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Biological Activities and Therapeutic Promises of Nigella Sativa L

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ABSTRACT

Nigella sativa L. (NS) is evident to have a number of important biological activities, such as antioxidant, anti-inflammatory, antibacterial, antifungal, anti-viral, anti-parasitic and anti-protozoal, cytotoxic, anticancer, neuro-, gastro-, cardio-, hetapto- and nephro-protective activities. In addition, the NS implies beneficiary effects on reproductive, pulmonary and immune systems as well as in diabetes mellitus (DM), fertility (male and female), breast cancer, dermatological complications, dehydration, dyspepsia, and osmotic balance and so on. Among the other isolated constituents, thymoquinone (TQ) is a vastly studied phytochemical in NS. A number of reports suggest that, the NS may be one of the potential herbs in health promotion. This paper will discuss the current scenario of NS activity in a mechanistic way.

Keywords: Nigella Sativa, Biological Activities, Health Promotion

List of abbreviations: 5-HIAA

5-HIAA: hydroxyindole acetic acid, 5-HT: serotonin, ACC: acetyl CoA carboxylase, AChE: acetylcholinesterase, ADA: adenosine deaminase, Akt: protein kinase B, ALT: alanine aminotransferase, AO: acid output, APAP: N-acetyl-p-aminophenol, AST: aspartate aminotransferase, bax/bcl-4: apoptosis regulator, bcl-1: cyclin b1, bcl-2: cyclin b2, bcl-xl: cyclin b xl, BUN: blood urea nitrogen, CAT: catalase, CDK-p16: cyclin-dependent kinase p16, CGD: conjugated diene, c-JUNK: c-Jun-amino-terminal kinase, CK: creatinine, COX-1: cyclooxygenase-1, COX-2: cyclooxygenase-2, CP: cisplatin, CVS: cardiovascular system, cyclin b1 (bcl-1), cyclin-dependent kinase p16 (CDK-p16), dcl-1: cyclin d1, DM: diabetes mellitus, FABPs: fatty acid binding proteins, FAS: fatty acid synthase, FGF: fibroblast growth factor, GPx: glutathione peroxidase, GSH: reduced glutathione, GSH-ST: glutathione-S-transferase, HbA1c: glycosylated hemoglobin, HDAC: histone deacetylase, HDL-C: high-density lipoprotein-cholesterol, HIV: human immunodeficiency virus, i.g.: intra-gastric, i.p.: intra-peritoneal, IFN-γ: interferon-gamma, IL: interleukin, LDH: lactate dehydroginase, LDL-C: low-density lipoprotein-cholesterol, LPO: lipid peroxidase, LPO: lipid peroxidase, LT4: leukotriene-d4, MDA: malonilealdehyde, MPO: myloperoxidase, NF-κB: nuclear factor-kappa-B, NK: natural killer, NLRP3: NACHT, LRR, and pyrin domain-containing protein 3, NO: nitric oxide, OSI: oxidative stress index, OXT: oxytetracycline, p.o.: per oral, PET: pulmonary function test, PGD: prostaglandin, PGE2: prostaglandin, ROS: reactive oxygen species, SCC: squamous cell carcinoma, SOD: superoxide dismutase, SP-1: protein expression in papiloma, TAC: total antioxidant capacity, TBARS: thiobarbituric acid substances, TC: total cholesterol, TG: thyroglobulin, TGF-β: transforming growth factor beta, TNF-α: tumor necrosis factor-alpha, TNO: nitric oxide, TOS: total oxidative status, TQ: thymoquinone, TSH: thyroid stimulating hormone, UI: ulcer index

Introduction:

This study is stimulated by the talks of the noble man, the last Prophet of the religion Islam, Hazrat Muhammad (PBUH); who told that the black seed (Scientific name: *Nigella sativa*; Urdu: Kalonji; Arabic: Habba-tu sawda/ Habba Al-Barakah; English: Black cumin/ Black seed; Persian: Shonaiz; Bengali: Kalajira;

Hindi/Nepali: Mangrail)1 contains all kinds of remedies except death. To date (March 2016) in the databases such as PubMed/ Medline, Science Direct, Web of Science, Scopus, and google, a total of 1290 published evidences were found on the following topics: morphology of the plant, isolated compounds and their derivatives, and pharmacological activities. One general revision of this plant was done by Ahmad et al[2], following to a dermatological revision by Aljabre et al[3], an immunomodulatory and anti-inflammatory revision by Majdalawieh and Fayyad[4], an anti-inflammatory, antioxidant, an immunomodulatory revision by Gholamnezhad et al[5], male fertility revision by Mahdavi et al[6], metabolic parameters in diabetes mellitus revision by Heshmati and Namazi[7], and thymoquinone and its therapeutic potentials by Darakhshan et al[8].

