CHAPTER 21

IMMUNOMODULATORY EFFECTS OF *NIGELLA SATIVA*

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SUMMARY

Nigella sativa, commonly referred to as black cumin or black seed, has drawn interest due to possible immunomodulatory properties. Thymoquinone is one of the bioactive chemicals found in *N. sativa* that has immunomodulatory qualities that can affect the immune system. It can boost immune cell activity, control inflammatory processes, and alter the generation of cytokines, among other immunological responses. *Nigella sativa* has immunomodulatory properties that may be related to its capacity to control the proportion of T lymphocytes to macrophages, among other immune cells, promoting a more robust and well-balanced immunological response. Thymoquinone has also been linked to decreased inflammation and oxidative stress, which lends credence to its possible immunomodulatory function. In this chapter, the immunomodulatory potential of cumin is discussed.

INTRODUCTION

Herbs have been commonly used for centuries for curing different ailments throughout the world. Different plant extracts and their byproducts possess different medicinal properties. Due to abuse and overuse of antibiotics and other allopathic medicines, different health insecurities are also raised. Modern science is leading towards less use of medication to avoid antimicrobial resistance and other health hazards due to prolonged use of antibiotics. Modern research is mainly focused on the use of herbal and natural extracts for curing and preventing diseases. Most of the herbal products have been proven to be beneficial for living beings. They possess specific properties that provide effective control of different organs and systems of the body (Ahmad et al., 2013).

Nowadays health issues due to technological advancements have led researchers to more innovative and conventional solutions. The most inclination in this field is towards alternative medicine and traditional methods by using products of natural origin. These products have become very popular around the globe because of the benefits they provide to human as well as animal health. In recent times, the daily usage of plant-originated food products has increased, which do not pose any health risks and has minimal side effects. Also, these products are being used as therapeutic and supportive supplements. Since the ancient era, various plants have been used to treat different pathological conditions. Similarly, nowadays, plants and their byproducts byproducts-related industry are flourishing rapidly from year to year. In developing countries, a major portion of the population is dependent on herbal medicines to cure different basic health-related problems. Contrary to chemical drugs, herbal medicines are way more effective, safer, less toxic, easy to use, and economical. Due to this reason, research is carried out on the therapeutic potentials and clinical uses of plants in human and veterinary medicine (Majdalawieh & Fayyad 2015).

NIGELLA SATIVA (BLACK SEED)

Among these herbal medicines, Black Seed *(Nigella sativa*) is the most common and is widely used in medicinal therapy. Many studies have revealed that black seed possesses multiple properties for the betterment of health. Black Seed and its metabolites which are some chemicals are the main source of treatment for different ailments. The properties of black seed are

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antibacterial, antiviral, anti-parasitic, anti-fungal, anti-inflammatory, immunomodulatory, Antioxidant, anti-cancerous, and hepato-protective activities (Boskabady et al., 2011). *N. sativa* also has beneficial effects on the immune system, pulmonary disorders, reproductive issues as well as diabetes mellitus. It also helps to promote osmotic balance and resolve dermatological complications, breast cancer issues, fertility, and dehydration. Black Seed is a little herbal medicine with a little diameter, it commonly belongs to the botanical family *Ranunculaceae*. It is commonly found in Middle Eastern countries South Europe, the Mediterranean region, India, Pakistan, Syria, Turkey, Saudi Arabia, North Africa, and Southeast Asia. Black Seed has tapered green leaves and rose-like pink, yellow, pale blue, white, and purplish flowers with 6-10 petals attached to them. The mature fruit contains multiple tiny seeds, dark black in color. The last Holy Prophet of religion Islam, Hazrat Muhammad (SAW) told us that the Black Seed *(Nigella Sativa)* has the property to cure all kinds of ailments except death (Hikmah et al., 2022).

