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NIGELLA SATIVA: A PLANT WITH MULTIPLE THERAPEUTIC IMPLICATIONS

Sara Darakhshan [*1, 2], Reza Tahvilian [2] and Abasalt Hosseinzadeh Colagar [3]

Department of Biology [1], Faculty of Science, Razi University, Kermanshah, Iran.
Department of Pharmaceutics [2], Faculty of Pharmacy and Pharmaceutical Sciences, Kermanshah University of Medical Sciences, Kermanshah, Iran.
Department of Molecular and Cell Biology [3], Faculty of Basic Sciences, University of Mazandaran, Babolsar, Iran.

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Correspondence to Author:
Sara Darakhshan

Department of Biology,
Faculty of Science, Razi
University, Kermanshah, Iran.

E-mail: darakhshan.sara@gmail.com

ABSTRACT: The medicinal plants have attracted great attention in recent years for better therapeutic management of diseases. *Nigella sativa* is one of the most extensively studied plants and has gained more and more interest in potential medical use in recent years. Historical and traditional uses of this herb have been widely documented in ancient documents; and *Nigella sativa* has been broadly used throughout the world for the promotion of health and the treatment of many acute, as well as, chronic conditions for centuries. The seeds of *Nigella sativa* have rich biological active compounds, and are used as a natural remedy to treat various ailments since it has a variety of pharmacological properties, which include anti-oxidant, anti-inflammatory, immuno-potentiating actions, anti-cancer, analgesic, anti-histaminic, anti-microbial, hypoglycemic, gastroprotective, nephroprotective, lung protective, cardioprotective, hepatoprotective, and neuroprotective. This plant also has positive effects on the reproductive system and skin diseases. On the other side, adverse effects and toxicity about the use of *Nigella sativa* were rarely reported. An ingredient isolated from seeds and evaluated mostly is thymoquinone, but since the beneficial effects of seeds not limited to this compound and it appears the all compounds in the seeds act synergistically; in this article, an attempt that the effects of *Nigella sativa are* addressed. In this review, we summarize and discuss the effects of *Nigella sativa* seeds in the treatment and prevention of various diseases. This review is mainly intended to provide a comprehensive knowledge regarding existing data of the pharmacological and toxicological actions of this plant.

INTRODUCTION: The use of plants as medicines dates from the earliest years of man's evolution, and there is a vast historical legacy regarding the use of plants in folk medicine.

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Herbal medicine is requiring special consideration due to their potential impact of people's health. Due to the widespread belief that "green medicine" is safe, the use of plant products is perceived as effective, better tolerated by patients, and less expensive [1]. In recent years, medicinal plants have stimulated the new interest of research in the light of scientific developments throughout the world, to find agents that are more efficacious. *Nigella sativa* Linn. (NS) is one of the most useful medicinal herbs that are considered a panacea to promote health and treat a wide range of diseases for more

than 2000 years. It has many common applications by people in the countries surrounding the Mediterranean Sea, the Arab world, Indians and Persians [2].

The historical tradition of black seed in medicine is substantial also religious. NS seeds were prescribed by ancient Egyptian and Greek physician to treat a variety of problems, including headache, nasal congestion, toothache, and intestinal worms, as well as a diuretic to promote menstruation and increase milk production [3]. In the Holy Bible is described as the Melanthion of Hippocrates and Dioscorides and as the Gith of Pliny [4], and later the Prophet Muhammad (PBUH) once stated the black seed could heal every disease except death [3]. It also suggested in "The Canon of Medicine" by Avicenna [5], as well Hebrew texts (lists of Materia Medica) found in the Genizah [6]. Avicenna refers to black seeds, as these stimulate the body's energy and helps recovery from fatigue [5].

The seeds of the plant, commonly known as black seed or black cumin, are the source of the active ingredients of this plant, and NS seeds is used in folk medicine as a natural therapy for a variety of ailments and symptoms such as headache, nasal congestion, toothache, fever, diarrhoea, dizziness, inflammation, cough, influenza, bronchial asthma, eczema, hypertension, diabetes, kidney and liver dysfunctions, lung diseases, rheumatism, parasitic infections, obesity, back pain, hyper-cholesterolemia, gastrointestinal disorders, and overall for general well-being, for more than two thousand years [7, 8]. It also has been shown to produce beneficial activities as a diuretic and galactagogue, as well for gynecological disorders, memory impairments, common cold, and joints stiffness [9, 10].

The nutritional and health improving properties of NS are very well known and NS is considered as an herbal food substance having diversified use for reducing the risk of maladies. Nowadays, a great deal of attention has been focused on black seed, and thus their consumption has increased in the world. Clinical and animal studies in recent years have shown that the NS extracts and its oil have a wide spectrum of medicinal properties including anti-oxidant, anti-inflammatory, immuno-modulatory actions, anti-cancer, analgesic, anti-histaminic, anti-microbial, anti-diabetic, gastro-protective, nephroprotective, pulmonary protective, cardio-protective, hepatoprotective, and neuro-protective. This plant also has a reproductive system and skin care assets. Thymoquinone is a constituent isolated from seeds, which accounts for most of the pharmacological properties of NS [7], but it appears the all compounds in the seeds act synergistically and a combination of these contributes to the pharmacological activities of seeds.

Therefore, it may be significant to use the whole oil or extract of the seeds in scientific studies [7, 11]. The present work is aimed at summarizing the valuable works done by several investigators on the effects of NS extracts, oil, and active principles. This review suggests a potential role of NS in the management of a vast array of problems; however, more studies should be conducted to evaluate their effectiveness. We hope this review will encourage interested researchers to conduct further pre-clinical and clinical studies to evaluate the positive activities of NS.

Characteristics and Chemical Components: *Nigella sativa* grows in the Mediterranean region, South Europe, Saudi Arabia, Syria, Turkey, Pakistan, India, and Iran13. NS is a 20-30 cm tall dicotyledon annual and herbaceous plant that belongs to the family of Ranunculaceae. It is a bushy, self-branching plant with white, pale yellow, pink or pale blue flowers. It reproduces with itself and forms a fruit capsule. The fruit is a large and inflated capsule composed of three to seven united follicles, each containing numerous white trigonal seeds. When the capsule has matured, it opens up, and the seeds contained within are exposed to the air, becoming black [14]. Seeds are angular, small size (1-5 mg) and dark grey or black color [15]. They taste slightly bitter and peppery with a crunchy texture. The seeds are employed as a spice and condiment also food preservative in various parts of the world. Both the seeds and its oil are used as nutritional supplements [7]. Seeds are added to some food products such as paste, pastry, cheese, pickles and bakery products for flavoring and preservation [16]. NS seed components have been used to prepare functional cosmetic and dietary supplement products. Commercial use of these seeds has recently been

extended too many products including shampoos, oils, soaps, *etc*. [15] *Nigella sativa* oil (NSO) also more yellow-colored than other vegetable oils and it can protect against UV light, which justifies its use in the cosmetic industry. NSO is very stable and could be conserved safely for a long time due to its considerable polyphenolic content [17]. Some properties of NS plant shown in **Table 1**, and **Fig. 1** is an illustration of whole NS plant, its flower, capsule, and seeds.

TABLE 1: SOME PROPERTIES OF *NIGELLA SATIVA* PLANT

Kingdom	Plantae
Order	Ranunculales
Family	Ranunculaceae (buttercups)
Genus	*Nigella*
Species	*N. sativa*
Common names	Black seed, black cumin, black caraway, panacea,blessed seed, Habbatu Albarakah, Habbah Al-Sauda, Kalanji, Al-Kammoon Al-Aswad, Shoneez, Khodhira, Sinouj

FIG. 1: A) THE *NIGELLA SATIVA* PLANT; B) THE FLOWER; C) THE PREMATURE FRUIT CAPSULE; D) THE MATURED FRUIT CAPSULE CONTAINS NUMEROUS SEEDS, AND E) THE SEEDS OF *NIGELLA SATIVA* (BLACK SEED)

The chemical composition of this species is very rich and diverse, and over 100 constituents have been identified, reported or isolated from seeds, though many of these have not been chemically identified nor have been pharmacologically tested. Seeds contain fixed oil (about 30%) and essential or volatile oil (VO) (0.5% w/w). This is also a rich source of proteins and amino acids (including eight of the nine essential amino acids), carbohydrates, alkaloids, organic acids, tannins, resins, mucilage, metarbin, glycosidal saponins, crude fiber, vitamins (Vitamin A, vitamin B, vitamin B_2, niacin, and vitamin C), enzymes and minerals [18, 19, 20]. The seeds are a good source of potassium, phosphorus, sodium, iron, zinc, calcium, magnesium, manganese, copper and selenium [21]. The fixed oil is composed mainly of unsaturated and essential fatty acids, arachidic (C20:2), palmitic (C16:0), stearic (C18:0), linoleic (C18:2) and oleic acids (C18:1) (both later cannot be synthesized in the body), while VO contains 18.4-24% thymoquinone and 46% monoterpenes such as *p*-cymene and pinene [22]. Four alkaloids have been isolated: nigellicine, nigellidine (indazoles), nigellimine and nigellimine N-oxide (isoquinolines) [7].