This text summarizes the above mentioned seven revision articles. Additionally, data from 2014 to March 2016 were also included in this revision. More emphasize was given to the action mechanisms.

Findings:

Nigella sativa (NS) in short

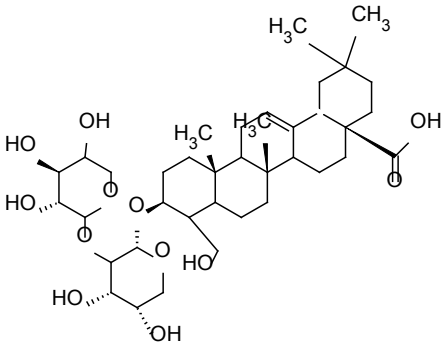
The NS is a small shrub (20 to 90 cm in tall) under the family, Ranunculaceae. It is native to Southern Europe, North Africa and Southeast Asia; cultivated in many countries in the world like Middle Eastern, Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, Saudi Arabia[2]. It has tapering green leaves and rosaceous white, yellow, pink, pale blue or purplish flowers with 5-10 petals. The ripe fruit (capsule: 3-7 united follicles) contains numerous tiny seeds, dark black in color. The seed and oil of NS was frequently used in ancient remedies (Unani, Ayurveda, Chinese and Arabic) in Asian countries and in the Middle-East. The use of black seeds (seeds of NS) had been mentioned by Ibne-Sina (980-1037) in his famous book Al-Qanoon fitt-Tibb[3].

Traditionally NS is used as a medicament of a variety of disorders, including the respiratory system, digestive tract, cardiovascular system (CVS), kidney, liver, and immune system. The use of NS in fatigue and dispiritedness is antique. The most common traditional uses belong to the ailments, including asthma, bronchitis, rheumatism and related inflammatory diseases, indigestion, loss of appetite, diarrhea, dropsy, amenorrhea, dysmenorrhea, worms and skin eruptions. It is also used as an antiseptic and local anesthetic[2].

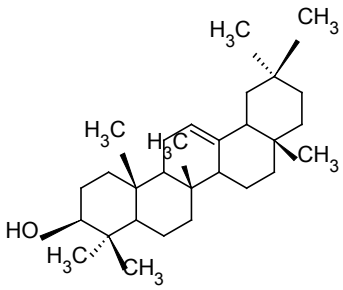
Chemical composition

The black seeds contain protein (26.7%), fat (28.5%), carbohydrates (24.9%), crude fiber (8.4%), total ash (4.8%), volatile oil (0.5-1.6%), fatty oil (35.6-41.5%)[2], cellulose (6.8-7.4%) and moisture (8.1-11.6%)[9]. The seeds are also rich in various vitamins (e.g. - A, B1, B2, B3 and C) and minerals (e.g. - Ca, K, Se, Cu, P, Zn, Fe). Carotene and vanillic acid are also reported in its seeds and root, and shoot, respectively. As fatty components, linolic acid (50-60%), oleic acid (20%), dihomolinoleic acid (10%) and eicodadienoic acid (3%) are the main unsaturated fatty acids. The palmitic acid and stearic acid belong to two main saturated fatty acids, in which α-sitosterol (44-54%) and stigmasterol (6.57-20.92%) are the pioneers[2]. Some other fatty acids such as myristic acid, palmitoleic acid, linoleic acid, linolenic acid, arachidonic acid, cholesterol, campesterol, β-sitosterol, Δ5-avenasterol, Δ7-stigmasterol, and Δ7- avenasterol are also reported by Gharby et al[9] in NS.