Nigella sativa seeds are known to be used to support healthy living, active and proper aging, improved standard of life, and most significantly for prevention of diseases, i.e., preventive medicine. Thymoquinone is an important component of *Nigella sativa* seed oil, which is the basic unit of supporting a healthy and sound life. It is preferable due to its potential for medical properties. According to recent research, the active ingredient which is found in the plant seed is a product that might be very beneficial to be utilized due to the vitamins and fatty acids in it. The clinical findings have scientifically revealed the pharmaceutical properties of the *Nigella sativa* seed that's why it is the preferred plant for treatment purposes. Besides all the features of *Nigella sativa*, the immunomodulatory effects of the substances in its composition (thymoquinone) are of great importance (Ojueromi et al., 2022). A few years ago, studies have been started to know that the black seed can enhance the immune response in humans. In recent years, the immunomodulatory effects and protein structures of all the extracts of black seed have been isolated and analyzed in vitro. As a result of these studies, it is revealed that black seed oil enhances T-cell proliferation and hence stimulates cellular immunity; on the other hand, it has been found that it suppresses B-cells, i.e. humoral immunity. These findings were obtained through *in vitro* as well as *in vivo studies.* According to recent data, it is evident that *Nigella* can increase cellular immunity while decreasing humoral immunity. However, critical experimental studies are needed to test this hypothesis. The immunomodulatory effects of black seed can be measured according to the natural body immune reaction mediators in different ailments (Islam et al., 2017).

Physical and chemical properties of black seed

Physically the black seed oils were the same no matter whether the oil was obtained by the cold press method or by the solvent extraction procedure, physical as well as chemical properties remained the same. In recent studies, it has been observed that black seed oil has a greater tendency to ultraviolet light absorption because of its color combination and the ability of sun protection effect. As far as oxidative stability of black seed is concerned, studies showed that by the use of a rancimat device, it was evident that it has 55 hours of durability. Hence it can be stored for a long purpose due to very little amounts of unsaturated fatty acids in it and a greater amount of naturally occurring antioxidants (Salem et al., 2023).

Considerations of *Nigella sativa* seeds

Environmental changes such as climate, sunlight, sea level, distance from the equator or poll, soil capability, soil interaction, purity of water, and minerals are proven to be crucial in the development of plants such as black seed. Also, factors such as the age of the plant, its development, time of harvest, processing, and drying procedures have positive as well as negative effects on the basic development of black seed. The development of crucial substances, both quantity and quality of the important oil obtained is of great importance in this regard. That's why the number of essential oils such as nigella and thymoquinone, which are active ingredients in the structure of black seed cultivated in different terrestrial regions, may also vary (Liang et al., 2021).

POTENTIAL ACTIVITIES OF BLACK SEED

Black Seed has a very good potential regarding its medicinal and supplementation properties. It provides a good metabolism of the body and maintains the normal functioning of cells in many ways. Following are some of the potential activities of black seed (Gholamnezhad et al., 2015a).

Nigella against bacteria

N. sativa has proved to possess a very good antibacterial property against gram-positive bacteria (*Staphylococcus aureus*) as well as gram-negative bacteria (*Pseudomonas aeruginosa* & *Escherichia coli*) species. It is evident that black seeds have synergistic effects with gentamycin and streptomycin. The major concern of modern medicine is antimicrobial resistance and hence black seed is very effective against resistant microorganisms of gram-positive and gram-negative nature. (Islam et al., 2017).

Nigella against virus

Herpes virus which is the causative agent of a very fatal disease in immunocompromised animals in the same manner as it is caused by human cytomegalovirus in immunocompromised

human beings. In a recent study, it has been found to be evident that the in vivo treatment with black seed oil has a promising anti-viral effect against MCMV infections reflecting the medicinal ability of N. *sativa* oil as an anti-viral treatment. The immune response against most of the viral infections is controlled by both specific and non-specific cells. All the cell populations play an effective anti-viral role at a given time period after the infection, where non-specific cells are crucial during the starting phase, in contrast, T cells are required for the elimination of the virus in the later stages (Kheirouri et al., 2016). The mediators that are synthesized by these cells mostly IFN-g are of prime importance for the antiviral impact. It was also observed that this antiviral response of the black seed oil is interlinked with the increasing response of CD4 and CD8 cells the nonspecific cells were also enhancing their ability of IFN-g synthesis which is considered a key factor for making animals more resistant to MCMV infections. It is also mentioned in a study that some of the viral infections may lead to apoptosis causing lymphocyte level decrease in the host, the anti-oxidative property of black seed can hinder virus-oriented apoptosis as well as the viral multiplication in target cells. Hence the anti-oxidative property of the black seed oil may impart an impact to its anti-viral activity. Eventually, the anti-viral response of N. *sativa* against MCMV infection creates a space for a novel anti-viral remedy. Therefore, more researches are required to advocate this effect in other viral models and also to explain the active ingredients possessing such anti-viral effects (Khazdair et al., 2021).