Three flavonoid glycosides and triterpene saponins were also identified from NS, together with four phospholipid classes: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol (from high to low level) [23]. By HPLC analysis of NSO, thymoquinone, thymo-hydroquinone, dithymoquinone (or nigellone; a dimer of thymoquinone), thymol, carvacrol,

nigellicine, nigellidine, and α-hederin are considered the main active ingredients [24, 25]. Thymoquinone (TQ) is the most abundant constituent of the VO, which is also present in the fixed oil and much of the biological activity of the seeds is due to it [7].

The composition and yield of NS oils differ depending upon geographical conditions (source), storage conditions, as well as the diverse methods employed for oil extraction. The common ways that have been used for delivery of NS extracts or NSO are orally [7, 11, 26, 27, 28], intravenously (i.v.) [29, 30], and intraperitoneally (i.p.) [31, 32, 33]. However, other methods also developed; NSO-PLGA microparticle [34], proniosome (niosomes) formulation [35] and biocompatible gold nanoparticles-NS extract [36]. LD_{50} values, obtained by single doses, orally and intraperitoneally administered in mice, were 28.8 ml/kg body weight and 2.06 ml/kg body weight, respectively[28].

Anti-oxidant, Anti-Inflammatory and Immuno-modulatory Effects: There is an increasing demand for anti-oxidants from natural sources, and nowadays they are gaining much attention. NS has been used for centuries for the treatment of many diseases, and amongst the effects of NS, at this time, the anti-oxidant and anti-inflammatory effects are the most prominent. One of the potential properties of NS seeds is its ability to reduce toxicity due to its radical scavenging and cytoprotective activities. Another side, experimental evidence demonstrated potent enhancing effects of NS and its active ingredients on immune responses.

Oxidative damage to biological structures has been involved in the toxicity-induced pathophysiology of some diseases[37]. In a study, Burits and Bucar[38] showed that thymoquinone, carvacrol, t-anethole, and 4-terpineol demonstrated respectable radical scavenging property. The essential oil and its constituents possessed anti-oxidant, but no pro-oxidant effect and the anti-oxidant activity of NSO is greater than that of TQ [38] showing that TQ is not the only radical scavenging composite in the oil. Minerals calcium, iron, copper, zinc, and phosphorous had chelating action, inhibited free radical generations by stabilization of transition metals thereby reduced free radical damage [39]. The fixed oil of NS inhibited non-enzymatic lipid peroxidation in ox brain phospholipid liposomes and had substantial free radical scavenging properties [40]. It inhibited both cyclooxygenase (COX) and 5-lipoxygenase pathways of arachidonic acid metabolism and membrane lipid peroxidation. However, the inhibition of eicosanoid generation and lipid peroxidation by NSO is greater than is expected from TQ, and showing that other components may contribute to its anti-oxidant and anti-eicosanoid activity [40]. In a study, thymol played a role as singlet oxygen quencher, while TQ and dithymoquinone showed superoxide dismutase (SOD)-like activity [41].

NS also had potential benefit in the prevention of toxicity from anti-cancer drugs or other cell-damaging agents through free radical scavenging activity and improved the antioxidant defense system. NS protected liver tissue from carbon tetrachloride (CCl_4)-induced toxicity [42, 43]. In gentamicin-induced nephrotoxicity, treatment with NSO produced scavenging free radicals activity including GSH level and the total antioxidant status in renal cortex [44].

Pretreatment of rats with NS undergoing ethanol-induced gastric ulcer caused an increase in GSH level [45]. NS extract also decreased the toxicity of cisplatin in mice [46]. NS supplementation reduced markedly immune system alterations induced by benzo(a)pyrene (B(a)P) [47]. NS has been shown to suppress the ferric-nitriloacetate-induced oxidative stress, so prevented renal carcinogenesis [48]. NS may suppress potassium bromate (KBrO3)-mediated renal oxidative stress and toxicity.

Treatment of rats with NS extract decreased renal microsomal lipid peroxidation, gamma-glutamyl transpeptidase (GGT), H_2O_2 and xanthine oxidase. There was the significant recovery of renal glutathione content and anti-oxidant enzymes [49]. NSO significantly reduced propoxur-induced oxidative stress in brain of rats via free radicals scavenging mechanism [50]. Pre-treatment of rats with NSO reduced the subsequent cyclosporine (CsA) injury in heart by improvement in anti-oxidant enzyme status. It caused an increase in the activities of SOD, catalase (CAT) and glutathione peroxidase (GPX) [51]. Also, NS had radio-protective effects against radiation-related oxidative stress and NS treatment meaningfully antagonized the effects

of radiation; therefore, NS may be a beneficial agent in protection against ionizing radiation-related tissue injury [52]. *Ex-vivo* treatment of mouse splenic lymphocytes with an ethanolic extract of NS before irradiation showed significant inhibition of the formation of lipid-peroxides and intracellular reactive oxygen species (ROS), which correlated with radiation-induced apoptosis.

Moreover, radiation-induced DNA damage was prevented in splenocytes pre-treated with NS ethanolic extract [52]. The liver tissue total oxidant status, lipid hydroperoxide level, and oxidative stress index were increased in the irradiated rats compared to the control rats, while total anti-oxidant status and sulfhydryl (-SH) levels were significantly declined. Treatment with NSO reduced oxidative stress markers, also enhanced the antioxidant capacity in the liver tissue of irradiated rats [53]. Also, NSO has been revealed to prevent oxidative injury during cerebral ischemia-reperfusion injury in rat [54].

Also, NS can attenuate the oxidative stress by increasing the expression of endogenous anti-oxidant content. After long-term administration of NSO, level of liver, kidney and cardiac enzymes in rats is normal [55]. NS increased activities of CAT, glutathione - S - transferase (GST), adenosine deaminase (ADA) and myeloperoxidase (MPO) by normalizing GSH and nitric oxide (NO) levels [56, 57]. Ilhan *et al*., [56] have reported the anti-epileptic effect of NS because of its ability to inhibit excessive ROS formation in pentylenetetrazol - induced seizures. They have reported that NSO elevated the level of GPX in pentylenetetrazol kindling seizures in mice compared to an untreated group. Oral administration of NSO (at 100 mg/kg body weight for 1 week) resulted in significantly increased levels of total antioxidant status in methionine-induced hyperhomocysteinemia in rats [58].

The NSO has also shown potent anti-inflammatory effects on some inflammation-based models through suppression of the inflammatory mediators. NS has been shown to have anti-oxidant/anti-inflammatory efficacy in models with asthma [59], tonsillopharyngitis [60], diabetes [61], and neuro-degeneration diseases [62]. Kalus *et al*., [26] reported that oral administration of NSO at 40-80 mg/kg/day for 8 weeks decreased the disease scores (*i.e.*, IgE and eosinophil levels) in patients with allergic rhinitis, bronchial asthma, and eczema. Administration of 100, 200, and 400 µl/kg doses of NSO injected intravenously considerably reduced the carrageenan-induced paw edema. The anti-inflammatory effect of NS is comparable to that of 100 mg/kg aspirin [62].

NS seems to be a promising candidate for nutritional interventions in humans with food allergy. Hexanic NS extract significantly decreased clinical scores of ovalbumin (OVA)-induced diarrhea and allergy-related immune markers. It decreased intestinal mast cell numbers and plasma mouse mast cell protease-1 (MMCP-1). NS extract significantly improved symptoms and immune parameters in murine OVA-induced allergic diarrhea [63]. Injection of NSO leads to a significant reduction in endotoxin shock, in response to lipopolysaccharide (LPS) administration [64].

In a placebo-controlled study, forty rheumatoid arthritis patients took two placebo capsules daily for 1 month. This was followed by a month of NSO capsules 500 mg twice/day. The disease activity score significantly decreased after receiving the NS capsules compared with before and after placebo. Likewise, the number of swollen joints and the duration of morning stiffness improved [65]. The effects of NS extract or its active ingredients on excessive inflammatory molecules may explain the effect of NS in ameliorating inflammatory diseases.

NS extract inhibited the production of 5-lipoxygenase products and 5-hydroxy-eicosa-tetra-enoic acid (5-HETE) production from poly-morphonuclear leukocytes. Nigellone caused a concentration-related inhibition of 5-HETE production. Inhibition of both COX and 5-lipooxygenase pathways is key factors mediating the anti-inflammatory effects of NS [66]. Thymo-hydroquinone, thymol, and TQ can participate in the general anti-inflammatory activity of NS. Thymol was the most active against COX-1, while thymohydroquinone and TQ exhibited the strongest inhibitory effect on COX-2 [67].