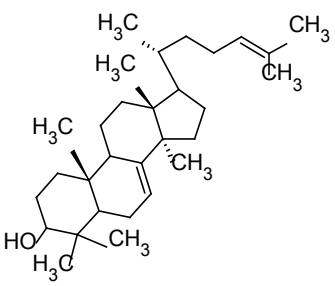
The seed contained alkaloids are: isoquinoline alkaloids (e.g. - nigellicimine, nigellicimine N-oxide), pyrazole alkaloids or imidazole ring bearing alkaloids (e.g. - nigellidine, nigellicine). It also contains terpenes (e.g. - α-hederin) and saponins. Evidences tell that thymoquinone (2-Isopropyl-5-methylbenzo-1,4-quinone, 30-48%), thymohydroquinone, dithymoquinone, p-cymene (7-15%), carvacrol (6-12%), 4-terpineol (2-7%), t-anethol (1-4%), sesquiterpene longifolene (1-8%), α-pinene and thymol etc. are the most important active components reported in NS. The other chemical components are: carvone, nigellicine1, nigellone, citrostradienol, cycloeucalenol, gramisterol, lophenol, ostusifoliol, stigmastanol, β-amyrin, butyrospermol, cycloartenol, 24-methylene-cycloartanol, taraxerol, tirucallol, 3-O-[β-D-xylopyranosyl(1→3)-α-L-arabino-pyranosyl]-28-O-[α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranosyl] hederagenin, esters of unsaturated fatty acids with ≥C15 terpenoids, esters of dehydrostearic and linoleic acid, aliphatic alcohol, β-unsaturated hydroxyl ketone, hederagenin glycoside, melanthin, melanthigenin, bitter principle, tannin, resin, reducing sugars, glycosidal saponin, 3-O-[β-D-xylopyranosyl(1→2)-α-L-rhamnopyrasyl(1→2)-β-D-glucopyranosyl]-11-methoxy-16, 23-dihydroxy-28-methylolean-12-enoate,stigma-5,22-dien-3-β-D-glucopyranoside,cycloart-23-methyl-7,20,22-triene-3β,25-diol, nigellidine-4-O-sulfite, N. mines A3, a4, A5, C, N. mines A1, a2, B1, and B2[2]. Chemical structures of some NS-derived phytochemicals are shown in Figure 1.