Nigella against fungi

The aqueous extract of Black seeds possesses a suppressive property against Candidiasis in different species of mice. The ether extract of Black seed and thymoquinone have anti-dermatophyte potential. A trial reveals that these both when used against eight species of dermatophytes in which four species of *Trichophyton rubrum* and one of each *Trichophyton interdigitale*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, and *Microsporum canis* by using agar diffusion method. As a result of these trials, it was evident that the ether extract of black seed and thymoquinone show inhibitory properties against different fungal strains. Such results show the effectiveness of *N. sativa* as a source for Anti dermatophyte medicine.(Majdalawieh & Fayyad 2015).

Nigella sativa boosts the immune system

It has been reported that black seed oil increased CD4 (auxiliary lymphocyte) / CD8 (suppressive lymphocyte) rate by 55% and the natural killer (NK) cells were reported to be increased by 30% because of using nigella sativa seed extract was taken for a week, the number of natural killer cells and cytotoxicity against YAC-1 tumor cells increased nearly double of that. Black seed oil directly promotes immune resistance in the old ones. It has been reported that it checks a decrease in the amount of hemoglobin and total leukocytes caused by "cisplatin" in mice. The total number of leukocytes was enhanced by 3.2% in mice that were shifted to black seed. Black seed also has the role of enhancing the potential of T cells in the immune system of the body. It is being evaluated that black seed triggers T lymphocytes for the release of IL-3 while it has no significant activating effect in the IL-2 release and its proteins proficiently increase the number of total lymphocytes, and hence in this way, it has an immune-modulatory effect in both humans as well as animals (Boskabady et al., 2011). In most of the studies conducted, it was found that black seed extract enhances the T cell population, CD3, CD4, and CD8 surface antigens, and a significant amount of immune system cells. As a result of this increase, it was found that thymoquinone triggers hematopoiesis and hence the immune system-related cells are promoted in the body. In allogeneic cell cultures and also in in-vivo studies using black seed extracts, it promotes a large amount of IL-1β and TNF-α expression. When triggering the T lymphocytes of the black seed in the same manner as IL-3 secretion, IL-2 has no activating effect on the release and their protein proficiently increases the total amount of lymphocytes hence in this way it modulates the immune response both in humans and in animals (Salem, 2005).

In some other studies on mice, it was suggested that the black seed prohibited a decrease in hemoglobin concentration and total leukocytes caused by Cisplatin. In the same study, black seed seeds enhanced CD3 + T cells, CD4 + helper T cells, CD8 + suppressor to cytotoxic T cells and their ratio to one another (CD4 + / CD8 +) and enhanced the total leukocyte count in the body. A number of researches have been brought out to show the impact of Nigella and thymoquinone on the immune system. In recent studies, thymoquinone has been shown to have a significant impact on the immune system. These effects are defined by the active substance as activation and inactivation of T cells, B cells, and cytokines implicated in the immune system (Islam et al., 2017). According to in vitro and in vivo data, it is most evident that black seed can enhance cellular immunity while decreasing humoral immunity. However, more advancements are needed to check this hypothesis. The immunomodulatory effects of N. *sativa* can be evaluated based on the natural immune reaction mediators in diseases. In most recent studies, different implementation methods (orogastric, intraperitoneal, subcutaneous, inhalation, etc.) of the active substances and their impacts were observed for the investigation of immunomodulatory effects of black seed (Abel-Salam, 2012).