Moreover, NSO inhibited the histamine released from mast cells [45]. NSO and nigellone inhibited histamine release from peritoneal mast cells by different secretagogues; antigen sensitized cells,

compound 48/80 and the Ca^{2+}-ionophore A23187. Nigellone lowered intracellular calcium in mast cells by inhibiting its uptake and stimulating the efflux as well as by inhibition of protein kinase C (PKC), is involved in the NO/iNOS production [68]. Moreover, aqueous extract of NS had anti-histaminic effects on precontracted guinea pig tracheal chains [69]. Additionally, the fixed oil decreased the release of PGE2 and inhibited the release of leukotrienes and histamine from normal and sensitized guinea pig lungs [70]. Treatment with aqueous extractor boiled fraction of NS caused a dose-dependent decline in NO production when activated with LPS in murine macrophages [71].

Treatment with NSO in sensitized rats ameliorated allergic airway inflammation by inhibiting T-cell proliferation. It showed attenuation of eosinophilic inflammation associated with significant inhibition of elevated mRNA expression of IL-4 and IL-5, and reduction of delayed AHR[72]. The NS aqueous extract enhanced splenocyte proliferation. Also, it favored the secretion of Th2, versus Th1, cytokines by splenocytes. The secretion of IL-6, TNF-α and NO, by primary macrophages is significantly suppressed by the aqueous extract of NS, indicating that NS exerted anti-inflammatory effects [73].

The majority of subjects who treated with NSO for 4 weeks showed an increase in CD4 to CD8 T cells ratio and an increase in NK cell function [74]. Oral administration of NS aqueous extracts (one week) increased the number of splenic NK cells, and their cytotoxicity against YAC-1 tumor targets when compared with control NK cells [75]. NS extract can enhance macrophages' innate immune functions that could control infectious diseases and regulate adaptive immunity[76]. Dietary supplementation with NSO improved the immune response of healthy old subjects, which is mediated by a change in the factors closely associated with T cell activation [77].

Furthermore, NS significantly reduced the number of peripheral blood eosinophils, the elevated serum IgG1 and IgG2 levels and Th2 cytokine profiles [78]. The effect of the volatile oil was studied on the antigen-specific response induced by vaccinating rats with the typhoid TH antigen. In that study, treatment with NSO reduced antibody production in response to typhoid vaccination as compared to the control rats [79]. Moreover, administration of NSO commenced 6 weeks after induction of streptozotocin (STZ)-induced diabetes significantly induced beneficial effect, coincided with rise in the phagocytic activity of peritoneal macrophages, and lymphocyte count in peripheral blood compared with control diabetic hamsters [27], indicating to the potential of NSO to enhance innate immunity. Salem [11] in a review summarized the immuno-modulatory properties of the NS.

These results suggest that NS is an effective free radical scavenger and could find clinical application against a variety of damages caused by oxidative stress. It is useful in diseases in which free radicals are involved, *e.g.*, anoxia and ischemia of brain and heart as well as arteriosclerosis, EAE, arthritis, and cancer. The studies have shown that NS inhibits key inflammatory mediators, and thus, may be useful in ameliorating inflammatory and immune conditions. Given the potent anti-inflammatory effects of NS on different inflammatory disease models, future studies are required to explore the effects of NS on the expression of chemokines, cytokines, and inflammatory molecules. If further studies were done on NS, it may consider as possible non-steroidal anti-inflammatory drugs and would be translated to the clinical settings in humans.

Anti-Cancer Effects: The consideration of the use of naturally derived drugs as co-adjuvants in the treatment of cancer is of growing interest. While many anti-cancer agents have been developed, unfavorable side effects and resistance are serious problems. In fact, due to concerns about the toxic adverse effects of conventional medicine, the use of natural products as alternatives have been increasing. NS has been shown to exert biological activity on various types of human cancers. NS extract containing fatty acids were found to completely inhibit the ehrlich ascites carcinoma (EAC) in mice [80]. NS caused cytotoxicity to EAC, Dalton's ascites lymphoma ascites (DLA), and Sarcoma-180 cells (S-180 cells) [81]. NS extract exhibited significant cytotoxicity and growth inhibitory in the ethyl-acetate fraction against different classes of cancer cell lines, P388, Molt4, Wehi 164, LL/2, Hep G2, SW620 and J82, and non-toxic against human umbilical cord endothelial cells [82].

Alpha-hederin and TQ caused apoptosis in four human cancer cell lines, lung cancer: A549, pancreatic cancer: HEp-2, colon cancer: HT-29 and larynx cancer: MIA PaCa2, in a dose- and time-dependent manner [83]. Alpha-hederin isolated from CC-5 (column fraction 5) of NS ethanolic extract, when given i.p. for 7 days at doses of 5 and 10 mg/kg body weight to mice with formed tumors, produced significant tumor inhibition rate against implanted murine P388 leukemia and implanted LL/2 (Lewis lung carcinoma) cells in BDF1 mice [84]. The chloroform extract of NS induced apoptosis in HeLa cells, human cervical cancer.

This led to up-regulated the expression of p53, caspase-3,-8 and -9. [85] It decreased the fibrinolytic potential of the human fibrosarcoma cell line (HT1080) *in-vitro*, implying that inhibition of tumor invasion and metastasis may be one anti-cancer mechanism of NS. NSO had a role in modulating the balance of fibrinolysis/thrombus formation by modulating the fibrinolytic potential of endothelial cells [86]. Injection of NSO into the tumor site significantly inhibited solid tumor development as well as the incidence of liver metastasis, thus improving survival in 815 mastocytoma tumor-bearing mice [87]. The alcoholic and aqueous extracts are cytotoxic to breast cancer cells, MCF-7 line cell and anti-tumor activity of doxorubicin was enhanced, when NS extract was involved as an adjunct compound; so doxorubicin-NS lipid nanoemulsion may be a promising anti-canceroption [88]. Methanolic extract of NS induced apoptosis in SiHa human cervical cancer cells through both p53 and caspase activation [89].

In addition, to direct anti-tumor effects, NS preparations may have potential for cancer chemoprevention as well as for reducing the toxic effects of anti-cancer agents. NSO had chemopreventive effects on the induction and development of 1,2-dimethylhydrazine (DMH)-induced aberrant crypt foci (ACF) and putative pre-neoplastic lesions for colon cancer. It could inhibit colon carcinogenesis in the post-initiation stage in rats, and that the inhibition may be associated, with suppression of cell proliferation in the colonic mucosa [90]. NS also had a preventive influence against azoxymethane-induced genotoxic effects and colon cancer in rats [91]. NS has a chemopreventive effect on potassium bromate ($KBrO_3$), a potent nephrotoxic agent-mediated toxicity and tumor promotion response in rats [49]. It also suppressed ferric nitrilotriacetate (Fe-NTA)-induced hyperproliferative response and renal carcinogenesis. Treatment of rats orally with NS (50-100 mg/kg body weight) resulted in major reductions in DNA synthesis and incidence of tumors [48]. Moreover, topical application of the NS extract inhibited 7, 12-dimethylbenz[a]anthracene-initiated and croton oil-promoted skin papillomagenesis in mice. The dose of 100 mg/kg body weight of NS delayed the onset of papilloma formation [80].

NS ethanolic extract may be an anti-tumor therapeutic approach against diethylnitrosamine (DENA)-induced liver carcinogenesis. Serum NO, TNF-α, and IL-6 levels were significantly increased in rats treated with DENA. Significant up-regulation of liver iNOS mRNA and protein expression as well iNOS enzyme activity was also observed. Subsequent treatment with NS ethanolic extract considerably reversed these effects and improved the histopathological changes in malignant liver tissue, without any toxic effect [92]. It is now considered that chemoprevention by the use of natural dietary agents may represent a better chance to avoid the problems of cancer by delaying, preventing, or even reversing the development of tumor cells.

As a final point, NS and its active ingredients have been shown to play potent anti-tumor activities in different cell lines and animal tumor models, explaining the traditional use of NS in treating various types of cancer. Nevertheless, the exact molecular mechanisms of NS on different cancers should be investigated, besides there are no clinical data regarding the use of NS for cancer management.

Anti-Microbial Activities: In recent years, existing interest in plants as a natural source for anti-microbial drugs. In various studies, anti-bacterial, antiviral, anti-parasitic and anti-fungal effects of this plant and its ingredients have been proven. The NS extracts and oil had powerful anti-bacterial properties against diverse genus of bacteria. Abou-Zied [93] tested the effect of different concentrations of NSO against pathogenic bacteria, *Streptococcus pneumonia*, *Proteus vulgaris*,

Shigella dysenteriae, *Acinetobacter sp.*, *Salmonella typhi*, *Gemella spp.*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermis* and *Pseudomonas aerugenosa.* Diethyl ether extract caused concentration-dependent growth inhibition of gram-positive also gram-negative; *Escherichia coli*, *Bacillus subtilis*, *Listeria monocytogens*, *Shigella sp.*, *Salmonella sp.*, *Streptococcus faecalis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The oil was also found active against *Micrococcus lysodeikticus* and *Sarcina lutea* [94].