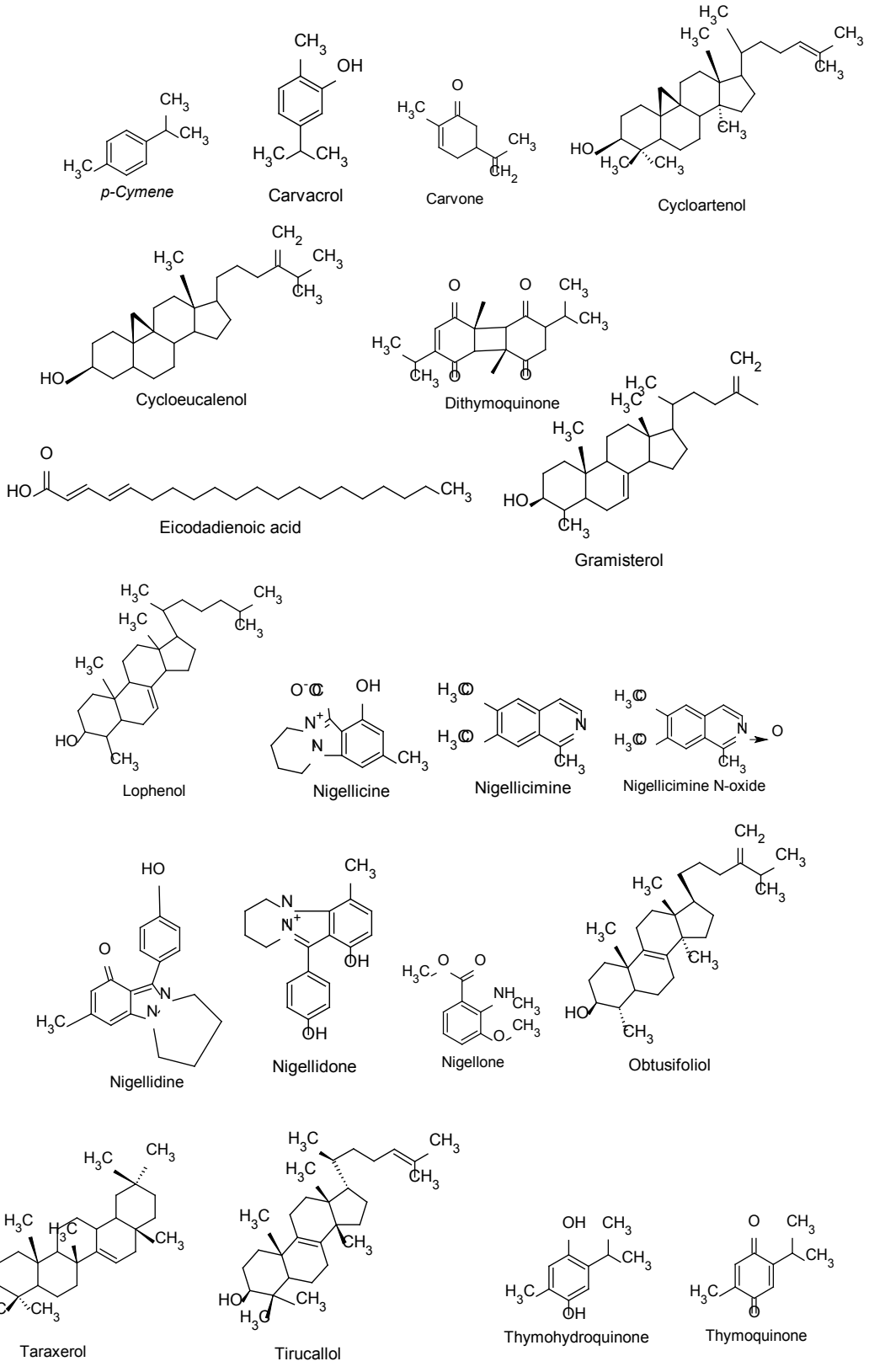
α–Hederin



β–Amyrin



Butyrospermol


p-Cymene
Carvacrol
Carvone
Cycloartenol
Cycloeucalenol
Dithymoquinone
Eicodadienoic acid
Gramisterol
Lophenol
Nigellicine
Nigellicimine
Nigellicimine N-oxide
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Thymohydroquinone
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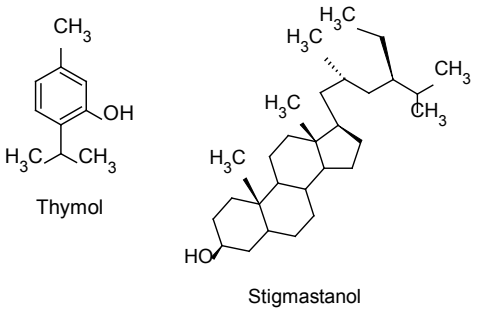

Figure 1. Some important chemical moieties isolated from *N. sativa.*

Pharmacological activities of NS:

NS against bacteria

The NS is found to act against gram positive (*Staphylococcus aureus*) and gram negative (*Pseudomonas aeruginosa* and *Escherichia coli*) species. It showed synergistic effects with streptomycin and gentamycin, while additive with spectinomycin, erythromycin, tobramycin, doxycycline, chloramphenicol, nalidixic acid, ampicillin, lincomycin and co-trimoxazole and similar to topical mupirocin. Moreover, the NS has potent inhibitory activity against antibiotic resistant microorganisms, including many multi-drug-resistant (MDR) gram positive and gram negative bacteria[3]. According to Manju et al[10] the EO from NS is able to protect Artemia spp. from Vibrio parahaemolyticus Dahv2 infection. According to Hariharan et al[11], thymoquinone (TQ) the well known NS compound has shown antimethicillin-resistant activity in S. aureus. TQ is also evident to act against a number of gram positive and gram negative pathogenic bacteria[8].

NS against fungi

The NS is found to inhibit the growth of *Candida albicans* and *Madurella mycetomatis*, while TQ by *Aspergillus niger*, *Fusarium solani* and *Scopulariopsis brevicaulis* more effectively than the amphotericin-B and griseofulvin. The TQ also acted against *Trichophyton* spp., *Epidermophyton* spp., and *Microsporum* spp. In addition TQ, *thymohydroquinone* and thymol are also demonstrated to have an antifungal effect against many clinical isolates, including dermatophytes, molds and yeasts3. Furthermore, the NS seed oil (10-200 µg/mL) was found to act against *Saccharomyces cerevisiae* and C. *utilis*[12].