Effect of black seed on cellular immunity: Black seed is being used as an anti-inflammatory, and anti-oxidant in nature, and anti-cancerous research activities are growing very rapidly to

assess the capabilities of black seed to interpret adaptive immunity. Researchers also examined the different aspects of the Nigella seed extracts on human peripheral blood mononuclear cells (PBMC) that have the potential to different mitogens in-vitro. Black seed extracts have a significant role in PBMC response to the whole allogenic cells but not to the Phytohemagglutinin (PHA) or concanavalin A (Con A) also of two T cells mitogens. Black seed extracts alleviate the secretion of interleukin IL-3 from the PBMCs cultured in both the availability and non-availability of pooled allogenic cells, but in contrast, there is no significant role in IL-2 secretion from mitogen-stimulated PBMCs. By using the combined lymphocyte cultures, it was revealed that whole and the purified protein extracts of Black seed impart a stimulatory role on the unstimulated lymphocytes and the Pokeweed Mitogen (PWM) activated lymphocytes respectively (Ahmad et al., 2013).

Black seed extracts and their constituents had a minor or no effect on the secretions of IL-4 from lymphocytes, both in the presence and absence of PWM. The interleukin IL-8 secretion was decreased by black seed extracts when the lymphocytes were left unstimulated, but somehow it increased in PWM-stimulated lymphocytes. All these findings and studies reflect that black seed have a significant stimulatory impact on cellular immunity (Majdalawieh & Fayyad, 2015).

Effect of Black Seed on Humoral Immunity: In a recent study by using an aqueous extract, splenic combined lymphocytes cultures and an ethyl acetate column chromatographic fraction of black seed in-vitro can significantly increase the multiplication of lymphocytes cultured in the presence of Con A. These studies indicate that black seed exerts a good suppressive effect on humoral immune responses. It is also evident that the volatile oil of black seeds as an immune modulator has a greater potential in Long-Evans rats who were supplemented with a specific antigen (Typhoid TH). Recent results revealed that oral usage of black seed oil in antigen-challenged rats profoundly decreases serum antibody titer. It also evaluated the immunomodulatory potential of black seed by interpreting its ability to enhance humoral-mediated immunological changes that supplement the treatment with chloramphenicol, an antibiotic. Similarly, oral administration of chloramphenicol in albino rats led to a very low haemagglutination titer (Salem, 2005).

IMMUNOMODULATORY PROPERTIES OF BLACK SEED

Immunomodulatory effects of black seed are of great importance in medical science, it is a perfect immune booster and a very effective herbal extract. Black seed is very commonly used in humans as a medicine and also used in daily usage but its properties are not yet defined to the consumers. There are many studies that reflect the usage of black seed as an immune modulator in humans as well as in animals. The two types of immunity, innate and adaptive are of great concern when describing the immune status of living beings. The pathogen-linked molecular pattern and the specific antigens both are defined by the innate and adaptive immunity concept. The granulocytes, NK cells, and the DCs are the prime examples of non-specific cells which are part of innate immunity whereas the cellular and humoral immunity which are part of adaptive immunity are mainly mediated by B cells, responsible for the secretion of antigen-oriented antibodies and helper T cells CD4+, cytolytic T cells CD8+ respectively. The T helper cells (CD4+) are mainly used to coordinate an immune response in the body. It helps the body against different ailments. The cytolytic (CD8+) perform the lysis of cells hence termed as killer cells. These are designated to perform their functions at the infectious site and also on the cancerous cells. Both of these T lymphocytes are crucial for the body and play a vital role in the eradication and spreading of infections and cancer (Said et al., 2022). Besides all the important features of black seed, the immunomodulatory effects of its metabolites or constituents are of great concern. Many studies reflect that it must be used in daily routine life for a healthy being, it can be used as an immune booster in humans as well as in animals. In most of the cases where black seed oil is used for treatment against any bacterial or viral infection, the ratio of CD4 to the CD8 T cells is increased by 55% within four weeks also the natural killer (NK) cell functions also proliferated by 30%. In a well-designed recent *in vitro* research, the protein constituents and the metabolites of Black seed oil and their immunomodulatory properties were elaborated. The PBMC (peripheral blood mono-nuclear cells) in humans and the reaction to different mitogens of different components of black seed extract were observed, the results were different, and there were no up-to-the-mark stimulatory responses on the PBMC. In some of the cultures where we used lymphocyte cultures in mixed form, there were good results it. Out of the four protein extracts, black seed showed good results. By using PWM (B cell) mitogens to react with lymphocytes, nigella has shown somehow better results. They have a restrictive nature in this aspect against different extracts of black seeds (Niu et al., 2021). By the continuous usage of nigella oil, the triggering property on different cells including the proliferation of T cells, the water level, and the chromatographic portion of ethyl acetate has increased the multiplicative effect to Con-A, in contrast to LPS (B cell) mitogen. All of the findings show that the nigella oil and its extract have the potential to promote strong immune outcomes in the body, this potential increases with the continual usage of black seed oil, and somehow cellular immunity is also reliable on the quality of immune effects (Said et al., 2022).