The extract successfully eradicated a non-fatal subcutaneous staphylococcal infection in mice when injected at the site of infection. Exposure of *Staphylococcus aureus*, *Pseudomonas aerugenosa* and *Escherichia coli* in concentrations of 250-400 μg/disc eradicated the microorganisms [2]. Fixed oil of petroleum ether extract of NS enhanced healing of staphylococcal-infected skin by reducing local infection and inflammation, bacterial expansion and tissue impairment [95].

NS had anti-bacterial effect against drug-resistant bacteria; *e.g.*, *Escherichia coli*, *V. cholera*, and *Shigella dysentriae* [96]. NS had an inhibitory effect on methicillin-resistant *S. aureus*. Some of methicillin-resistant *S. aureus* strains were sensitive to NS ethanolic extract at a concentration of 4 mg/disc [97]. Furthermore, the ether extract showed synergistic and additive anti-bacterial effect with antibiotics. It showed anti-bacterial synergism with streptomycin and gentamycin and showed additive antibacterial action with spectinomycin, erythro-mycin, tobramycin, doxycycline, chloramphenicol, nalidixic acid, ampicillin, lincomycin and co-trimoxazole combination [2]. Combination of TQ and thymohydroquinonewith ampicillin, cephalexin, chloramphenicol, tetracycline, gentamicin, and ciprofloxacin exerted synergism in *S. aureus* [98]. NSO entirely inhibited aflatoxins B1, B2, G1, G2 production [99]. In an *in-vitro* study, NS extract produced within 60 minutes, a 100% growth inhibition on some strains of *Helicobacter pylori* [100]. NSO is also reversed. *Pylori* - induced gastric mucosal injury [101].

NS has been reported to have activities against viruses. *In-vivo* treatment with NSO induced a striking anti-viral effect against murine cytomegalovirus (MCMV) infection. It decreased the number and cytolytic function of NK cells after infection, and increased numbers of macrophages; and $CD4^+$ T cells, also up-regulated the suppressor function of macrophages in spleen[31]. The anti-viral effect of NSO is associated with enhancing the response of CD4 and CD8 cells, and macrophages [31], enhancing their ability of IFN-γ production that is known to reduce mice more resistance to MCMV infection. NS also induced anti-viral activity against hepatitis B virus [31]. Treatment of typhoid-antigen-challenged rat with the volatile oil revealed an immunosuppressant action as evidenced by the substantial decreases in the antibody titer and the splenocytes and neutrophils counts [102].

Ethanolic extract of NS reversed the systemic inflammatory reaction to polymicrobial sepsis and thereby reduced multiple organ failure. NS treatment significantly decreased pro-inflammatory cytokine levels in serum; lipid peroxidase level, MPO activity, and pathological changes in lung tissues, in cecal ligation and puncture-induced sepsis, while considerably augmented GSH levels and SOD activity in the lung tissue. NS treatment after ligation and puncture potentially reduced mortality and minimized the histopathological changes in lung tissue, under sepsis conditions [103].

In addition, to its anti-bacterial and anti-viral effects, NS showed also anti-helminthic activity. Treatment with NS had a protective effect on the *Schistosoma mansoni*-infected mice.NSO was considered as a protective agent against the genotoxicity, evidenced by a reduction in the percentage of chromosomal aberrations and the incidence of chromosome deletions and tetraploidy induced as a result of schistosomiasis [104].

Administration of NSO markedly reduced the number of worm and egg burden, coincided with amelioration of the *Schistosoma*-induced liver fibrosis and changes in ALT, GSH, alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and SOD activity and/or content [105]; suggesting that the anti-schistosomal effect of NSO might be induced partly by its anti-oxidant effect. NSO possessed anti-nematodal properties comparable to those of piperazine and showed additive effects with praziquental [105], the drug for the treatment of schistosomiasis.

Studies revealed that NS has potent antifungal activity against some pathogenic fungi. Diethyl ether extract inhibited *Candida albicans*, a pathogenic yeast [94]. The aqueous extract of NS exhibited inhibitory effect against candidiasis. An inoculum of *Candida albicans* into mice produced colonies of the organism in the liver, spleen, and kidneys. To study the anti-fungal effect of the NS aqueous extract using this model, oral treatment of the infected mice once daily for 3 days starting 24 h after inoculation of *C. albicans* markedly inhibited the growth of the fungus in all studied organs, and these effects confirmed by histological examinations [106]. The ether extract of NS showed *in-vitro* inhibition of the growth of eight clinical isolates of dermatophytes (four species of *T. rubrum* and one each of *Trichophyton interdigitale*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, and *Microsporum canis*) [107].

In addition, the essential oil has potent anti-fungal effects on pathogenic dermatophyte strains; T. *mentagrophytes*, *M. canis*, and *M. gypseum* [108]. The hydroalcoholic extract of NS had anti-parasitic in patients with cutaneous leishmaniasis [109]. Methanolic extract of NS had anti-malarial activity against established malaria infection *in-vivo*. *Plasmodium yoelli nigeriensis* (*P. yoelli*) infection caused a significant increase in the levels of red cell and hepatic MDA, an index of lipid peroxidation in the mice. Serum and hepatic lipid peroxidation levels were increased in the untreated infected mice.

Furthermore, *P. yoelli* infection caused a major decrease in the activities of SOD, CAT, and GSH in tissues of the mice. NS extract reversed all changes to normal. Moreover, NS was found to be more effective than chloroquine, the reference drug, in parasite clearance and, in the restoration of altered biochemical indices by *P. yoelli* infection [110]. These results disclose that NS possesses anti-microbial effects against different pathogens, along with the potential of NS as a source for the production of new anti-microbial drugs. However, further studies are required to assess the mechanisms of the anti-microbial effects of NS, alone or in combination with other drugs, and on other bacterial, viral, and parasitic models to measure and validate its potential therapeutic effects.

Hepatoprotective Effects: The plants have been used in herbal medicine for the treatment of liver complications, and one of these plants is NS, which has been considered as a therapeutic for liver damages. NS was prescribed by Avicenna to treat jaundice and ascites, and in Unani Medicine, it is used as an anti-bilious [5, 111]. The protective effect of NS on liver injuries has been demonstrated by some experimental studies. Treatment with NS (at a dose of 5 ml/kg) has been reported to attenuate liver injury induced by carbon tetrachloride (CCl_4) in rats [112].

In CCl_4-induced toxicity, NSO decreased the elevated serum K^+ and Ca^{2+} levels, ameliorated the reduced RBC, WBC, and Hb levels [113], as well reduced the elevated liver enzyme levels, and increased the reduced anti-oxidant enzyme levels [114]. NS countered the elevations in serum ALT activity, oxidized glutathione level, and stress ratio caused by CCl4. NS ameliorated the reductions in GSH, NADPH-quinone oxidoreductase, and microsomal epoxide hydrolase, as well as the reductions in glutathione and cysteine levels produced by CCl_4 [115].

Fathy and Nikaido [92] showed the chemopreventive effects of NS that protect from DENA-induced hepatocarcinogenesis in rats. NS relieved the deleterious effects of ischemia-reperfusion (I/R) injury on the liver. Total antioxidant capacity in liver tissue was noticeably higher in the NS group. Total oxidative status, oxidative stress index and MPO in hepatic tissue were significantly lower in the NS group. NS treatment mitigated ALT, AST and LDH activities. Histological tissue damage was milder in the NS treatment group than that in the control group [116]. Moreover, bile-duct ligation-induced liver damage reduced by NS administration in rats. Data showed a decrease in GGT, ALP, AST, ALT, and LDH activities in the NS-treated rats when compared with bile-duct ligation group.

The NS-treated rats' tissue levels of total oxidant status, oxidative stress index, and MPO was significantly lower than that of the bile-duct ligation group. NS exerted a therapeutic effect on cholestatic liver injury in bile-duct ligated rats through attenuation of enhanced neutrophil infiltration and oxidative stress in the liver tissue

[117]. NSO may play a role against the hepatosplenic damaging caused by *S. mansoni* infection and reduced the number of worms especially in the liver. These effects may be induced partly by improving the immunological host system and with anti-oxidant effect [118].

Hypoglycemic and Anti-Diabetic Effects: Diabetes is a common chronic disease affecting millions of people worldwide. Hyperglycaemia, or high blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves, kidney, and blood vessels. NS seeds have been used traditionally for centuries aimed at treating diabetes. During the last years, several studies have shown that NS have anti-diabetic effects. It had great potential in the treatment of diabetic animals because of its combined hypoglycaemic [119] and immunopotentiating properties.