NS against viruses

In a study, the NS enhanced helper-T-cell (T4) and suppressor-T-cell (T8) ratio and increased the natural killer (NK) cell activity in human. Furthermore, it significantly inhibited the human immunodeficiency virus (HIV) protease and murine cytomegalovirus. In the latter case, it was found to increase in number and function of the M-phi and CD4+ve T cells with the production of interferon-gamma (INF-γ)[3].

NS against parasites

The NS is evident to have anti-*leishmaniasis*, anti-*miracidia*, anti-*cercariae* and anti-*Schistosoma mansoni* potentials. In the latter case the oil of the black seed showed a strong activity as compared to the anti-*schistosomal* and anthelmintic drug for domestic animals, prazequantel; where it produced a potentiating effect with the co-treatment[3]. Moreover, Simalango and Utami[13] suggested that the ethanol extract of NS (0.5-8%) has significant anti-*Ascaris suum* activity.

NS in wound infection

The wound healing capacity of NS was evaluated in farm animals, mice and human gingival fibroblast. The accumulated result suggests that there is a reduction in absolute differential leukocytes (WBC) counts, local infection and inflammation, bacterial expansion and tissue impairment, and free radical production. An elevation of basic fibroblast growth factor (FGF) and transforming growth factor beta (TGF-β) were also reported (Aljabre et al. 2015).

Antioxidant capacity of NS

A number of *in vitro*, *ex vivo* and *in vivo* antioxidant studies have been conducted with NS extracts, seed oil and TQ. The finding suggests that, NS and its derived components have potent radical scavenging as well as oxidative stress inhibitory capacities. TQ significantly changed the parameters including adenosine deaminase (ADA), catalase (CAT), myloperoxidase (MPO), lipid peroxidase (LPO), reduced glutathione (GSH), glutathione-S-transferase (GSH-ST), glutathione peroxidase (GPx), superoxide dismutase (SOD) and nitric oxide (NO) in the favor of reducing oxidative stress. It also reduced the malonilealdehyde (MDA), conjugated diene (CGD) levels and pro-inflammatory mediators interleukin (IL)-1-beta, IL-6, tumor necrosis factor-alpha (TNF-α), IFN-γ, and prostaglandin (PGE_2) rather than IL-10[2,14]. Figure 2 tells the basic antioxidant pathways of NS.