The T cell immunity of the body tends to increase because of the usage of the black seed, *in vivo* and in-vitro studies

implicate this in many researches. One study suggests that the continuous usage of extracts of nigella for 7-8 days orally tends to increase the amount of NK (splenic) cells by twofold and its toxic effects against tumorous target cells are also affected when studied with NK control group cells. The oral usage of Nigella seed for six weeks has favorable effects against diabetes. Nigella has immunomodulatory effects in different animals against different diseases. It enhances cellular immunity and tends the body to perform different metabolic phenomena effectively. It also has antibacterial, anti-fungal, anti-inflammatory, and anti-cancerous effects when used regularly and also enables the body to fight different toxins released from different sources. Black seed must be the part of human diet as well as for animals at least once a week. It promotes health and decreases disease incidence. It has anti-aging effects on the body by activating cellular immunity and providing a shield against different ailments (Khazdair et al., 2021). Nigella is a rich source of chemicals and metabolites which provide anti-toxic effects in living beings. The nutritional and dietary supplementation of Nigella enhances body condition and ensures good resistance to different metabolic disorders as well. The quantity, as well as quality of immune responses, are reportedly increased because of the transmission of the basic operations of DC. The modulation of DC functions of black seed might facilitate the immunomodulatory and anti-inflammatory mechanism potential. Many studies have been carried out on black seed but still, there lack of research on the influence of black seed and its metabolites on the physical and chemical effects on the cellular immunity of the body. There is a need to research the immunomodulatory effects and immune responses of black seed both in humans as well as in experimental models (Niu et al., 2021).

The oil of Nigella seeds is very beneficial for children as well as for adults, its capsules have also shown very good therapeutic responses against different diseases. It cures many problems like chronic asthma, weakness, skin infections, and also allergy-borne rhinitis when it is used orally @ 40-80mg/kg daily for 3-4 weeks continuously. It was also seen by giving black seed oil for continuous time @ 750mg/kg of body weight, the level of prostaglandin in the body is markedly reduced. These are some of the effects of black seed when given orally for some duration at a given specific dose rate (Khazdair et al., 2021). The intestine contains micro-flora which is beneficial for animal health. It provides help regarding the digestion of fodder or any feed material. The intestinal micro-flora is greater in numbers; multiple types are present there which makes them unique because of their population size as well as their function for promoting the normal physiology of individuals. The intestine also plays an important role in the regulation of the immune system in the body. The immune system which is linked with the intestine is one of the major immune modulators of both humans and animals. The microflora of the intestine has a pivotal role in the body's immune response to different ailments, it has a direct effect on the B and T cells to enable the body against different pathogenic conditions (Liang et al., 2021). The role of macrophages is also of great importance regarding the body's defense system. These are the fighting cells of the immune system. The stimulation of macrophages ensures the protection of the body against different ailments and febrile conditions and somehow the immune status of the body is also improvised. Black seed oil is directly involved in the activation of the macrophages to produce immune responses in the body. Nigella directly or indirectly mediates the body's defense mechanism and has an impact on body regulation. Continual usage of black seed ensures the proper regulation of the body's immune system and the mediation of different pathogenic conditions (Majdalawieh & Fayyad 2015).