Houcher *et al*., [120] showed that the use of commercial oil significantly reduced blood glucose. After 10 days of treatment with NS aqueous extract, a significant diminution in serum glucose levels was shown. NS directly inhibited the electrogenic intestinal absorption of glucose *in-vitro*. The aqueous extract (0.1 pg/ml to 100 ng/ml) exerted inhibition of the intestinal absorption and sodium-dependent glucose transport across isolated rat jejunum [121]. This is together with the improvement of glucose tolerance and body weight in rats after chronic oral administration. Chronic oral administration *in-vivo* of NS improved glucose tolerance as efficiently as metformin.

Indeed, a histological study of kidney, pancreas and liver samples after aqueous extract administration over 6 weeks showed these tissues to be normal and healthy [122]. El-Dakhakhny *et al*., [123] suggested that the hypoglycemic effect of NSO and nigellone may be mediated by extrapancreatic actions rather than by stimulated insulin release. Oral administration of NSO commenced 6 weeks after induction of STZ-induced diabetes (at a dose of 400 mg/kg body weight by gastric gavage) induced beneficial effect, coincided with elevation in the phagocytic activity of peritoneal macrophages, and lymphocyte count in peripheral blood compared with untreated diabetic hamsters.

Hepatic glucose production from gluconeogenic precursors (alanine, glycerol, and lactate) was significantly lower in treated hamsters. These indicated that the NSO hypoglycemic effect is due to, at least in part, a decrease in hepatic gluconeogenesis and that the immunopotentiating effect is mediated through stimulation of macrophage phagocytic activity either directly or via activation of lymphocytes [27]. Awadi *et al*., [124] found that a mixture of medicinal plants including NS, decrease hepatic gluconeogenesis through decreasing the activity of phosphoenolpyruvate carboxykinase and pyruvate carboxylase enzymes in STZ-induced diabetic rats. So, that the hypoglycemic action of NSO is mediated through a combined action of decreasing hepatic gluconeogenesis and activating pancreatic beta-cells with an increase in serum insulin level.

Farrah *et al*., [125] demonstrated that NSO exhibit a significant increase in serum insulin levels and hypoglycemic effect in STZ plus nicotinamide-induced diabetic hamsters. They also suggested that the observed decline in glucose after the first week of treatment with NSO might be due to decreased hepatic gluconeogenesis. However, after activation of beta-cells (after 4 weeks) in response to increased insulin levels, a meaningful decrease in glucose levels to normal was observed, which could be due to the combined action of decreased hepatic gluconeogenesis and activation of beta-cells. Treatment of STZ-diabetic rats with NS aqueous extract suppressed the pancreatic tissue lipid peroxidation, MDA levels and increased the level of SOD.

STZ-diabetes-induced an increase in heart and brain NO and MDA, also decrease in GST, GSH and CAT concentrations and these changes were reversed by post-treatment of rats with NSO. Serum cardiac creatine kinase muscle and brain types (CK-MB) was decreased in the diabetic rats, which recovered with NSO administration. During diabetes, there was a marked rise in norepinephrine and dopamine concentrations and a patent decrease in serotonin level; these were reversed by oral administration of NSO [126]. NS protected and preserved beta-cell integrity by decreasing oxidative stress. Thus, the anti-diabetic action of NS could be due to, in part, amelioration of the cellular and subcellular structures of beta-cells [61].

NS petroleum ether extract significantly lowered fasting plasma levels of insulin and triglycerides and normalized high-density lipoprotein (HDL)-cholesterol. Treatment of normal rats with the petroleum ether extract of NS (intragastric gavage) for 4 weeks induced a transient initial weight loss and a sustained reduction in food but not water intake; also, it had insulin-sensitizing actions. The decrease of blood glucose was associated with a significant reduction in insulin resistance. *In-vivo* NS treatment resulted in greater activation of MAPK and PKB in response to insulin [127].

Diabetes is usually associated with many problems. Osteoporosis is a major complication in patients with diabetes mellitus. NS might be used similarly to insulin as a safe and effective therapy for diabetes and might be useful in the treatment of diabetic osteopenia. NS had a greater anabolic effect on STZ-induced osteopenia by dramatically increasing the bone formation rate [128]. A study found that combined treatment of NS and parathyroid hormone was more effective in reversing the osteoporotic changes and in improving the bone strength of STZ-induced diabetic rats than either treatment alone [129]. NS may have potential use against obesity and the metabolic syndrome. Ethanolic extract induced an insulin-like stimulation of glucose uptake in C2C12 skeletal muscle cells and 3T3-L1 adipocytes [130].

It exhibited the remarkable ability *in vitro* to concomitantly increase insulin secretion, induce proliferation of pancreatic beta-cells, and stimulate glucose uptake in skeletal muscle and fat cells. NS ethanolic extract in C2C12 skeletal muscle cells as well as in H4IIE hepatocytes, increased activity of Akt, a key mediator of the effects of insulin, and activity of AMP-activated protein kinase (AMPK), a master metabolic regulating enzyme [131]. STZ induced a noteworthy decrease in the area of insulin immunoreactive beta-cells, and NS treatment resulted in an increasing area of insulin immunoreactive beta-cells considerably. NS has been associated with the recovery of the histopathologic changes in sciatic nerves three, and myelin breakdown decreased after treatment with NS in diabetic rats [132].

NS markedly increased body weight gain. Histopathological examination showed that the NS (5 mg/kg body weight) partially recovered hepatic glycogen content and protected a great deal of the pancreatic islet cells. The number of islets, cells and islets diameter were found statistically significant when compared to the control. The hydroalcoholic extract of NS at low doses had a hypoglycemic effect and ameliorative effect on regeneration of pancreatic islets. This effect could be due to the amelioration of beta-cell, thus leading to increased insulin levels [133].

These results indicate that NS affects blood glucose, insulin level and overall has hypoglycemic effects. These findings might provide a scientific validate for the evidence that NS seeds are widely used as an anti-diabetic remedy in Middle East folk medicine [119]. Consequently, NS may prove clinically useful in the treatment and management of diabetes and diabetic complications.

Neuro-Protective Effects: Many beneficial neuro-protective properties of NS have been reported. ROS are implicated in the mechanism of several neurodegenerative diseases, therefore, NS could be used as a possible remedy for cognitive disorders. People in different parts of the world take NS alone or oil of NS with either honey or boiled mint for various health benefits including memory improvement [134]. A survey showed the positive modulating impact of NS on memory, attention, and cognition in healthy elderly volunteers [135]. Another study showed chronic oral administration of NS could enhance the consolidation and recall capability of stored information and spatial memory in rats [136].

The hydroalcoholic extract of NS have protective effects on hypothyroidism-associated learning and memory impairment during neonatal and juvenile growth in rats; the effects might be due to the protective effects of NS extract against brain tissue's oxidative damage [137]. NS had anti-cholinesterase properties. The hydroalcoholic extract of NS prevented scopolamine-induced spatial memory deficits and decreased the acetylcholinesterase activity as well as oxidative stress of brain tissues in rats [138].

The methanolic extract of NS modulated the neuronal release of amino acid neurotransmitters including GABA, glycine, aspartate, and glutamate

at the synaptic terminals on cultured cortical neurons [139]. The sedative and depressive effects of NS could be based on changes of inhibitory/ excitatory amino acids levels. NS dry methanolic extract modulated amino acid release in primary cultured cortical neurons; GABA was significantly increased whereas secretion of glutamate, aspartate, and glycine was diminished. The sedative effect of the methanolic extract was due to the release of GABA and glycine in the cultured neuron medium, and therefore, exerted an increase in the agonist action over their receptors [139]. Al-Naggar *et al*., [140] studied the effects of aqueous and methanolic extract of NS on the central nervous system (CNS) in mice.

The results showed that it produced a sedative and depressive effect on CNS, significant reduction of spontaneous motility and decrease in body temperature. Administration of NSO produced anti-nociceptive effects through indirect activation of the supraspinal μ1- and κ-opioid receptor subtypes. The NSO (50-400 mg/kg) dose-dependently suppressed the nociceptive responses caused by thermal, mechanical and chemical nociceptive stimuli in mice; and that the supraspinal opioid systems, particularly μ1- and κ-opioid receptor subtypes are involved in the antinociceptive action of NSO [141].

In pentylenetetrazole and ciprofloxacin treated rats, the oral administration of NS prevented an elevation in aspartate and glutamate contents, whereas the levels of GABA and glycine were increased. The treatment with NS was found to ameliorate neurological defects which may lead to the initiation of epileptic seizures that reflect its potent anti-epileptic activity [142]. Akhondian *et al*., evaluated the efficacy of the aqueous extract of NS in reducing the frequency of seizures in childhood refractory epilepsy.

