decrease serum IgG. After administration of NSO, IgA, IgM, C3, returned to normal and the IgG level did not change, which indicates that NSO can play an important role in acute immune response[22].

A study tested the radioprotective effects of NSO against hemopoietic damage and immunosuppression in gamma-irradiated rats where Sixty male Wistar rats, were divided into 4 groups; I-control rats, II-rats orally intubated with NSO (1 ml/kg b.wt./day) for 5 days/week, III-whole body gamma irradiated rats with the estimated LD50/30 (4 Gray) and IV-rats daily intubated with NSO then subjected to whole body gamma irradiation. Irradiation resulted in significant decrease in hemolysin antibodies titers and delayed type hypersensitivity reaction of irradiated rats, in addition to significant decrease in white blood cells, plasma total protein and globulin concentrations with depletion of lymphoid follicles of spleen and thymus gland. Furthermore, there was a significant increase in malondialdehyde concentration with a significant decrease in plasma glutathione peroxidase, catalase and erythrocyte superoxide dismutase activities. Oral administration of NSO before irradiation considerably normalized all the above-mentioned parameters and produced significant regeneration in spleen and thymus lymphoid follicles. The study recommends NSO as a promising natural radio-protective agent against immunosuppressive and oxidative effects of ionizing radiation[23].

The above literature shows a very promising immunomodulating effect of NS as well as antimicrobial, anti-toxic and protective effects. However, the immunopotentiation effect of this miracle plant has not been investigated in normal humans. Hence this study is going to evaluate the impact of different doses of NS on the immune response in healthy humans.

Aim of the Study:

This study aims to assess the effects of different doses of Nigella Sativa (NS) on immunity of healthy male students in Imam Abdulrahman Bin Faisal University (IAU).

Objectives:

1. To assess the effect of NS on cytokines (IL-1, IL-4, IL-6, IL-10 and TNF)
2. To evaluate the effect of NS on immunoglobulins (IgG, IgM)
3. To assess the effect of NS on cellular immunity (CD4 & CD8)
4. To determine the best dose of NS to enhance immunity in healthy humans.

Methodology:

This is a double blinded random clinical trial. The study will be conducted on healthy male students studying in IAU and blood extraction will be carried out in the main campus university clinic. Students will take the intervention for one month and will be divided into four groups; 3 will take different doses of black seed and the fourth will serve as a control.

Participants:

The participants are going to be divided into 4 groups, 30 participants will be randomly allocated to each group. The first group is the control group – placebo - and they will be given 162mg of activated charcoal oral capsule, second group will receive 500 mg NS oral capsules, third group 1g and fourth group 2g.

Inclusion Criteria:

1. Healthy male IAU students
2. Age between 18-25
3. BMI = 18.5-29.9 kg/m^2

Exclusion Criteria:

1. Students with any acute or chronic illness (unless acute illness occurred during the study)
2. Students with abnormalities in the basic laboratory investigations
3. Participants with less than 90% compliance

Material:

Ethiopian NS, bought from the local market, will be cleaned, grinded and assembled in 500 mg capsules, in the pharmaceutics laboratory in the college of clinical pharmacy at IAU. Activated charcoal capsules **(**162 mg) similar in size and color to the capsules of NS (Arkopharma Pharmaceutical Laboratories Carros, France) will be used as placebo. The placebo capsules will be given in the same bottles of black seed. Each participant will be given enough capsules for the period of 4 weeks. Each bottle in the concerned group will be coded by the technical staff in the laboratory to achieve the double blindness in the study.

Study protocol:

After applying the inclusion and exclusion criteria mentioned above, recruited participants will be given a full explanation of the study and required procedures that will be done and those agree to join will sing a written consent. Full history and physical examination will take place to rule out any acute or chronic illnesses. Two blood samples will be collected from participants, in the Family and Community Medicine (FAMCO) center in the IAU campus, before initiation of the study and at the end of the 4 weeks study duration. The first sample will be assessed in the center's laboratory for basic tests which include; complete blood count (CBC), renal function test (RFT) and liver function test (LFT) to assure the general health of the participant. The second sample will be assessed in the microbiology laboratory in college of medicine at IAU to determine baseline cytokines level by enzyme-linked immunosorbent assay (ELISA). Participants will be followed for the study period (4 weeks) to ensure taking the capsules. At the end of the study duration (4 weeks) blood samples will be obtained for the analysis of both basic lab investigations and cytokines. Immunoglobulins and cellular immunity will be evaluated in both baseline and postintervention blood samples in hospital laboratory if the administration agrees.

ELISA kits for IL-1, IL-4, IL-6, IL-10 and TNF will be bought from Origin company, USA, and the cytokines levels will be measured according to the manufacturer recommendation.

Statistics:

Statistical analysis will be performed using the Statistical Package of Social Science (SPSS) version 16. Data will be presented as means ± SD (standard deviation). In each group, readings will be compared to their corresponding baseline values using Student's t- test for paired data. Results in the four groups will be compared using ANOVA. P value <0.05 is considered as significant.

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