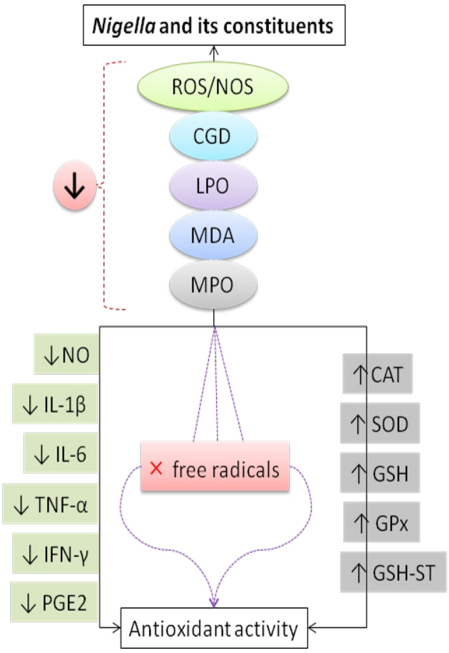

Figure 2. Antioxidative action pathways of *Nigella* and its constituents

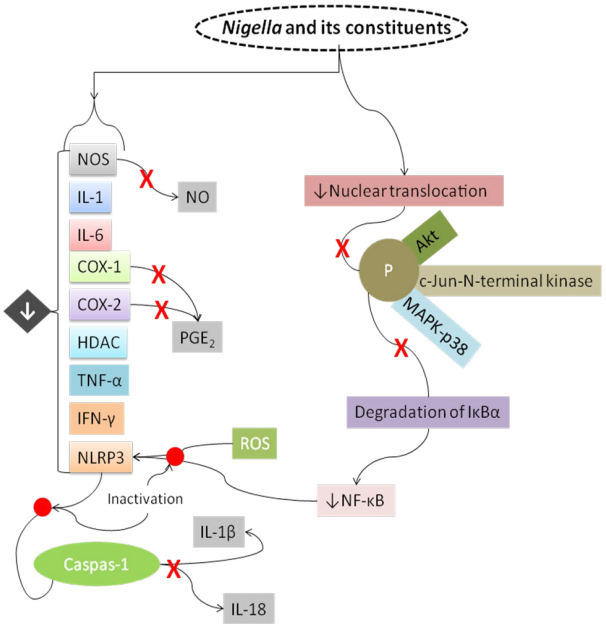

Figure 3. Antiinflammatory capacity pathways of Nigella and its constituents.

NS in inflammation:

Findings from different animal models suggest that, the NS extracts, seed oil and TQ have strong anti-inflammatory activities. In some studies, they were found to reduce the synthesis of NO, IL-1, cyclooxygenase (COX)-1, COX-2, histone deacetylase (HDAC) along with other pro-inflammatory mediators such as - IL-1β, IL-6, TNF-α, IFN-γ, and PGE_2[2]. The topical application of TQ was found to induce an expression of hemeoxygenase (HO)-1, NAD(P)H-quinoneoxidoreductase-1, GSH-ST and glutamate cysteine ligase in mice; while the seed oil inhibited COXs, 5-LPO in the pathways of arachidonate metabolism in rats[3]. TQ is also evident to down-regulate the nuclear translocation and the DNA binding of nuclear factor-kappa-B (NF-κB) via the blockade of phosphorylation and subsequent degradation of IκBα in mice. Moreover, the TQ was also attenuated the phosphorylation of Akt (protein kinase B), c-Jun-amino-terminal kinase (c-JUNK) and p38 mitogen-activated protein kinase (MAPK-p38). In another study, a decrease in expression of the NLRP3 (NACHT, LRR, and pyrin domain-containing protein 3) in B16F10 mouse resulted in inactivation of caspase-1 followed by the inhibition of IL-1β and IL-18. In addition, the inhibitory effect of TQ to NF-κB and reactive oxygen species (ROS) resulted in the partial inactivation of NLRP3 inflammasome[3,4,14]. Figure 3 tells the basic anti-inflammatory activity pathways of NS.

NS in cancer:

The black seed oil can stimulate the NK cells, which is a potential applicability in immune therapy. Otherwise, the oil components may induce pro-oxidative effects thus the carcinogeneticity. The TQ tested in a number cancer cells derived from mice, suggesting its ability to arrest G_0/G_1 phases of cell-cycle, which correlated with sharp increases in the expression of the cyclin-dependent kinase p16 (CDK-p16) and a decrease in cyclin d1 (dcl-1) protein expression in papiloma (SP-1) cell line and G_2/M arrest associated with an increase in the expression of the tumor suppressor protein p53 with a decreased level of cyclin b1 (bcl-1) protein. The chemopreventive potential of TQ may be due to its ability to increase the ratio of apoptosis regulator (bcl-4)/cyclin-2 (bax/bcl-2) expression and decreasing cyclin-xl (bcl-xl) protein. The antitumor activity of TQ was also reported in squamous cell carcinoma (SCC VII), FsaR and murine tumor models of fibrosarcoma and SCC. TQ showed potent anticancer activity in A431 and Hep2 cells via apoptosis by increasing the sub-G1 population, live/dead cytotoxicity, chromatin condensation, DNA laddering and Tunel-positive cells. Along with an increase in bax/bcl-2 ratio activation of cell proliferation of caspases and cleavage of poly ADP ribose polymerase was also observed[3].

A research done by Khalife et al[15] suggesting that TQ induced apoptosis through p53-independent pathway with an expression of p21 and cell-cycle S phase arresting in human colon cancer cells. TQ also exerted an anticancer effect in a number of cancer cell lines, including MCF-7/Topo breast carcinoma cells. It was found to down-regulate the NF-κB and MMP-9 in Panc-1 cells and bcl-2 in gastric cancer cells, while up-regulator of caspase-3 and caspase-9 in the later one. A number of derivatives of TQ namely 6-menthoxybutyryl, 6-hencosahexanyl conjugate, 4-acylhydrazones and 6-alkyl derivatives are also evident to produce anticancer activity in cancer cell lines[2].