This study was a double-blinded crossover clinical trial conducted on children with refractory epilepsy. The extract was administered as an adjunct therapy, and the effects were compared with those of a placebo. They received extract (40 mg/kg/8 h) or placebo for four weeks. The mean frequency of seizures decreased significantly during the treatment with the extract so the water extract of NS had anti-epileptic effects in children with refractory seizures [143]. NS could prevent the cerebral edema in neurons and supportive neuronal tissue of hippocampus; consequently was able to prevent/R in the hippocampus tissue [144].

Aqueous and hydroalcoholic extracts of NS (400 mg/kg) for 7 days were administered orally showed neuroprotective effects on focal cerebral ischemia in middle cerebral artery occlusion (MCAO) model rats. MCAO followed by reperfusion is a model of focal ischemia in rats, which resembles that of stroke inhuman. Pretreatment with both extracts showed improvement in grip strength, and infarct volume was reduced. An elevation of thiobarbituric acid-reactive substances (TBARS) and a reduction in GSH, SOD, and CAT levels were observed following MCAO; and pretreatment of NS extracts showed the reduction in TBARS, elevation in glutathione, SOD and CAT levels.

The neuroprotective effects could be due to its free radical scavenging & anti-inflammatory properties [145]. Additionally, NSO protected hippocampal cells of rats subjected to transient cerebral ischemia via four-vessel occlusion procedure for 20 minutes, and I/R model of brain insult. Pretreatment with NSO was resulted in a significant decrease in MDA level and have protective effects on lipid peroxidation during I/R injury in hippocampus [54]. Neuroprotective effects of NS in an experimental model of spinal cord injury in rats have also been attributed to its antioxidant capability. NSO treatment decreased tissue MDA and protein carbonyl levels and prevented inhibition of SOD, GPX, and CAT enzyme activities following experimental spinal cord injury. The morphology of neurons in NS-treated groups was well protected, and the number of neurons in the spinal cord tissue was meaningfully more than the spinal cord injury tissue [146].

NSO could protect brain tissue against tramadol-induced tolerance and dependence in mice. Administration of NSO (4 ml/kg, orally) along with morphine (5 mg/kg, S.C.) attenuated the development of tolerance [147]. It appears to have therapeutic potential in opioid tolerance and dependence through blockade of NO overproduction and oxidative/nitrosative stress induced by morphine [148]. Interaction of NS with the neurotransmitters such as dopamine, glutamate,

acetylcholine, GABA, histamine, and NO on the rewarding properties of morphine has been reported [139, 148]. The aqueous extract of NS suppressed penicillin-induced epileptic activity in rats. This anti-convulsant effect is a consequence of selectively altering the monoamine level in different brain regions [149].

NSO reduced propoxur (a carbamate insecticide)-induced toxicity and oxidative stress in brain regions [50]. NS extract was shown to protect against cisplatin-induced myelosuppression in mice [46]. NS therapy caused a morphologic improvement on neurodegeneration in the hippocampus after chronic toluene exposure in rats [62]. Significant histological improvement was found in the morphology of cerebral cortex and brain stem sections of rats chemically intoxicated with toluene upon using NS treatment [150]. According to these results, NS can widely use in nerves disorders like ischemia, Parkinson and Alzheimer disease, also depression and pain, and for memory enhancement.

Gastroprotective Effects: In the ethno-pharmacology, NS seeds are used for healing various gastrointestinal disorders. El-Dakhakhny *et al*., found that NSO had a gastroprotective action in an ethanol-induced ulceration model. NSO increased mucin production and protected against ethanol-induced ulcer in rats. When animals were pretreated with NSO before induction of ulcer, there was a significant increase in glutathione level, mucin content and free acidity, and a major decrease in gastric mucosal histamine content [45]. NSO as an anti-ulcer agent acted against acute alcohol-induced gastric mucosal injury in rats [151]. NS could protect the gastric mucosa against the injurious effect of absolute alcohol and promote ulcer healing as evidenced by the ulcer index values. The NS treatment decreased the number of mast cells as well as the area of gastric erosions.

Gastric tissue histamine levels and MPO activities were found to be increased in ethanol-treated rats, and NS reversed these rises [152]. NS prevented an alcohol-induced increase in TBARS, an index of lipid peroxidation. NS also increased gastric GSH content, enzymatic activities of gastric SOD and GST. It also affected CAT activity in gastric tissue [151]. NSO had beneficial effects on rats with necrotizing enterocolitis and reduced the severity of intestinal damage. Apoptosis and severity of bowel damage were significantly lower in the necrotizing enterocolitis plus NOS. Tissue GPX and SOD levels were preserved in the NSO treated group, whereas, tissue MDA, MPO levels of the NSO treated group was significantly lower than those in the necrotizing enterocolitis group [153].

NS-EA 51, a fraction of NS successfully prevented the histamine effect on gastric juice volume, pH, acid-output, ulcer formation, and pepsin activity. Fraction also inhibited gastric ulcer formation induced by hypothermic-restrained stress. The anti-ulcer effects of NS-EA 51 found comparable to the Famotidine, a reference anti-ulcer agent [154]. NSO had a marked protective action against I/R-induced gastric mucosal lesions, an effect that was associated with suppression in the levels of lipid peroxidase and LDH, and an increase in GSH and SOD levels [155]. The volatile oil of NS may block the Ca^{2+} *via* voltage-dependent and receptor-operated Ca^{2+}-channels.

It inhibited the spontaneous movements and acetylcholine-induced contractions of rabbit and guinea pig intestines [156]. NSO was also reported to produce a marked inhibition in the release of leukotrienes, which cause mucosal tissue injury and hypoxemia [157]. Furthermore, the high content of polyunsaturated fatty acids in NSO [40] could protect the gastric mucosa by increasing the bioavailability of arachidonic acid, resulting in the biosynthesis of the cytoprotective prostaglandins in the stomach [158]. Administration of the aqueous extract in a dose of 2 g/kg orally to rats induced significant protection against aspirin-induced increases in the volume of the gastric juice, the acid output, and the gastric ulcers [159].

Nephroprotective Effects: Some studies have reported the nephroprotective effects of NS toward kidney damages. NS protected against agents-induced nephrotoxicity. It also attenuated a variety of renal toxicities that are the consequence of oxygen free radical damages. Administration of NS extract decreased cisplatin toxic side effects, which is a chemotherapeutic drug poisonous to the kidney, on the kidney of rats [46]. Co-treatments gentamicin with NS considerably decreased renal damage. NS acted in the kidney as a potent to prevent the toxic effects of gentamicin in both

biochemical and histopathological parameters [160]. In gentamicin-induced toxicity, treatment with NSO produced scavenging free radicals activity, including GSH level and the total antioxidant status in renal cortex [44]. NSO also was found to inhibit CsA-induced oxidative stress in rat kidney [161].

CsA caused deterioration in the renal function, morphology and gave rise to severe oxidative stress in the kidney. NSO significantly improved the functional and histological parameters and attenuated the oxidative stress induced by CsA. Urine and serum creatinine levels, tissue SOD, GPX and CAT enzyme activities, and NO and MDA levels reverse to normal levels[161]. Oral administration of NS had a protective effect on renal toxicity-induced by potassium bromate (KBrO3) thought to diminish of oxidative stress that coincided with the reversal of renal glutathione content. Prophylaxis of rats orally with NS extract (50 mg/kg and 100 mg/kg body weight) resulted in a considerable decrease in renal microsomal lipid peroxidation, GGT, H2O2 and xanthine oxidase. There was also a reversal in the enhancement of blood urea nitrogen, serum creatinine, renal ornithine decarboxylase activity, and DNA synthesis [49].

NS has a chemopreventive effect against ferric nitrilotriacetate (Fe-NTA)-induced renal oxidative stress, hyperproliferative response, and renal carcinogenesis. Treatment of rats orally with NS (50 and 100 mg/kg body weight) resulted in a decline in GGT, lipid peroxidation, xanthine oxidase, H_2O_2 generation, blood urea nitrogen, serum creatinine, renal ornithine decarboxylase activity and incidence of tumors [92]. Renal ischemia followed by reperfusion leads to acute renal failure in both native kidneys and renal allografts. NS moreover had a protective effect against renal I/R injury in rat kidneys. NS was effective in reducing serum urea and creatinine levels as well as decreasing the tubular necrosis score. NS reduced total oxidant status levels and increased total antioxidant capacity levels in both kidney tissue and blood [162].

Pre- and post-treatment with NSO produced a reduction in serum levels of blood urea nitrogen and creatinine caused by I/R and significantly improved serum enzymatic activities of SOD and GPX and also tissue enzymatic activities of CAT, SOD, and GPX. NSO treatment resulted in a significant reduction in serum and tissue MDA, NO and protein carbonyl content that were increased by renal I/R injury. The kidneys of untreated ischaemic rats had a higher histopathological score, while treatment with NSO nearly preserved the normal morphology of the kidney [163]. Ethanolic extract of NS may also inhibit renal calculi induced by ethylene glycol and improved poultry quality[129].