The use of antibiotics in our everyday lives has become compulsory now. Nowadays bacteria have been resistant to multiple drugs due to their over and misuse. Also, their usage has multiple side effects on the body. Some of them are contraindicated in some pathological conditions and some cause severe immunosuppression. The defense mechanism is also compromised due to their continuous uptake, which is compulsory in some conditions. It is the requirement of disease being cured to give antibiotics on a regular basis for the wellbeing of animals as well as of humans. The long-term usage of antibiotics also deteriorates the normal functioning of the body; in some cases, we have to use broad-spectrum antibiotics for a longer duration therefore they cause immunosuppression in the body. In pigeons, Oxytetracyclines are used for many infections but prolonged use has negative impacts (Kheirouri et al., 2016). In a recent study, the pigeons were treated with black seed along with oxytetracycline for a longer duration to cure chronic infections. It was observed that the leukocytic count and other immune cells were intact, their level was increased in the body and the drastic effect of antibiotics was remarkably reduced. This approach is very beneficial for the treatment of various disorders and this advancement creates an ease for the medical professionals to prescribe antibiotics irrespective of their complications when used in combinations. In this way, Nigella seeds have a part in the immunomodulation of body functions and mechanisms. The major contribution black seeds have played in the immunomodulatory and immunoregulatory mechanisms of the body by mediating several immune responses in the body (Alshatwi, 2014). The defense mechanism of the human, as well as animal body, is pretty much supervised by the use of nigella extract accompanied by different antibiotics. Both work synergistically to cure different ailments and provide immunity for a longer duration. Black seed extract also augments the function and absorption of different essential vitamins and minerals in the body. It provides soothing effects to the intestinal mucosa and relieves animals from pain. Nigella extract is reported to have an extraordinary ability to provide a

cure for inflammatory bowel disease in animals. It also has an effect on different gastrointestinal issues and is widely used prophylactic to cure multiple digestive complications (Ojueromi et al., 2022).

Besides many miraculous properties and effects of black seed, it is also in many cooking and baking products as a spice. Due to this reason and its wide usage especially in the sub-continent makes black seed a complete medicinal product. It is also easy to cultivate and readily available products. It is also cheap in price and easily accessible from the market and superstores. The dosage of *Nigella Sativa* is also very low which makes it a very compatible medicine to be used in multiple doses for prolonged duration. It is also very easy to extract different constituents of black seed for its use in pharmaceutical industries. It is available in different forms of capsules, tablets, oils, and syrups to be used in both animals and humans. Black seed has been widely used as herbal medicine since ancient times, physicians use it for oral medication as well as to apply it on wounds because of its anti-bacterial, anti-oxidative, and anti-inflammatory properties. It can directly or indirectly act as an immune booster for the body against many viral and bacterial infections, its anti-parasitic properties are also of great concern which makes black seed a perfect combination to be used in pharmaceuticals (Alshatwi, 2014). Many extraction procedures are being widely applied to procure purified forms of *Nigella* so that the dosage and efficacy of black seed can be modified. The doubts or side effects of its usage are also being nullified during the coronavirus COVID-19 outbreak, Nigella was very commonly used in countryside by the farmers and many homeopathic physicians. It proves to be very beneficial in retaining the normal functioning of the body as well as immune modulation. Its antiviral activity was also very phenomenal. Hence black seed is a versatile and research-based product, many clinical trials proved its significance in human and veterinary medicine. More advancements are needed to ensure its availability in medicines (Gholamnezhad et al., 2015b).

CONCLUSION

All the literature and research work whether it is ancient study or the latest research reflects the significance of black seed as a great advancement in human and veterinary medicine. The main focus of our discussion was to elaborate on the immunomodulatory effects of black seed as well as its application in other infections as well. In this era, it is just like a blessing to have a product like black seed which possesses a numerous advantage and a wide application as a preventive medicine. More study is required to unveil its mysterious properties as a medicine for living beings.

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