Cardio-vascular Protective Effects: Cardio-vascular diseases are one of the major cause of death worldwide. NS has been used as a traditional remedy for circulatory diseases including hyper-tension. Results suggest an important therapeutic use of NS seeds in the prevention of cardiovascular ailments.

The VO seemed to possess the potential of being a potent centrally-acting antihypertensive agent. Intravenous administration of the volatile oil in doses of 4-32µl/kg induced dose-dependent increases in the respiratory rate and increased the intra-tracheal pressure. These effects of NS were reversed by cyproheptadine (a non-selective serotonin receptor blocker) and atropine (anti-muscarinic M2 agent). The results suggested that VO-induced cardiovascular depressant effects were mediated *via* mechanisms that involved both serotoninergic and muscarinic mechanisms [30].

In a double-blind, randomized study, 70 healthy volunteers randomly allocated to receive 2.5ml NSO or placebo two times a day for 8 weeks. Results showed that in NSO-treated group the systolic and diastolic blood pressures decreased significantly compared with baseline and placebo group at the endpoint. Also, no adverse effects were reported [164]. A study conducted a randomized, double-blind, and placebo-controlled trial to check the efficacy of oral NS extract supplement in patients with mild hypertension and results suggested that the daily use of NS for 2 months may have a blood pressure lowering effect [165].

Supplementation with NS (800 mg/kg orally for 12 weeks) can provide sufficient protection for the myocardium against I/R insult. The NS-treated group showed enhanced post I/R contractile and vascular recovery, which was accompanied by

elevated NAD^+ and decreased MDA. NS afforded substantial recovery of the post I/R cardiac functions *via* inhibition of mitochondrial permeability transition pore (MPTP) opening, which finally results in cardiomyocyte death[166]. El-Bahai *et al*., reported evidence of physiological and beneficial effects of long-term NS supplementation on cardiac hypertrophy in rats [167]. A study showed a potent inhibitory effect of aqueous and macerated extracts from NS on both heart rate and contractility of a guinea pig heart.

It may be due to calcium channel inhibitory or an opening effect for NS on potassium channels of the isolated heart [168]. NS had smooth muscle relaxant [69] and ameliorative effect of endothelial dysfunction [58]. It seems that the most important mechanisms involved in vasorelaxation of NS extract are inhibition of extracellular Ca^{2+} influx, blockade of KATP channels, also suppression of IP3-mediated receptors [169]. NSO was found to contain a modulator of Na^+/K^+-ATPase. Oleic and linoleic acids isolate from NS have a specific effect on ouabain interaction with the pump, which may be an element in the blood pressure lowering effect of these fatty acids. In Na^+/K^+-ATPase, K^+-dependent reactions were strongly modified after treatment by oleic and linoleic acids [170].

Pretreatment with NSO reduced the subsequent CsA injury in rat heart, demonstrated by normalized cardiac histopathology, and improvement in anti-oxidant enzyme status [51]. The state of hyperhomocysteinemia (HHcy) appears to be associated with higher risks of coronary, cerebral and peripheral vascular disease as well as with some other clinical conditions. Pretreatment with NSO produced strong protection against the development of methionine-induced HHcy and its associated state of oxidative stress [58]. Clinical studies should clarify if this plant is operative on the cardiovascular system, but overall it seems that NS can contribute to prevention and treatment of cardiovascular complications.

NS and Respiratory Diseases: In traditional medicine, NS seeds are used to treat a wide range of respiratory diseases including diarrhea, bronchitis, and asthma [171]. Therapeutic effects including the effect on asthma and dyspnea have been described for the seeds of NS in Iranian ancient medical books [172]. Mahfouz *et al*., [173] investigated the effectiveness of seeds' extract on the suppression of cough and bronchial asthma in adults.

The crude extract of NS exhibited spasmolytic, and bronchodilator activities mediated possibly through calcium channel blockade and this activity is concentrated in the organic fraction. NS boiled extract had a potent anti-asthmatic (broncho-dilatory) effect on airways of asthmatic patients [174]. Boiled extract of NS had a prophylactic effect on asthmatic disease. The asthma symptoms, frequency of asthma symptoms/week, chest wheezing, and pulmonary function tests values in the study group significantly improved [175].

Topical application of NSO was effective in the treatment of allergic rhinitis, with minimal side effects. After the 6 weeks treatment course, all of the patients in the mild active group became symptoms free; while in the moderate active group, 69% became symptoms free and 25% were improved; although in the severe active group, 58% became symptoms free and 25% were improved. Also, 92% of total patients in the active group demonstrated improvement in their symptoms or were symptoms free, and at the end of 6 weeks of topical use, the improvement in tolerability of allergen exposure in the active group was significant [176]. Nigellone suppressed symptoms in the majority of patients suffering from bronchial asthma when given orally [70].

Kalus *et al*., used a high dose of NSO (40 to 80 mg/kg/day) in 152 patients with allergic rhinitis and asthma and found that complaints related to allergic rhinitis decreased noticeably. NSO also decreased the IgE and eosinophil count. In this report, 80% of the cases had improvement of allergic rhinitis symptoms compared to those who received placebo [26]. In another clinical study, 66 patients with allergic rhinitis exposed to NSO. The results showed that NS could reduce the presence of nasal mucosal congestion, nasal itching, runny nose, sneezing attacks, turbinate hypertrophy, and mucosal pallor during the first two weeks. Moreover, NS should be considered for treating allergic rhinitis when the effects of other anti-allergic drugs need to be avoided [177].

The VO of NS, with TQ removed, acted as a central respiratory stimulant in guinea pigs [29]. NSO-induced respiratory effects were mediated via direct involvement of histaminergic mechanisms and indirect activation of muscarinic cholinergic mechanisms. Intravenous administration of VO induced dose-dependent increases in the respiratory rate and the intratracheal pressure in urethane-anesthetized guinea pig [29]. NS treatment inhibited the pulmonary inflammatory responses and reduced peribronchial inflammatory cell infiltration, alveolar septal infiltration, alveolar edema, alveolar macrophages, interstitial fibrosis, and granuloma formation in different pulmonary aspiration models. It indicated a significant reduction in the activity of iNOS and a rise in surfactant protein D in lung tissue after NS therapy [178].

NSO notably reduced the severity of lung damage due to hyperoxia. The severity of lung damage was lower in the hyperoxia plus NSO group. Tissue GPX and SOD levels were significantly preserved; MDA and MPO levels were lower in the hyperoxia adding NSO group [179]. Oral treatment with NSO showed a major decrease in airway hyper-responsiveness in bronchial asthma, the number of total leukocytes, macrophages, and eosinophils, levels of IL-4, -5 and -13 in bronchoalveolar lavage fluid, serum levels of total IgE, and a significant increase in the level of IFN-γ, indicating restoration of local Th1/Th2 balance. Furthermore, it significantly abrogated the histopathological changes of the lungs to nearly normal [180].

Pretreatment of sulfur mustard-exposed animals with NS, dexamethasone and their combination prevented increased tracheal responsiveness to methacholine, which was more pronounced in pretreated with both NS and dexamethasone. These results indicated a preventive effect of NS only and in combination with dexamethasone on tracheal responsiveness and lung inflammation of sulfur mustard-exposed guinea pigs [181]. Also, NS had an inhibitory effect on histamine (H1) receptors [182], inhibitory effect on calcium channels [183] and opening effect on potassium channels [69] in guinea pig tracheal chains. These studies indicate that NS treatment might be beneficial in respiratory diseases and supporting the use of it's in clinical phase. It could be a promising adjuvant therapy for bronchial asthma and allergic rhinitis in humans.

Effects of NS on the Reproductive System: NS is one of the components of traditional Iranian remedies for the treatment of infertility[184]. NSO had favourable effects on abnormal semen parameters in male fertility in normal and hyperlipidemic rats [185]. NSO improved abnormal semen quality in infertile men without any adverse effects. The patients (n=34) in NSO group received 2.5 ml NSO, and the placebo group received 2.5 ml liquid paraffin two times a day orally for two months. Sperm count, motility and morphology, and semen volume, pH and round cells were improved significantly in NSO treated group compared with the placebo group after this period[186].

The volatile oil inhibited the spontaneous movements of rat and guinea pig uterine smooth muscle also the contractions induced by oxytocin stimulation. These effects were concentration-dependent and reversible by tissue washing [187]. The administration of NS was effective in reversing tissue damage induced by ischemia and I/R in ovaries. The 500 mg/kg dose of NS before I/R reversed the trend in MDA and GSH levels, MPO and SOD activity. Ischemia and I/R increased the serum levels of IL-1β, IL-6, and TNF-α, while the administration of NS decreased the serum levels of this cytokines [188]. Ovariectomized rats showed a significant reduction in plasma Ca^{2+}, accompanied by a significant increase in plasma ALP, amino-terminal collagen type 1 telopeptide, MDA, nitrates, TNF-α and IL-6. These changes were reversed by NS supplementation in the ovariectomized-NS group.

Histological examination of the tibias revealed discontinuous eroded bone trabeculae with widened bone marrow spaces in ovariectomized rats accompanied by a decrease in both cortical and trabecular bone thickness. These parameters were markedly reversed in rats treated with NS. Histological examination of the liver showed mononuclear cellular infiltration and congestion of blood vessels at the portal area in ovariectomized rats, which were not found in NS-treated rats [189].

NS is used orally as a galactagogue in traditional medicine (during lactation time increase milk production in nursing mothers) [190]. However, no scientifically valid clinical trials support this use,

and no data exist on the safety and efficacy of NS in nursing mothers or infants.

Anti-Dyslipidemic Effects: Oral administration of 2 mg/kg of NS for 12 weeks decreased triglyceride and cholesterol levels [28]. In a survey, TQ and limonene showed hypolipidemic activities in atherogenic suspension fed rats. The oral feeding of 100 mg methanolic extract or 20 mg VO per rat/day effectively reduced the plasma triglycerides to a normal level, while HDL-cholesterol and its subfraction along with arylesterase activity levels were significantly increased.

The test fractions elicited a significant decrease in hepatic HMG-CoA reductase, a key enzyme in the cholesterol biosynthesis, activity. Methanolic extract possessing omega-6, linoleic acid along with palmitic acid was more effective than VO containing thymol and is thymol, in the reduction of hepatic HMG-CoA reductase activity as well as anti-oxidant mechanisms. This hypolipidemic and anti-atherogenic agent may be used in the protection of free radical-induced oxidative damage, hyperlipidemia / dyslipidemia, and atherosclerotic complications [191].

NS was evaluated for its hypolipidemic effects among menopausal women, and the results showed that NS improved lipid profiles. It was shown noteworthy decrease in total cholesterol, low-density lipoprotein (LDL) and triglycerides, and increase in HDL among menopausal women receiving NS powder at a dose of 1 g daily for two months compared to placebo group [192]. In treatment with NSO, the serum cholesterol and triglycerides levels decreased. In parallel, the significant slowdown of the body weight evolution was observed in NS-treated animals [193]. NS petroleum ether extracts considerably lowered fasting plasma levels of triglycerides and normalized HDL-cholesterol [127].

Hypercholesterolemia is the most important risk factor for atherosclerosis. In a randomized, placebo-controlled clinical trial, a patent dwindle was observed in the concentration of total cholesterol, LDL and triglycerides [194]. In CCl_4 hepatotoxicity, NSO showed a favorable effect on the serum lipid pattern where the oil administration (800 mg/kg orally for 4 weeks) caused a significant decrease in serum total cholesterol, LDL, triglycerides and an elevation of serum HDL level [195]. Oral pretreatment of rats with NSO protected against methionine-induced hyperhomocysteinemia (HHcy) through amelioration of the plasma levels of triglycerides, lipid peroxidation, cholesterol [58]. Concluding these data, NS may have positive therapeutic effects in the treatment of dyslipidemia and related abnormalities.

Safety and Adverse Reactions: About every drug or substance, the safety assessment is required before recommending its use. The various kind of seed extracts, oil and its constituents of NS are characterized by a very low degree of toxicity [7]. Studies indicated that the administration of NS by oral or intraperitoneal route has a low level of cytotoxicity in rats and mice [32, 33]. No evidence of NSO toxicity was observed, when administered in different doses up to 10 ml/kg body weightorally [33]. Chronic oral administration of NS improved glucose tolerance and reduced body weight without any toxic effect. Indeed, kidney, pancreas and liver tissues after chronic administration of aqueous extract over 6 weeks showed normal and healthy histology [122].

The high values of oral and intraperitoneal LD50 of NSO (LD_{50} value = 28.8 ml/kg body weight, orally; LD_{50} value = 2.06 ml/kg body weight, i.p.) show its low acute toxicity; and the key hepatic enzymes stability and organs integrity during and after 12 weeks of daily treatment show its low chronic toxicity [196]. Significant histopathological modifications in organs (heart, liver, kidneys, and pancreas) of NS-treated rats were not shown. Key hepatic enzyme concentrations did not change noticeably, while serum glucose, triglyceride and cholesterol levels reduced pointedly and HDL concentration increased, in NS-treated rats [28].

Tauseef *et al*., indicated that fixed oil 4.0% and essential oil 0.30% are safe as serological indices, like liver and kidney function tests, serum protein profile, level of cardiac enzymes and electrolytes balance remained in the normal ranges even after 56 days of study. Moreover, diets were insignificant in their impression regarding organs to body weight ratio [55]. Injection of an emulsion of NSO induced considerable reduction in endotoxin shock in response to LPS [64]. Also, externally in an

ointment form, the anti-inflammatory activity of NS was found to be the same range as that of other similar commercial products without induction of skin allergy [197]. On the other side, there are some data that indicated negative effects. The use of NSO in high doses and prolonged duration might be unsafe due to the presence of some toxic components such as glucoside in its oil [28]. The aqueous extract may induce chronic toxic effect and muscle relaxant effect at high dose [28, 187]. The changes in hemoglobin metabolism and the fall in leukocyte and platelet count also must be taken into consideration in NS treatments [28]. El-Shabrawy and Nada [198] reported no toxic symptoms in mice or rats after oral administration of NS extract doses up to 25 g/kg.

However, when 25 g/kg was injected either rats or mice, some toxic symptoms were observed and examination revealed circulatory failure as the most likely cause of death. Khader *et al*., reported that NS aqueous extract exhibit a clastogenic potential in primary rat hepatocyte cultures. TQ, like other quinone compounds, can be considered to be a redox-cycler which is metabolized to hydro-quinones or semiquinone radicals by cellular oxidoreductases leading to the production of ROS. Via this mechanism, TQ could lead to adverse effects and thus be responsible for the effects of aqueous extracts of NS found in primary rat hepatocytes [199].

Furthermore, NS extract should be used with precaution in pregnant women and children due to its hypoglycemic properties. In children, it was recommended that NS should be administered in weight-adapted doses. Consequently, it concludes that the NS seeds and their extracts and oil appear to have a low level of toxicity and could be considered safe at normal concentrations in cells. However, in spite of a large number of experimental studies, there have been few studies on humans. Besides exhibiting potentially therapeutic activities, acute and chronic toxicity need to be recorded and thus require further studies on its effects and metabolism.

DISCUSSION & FUTURE PERSPECTIVES: Plants have been used as a source of therapeutics since ancient time. Medicinal plants and plant extracts have been traditionally used against a wide range of diseases or to promote general health. Medicinal plants nowadays are a vital source of drug synthesis, and at least a third of current drugs are derived from plants. Despite the move toward synthetic medicine, traditional plant-based remedies still play an important role in the world's medicine and nowadays, there is an increasing demand for bioresources in the management of chronic diseases, instead of chemical drugs, to avoid undesirable effects.

Nigella sativa has been used for centuries by diverse human cultures around the world, especially in Asian countries. It has been extensively studied in recent years, and its beneficial effects against an assortment of illnesses are well established [10]. Current researches on dietary anti-oxidant components are proving to be of great interest because these substances may protect important biological molecules from oxidative damage, and consequently reduce the risk of several maladies including inflammatory and immune conditions, cancer, liver and kidney problems, and cardiovascular diseases. *Nigella sativa* is a respectable example of dietary anti-oxidant whose seeds is now recognized by some pharmacopeia.

Clinical and animal studies have demonstrated many therapeutic effects of NS, but further investigations on the mechanisms of action are required and may have a considerable impact on future clinical treatments of patients with various failures. More human studies are needed before *Nigella sativa* can be recommended for any indication. Improving the productivity and quality of medicinal plants should be an ultimate goal, in the latter years, to meet the increase of population and to avoid chemical therapy side effects on human health.

Although, *Nigella sativa* does not have a noteworthy economic world market share, yet, it nevertheless constitutes a niche market whose size is constantly growing due to its alleged pharmacological properties, and to reasons resulting from its mention in sacred texts. Coupling these beneficial effects, *Nigella sativa* seed is a promising source for active ingredients that would be with potential therapeutic modalities in different clinical settings; the efficacy of its, however,

should be measured by the nature of the diseases. Overall, results suggested that *Nigella sativa* seed oil, extracts and its active ingredients possess remarkable healing activities and could deserve further consideration as a potential multi-purpose product for pharmaceutical, industrial and cosmetic uses. Furthermore, it recommends that *Nigella sativa* incorporated into diets and everyday lifestyles for health improvement. At this moment, there are many experimental data that hopefully, may stimulate the beginning of the era of clinical studies to evaluate the potential of seeds.

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