Recent evidence suggests that the nanoemulsion of NS oil at a concentration of 20-80 µL/mL caused cell membrane blebbing, cytoplasmic vacuolation, marginalization of chromatin, and fragmentation of the nucleus in MCF-7 cells[16]. A recent evidence suggests that topical use of black seed oil (600 mg) reduced cyclic mastalgia in woman (n = 52), where the activity was found significant when compared to the painkiller, diclofenac[17]. A basic NS-anticancer traits has been sketched in Figure 4.

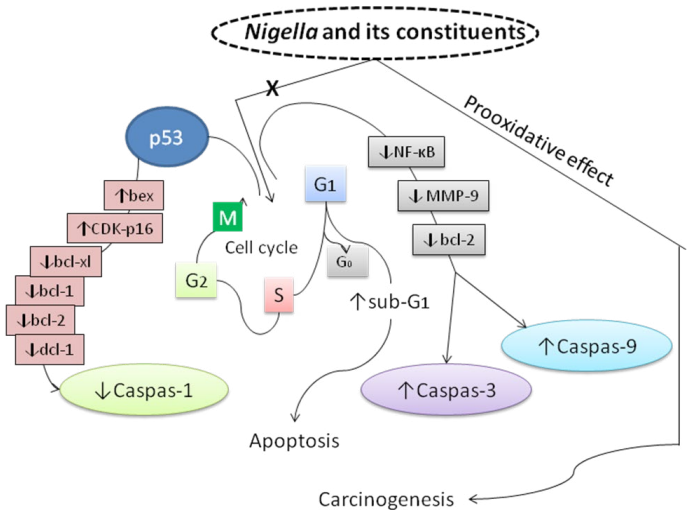

Figure 4. Basic anticancer pathways of Nigella and its derivatives.

NS in diabetes

The NS was found to reduce blood glucose level with an augmenting insulin level and C-peptide in rats. Whereas, TQ reduces the tissue MDA levels, DNA damage, mitochondrial vacuolization and fragmentation, and preserves pancreatic β-cell integrity via antioxidant capacity. In a study TQ increased the levels of insulin, Hb with a significant decrease in glucose and glycosylated hemoglobin (HbA1c) levels. The NS showed a synergistic activity with parathyroid hormone in improving bone mass, connectivity, biomechanical behavior and strength in T2D rats. The NS is also evident to show some advantages in insulin resistance syndrome and dislipidemic patients. Furthermore, an insulin-sensitization action via enhancing ACC phosphorylation (mainly MAPK signaling pathway) and muscle GLUT4 content as well as progressive normalization of glycaemia are also seen in NA-treated diabetic Meriones shawi[2,7]. In a study, the lipid (4%) and volatile oil fractions (3%) of NS in streptozotocin-induced diabetes mellitus (DM) rats reduced toxicological and adverse consequences significantly[18]. In addition, an improved glycemic status and lipid profile with NS oil treatment at 3 g/3-times/day in DM patients (n = 72) were also reported by Heshmati et al[19].

TQ when tested in clonal β-cells and rodent islets it caused a protective effect with normalization of chronic accumulation of malonyl CoA, and elevation of acetyl CoA carboxylase (ACC), fatty acid synthase (FAS) and fatty acid binding proteins (FABPs) following a chronic glucose overload, suggesting the modulation in β-cell redox circuitry and enhancing sensitivity of β-cell metabolic pathways to glucose and glucose-stimulated insulin secretion (GSIS) under both normal conditions and hyperglycemia[20]. Generally, the MAPK regulates a number of transcriptional factors, altering of which interferes in cell-cycle. Thus, NS and TQ may be a good remedy for both type 1 and 2 DM patients, as in this consequence maintenance of beta-cell integrity and secretion of insulin sufficient for glycogenesis and phosphorylation of raised glucose in the blood are crucial. Otherwise, along with ingested food, oxidative stress, infection and trauma are the factors that increase in blood sugar levels. Thus, the antidiabetic activity of NS and TQ may connect with their antioxidant, antimicrobial, cytotoxic and anti-inflammatory activities. Otherwise, the decreasing level of HbA1c is one of the remedy for cardiovascular disease, nephropathy, neuropathy, and retinopathy. Figure 5 tells the possible anti-diabetic action pathways of NS.

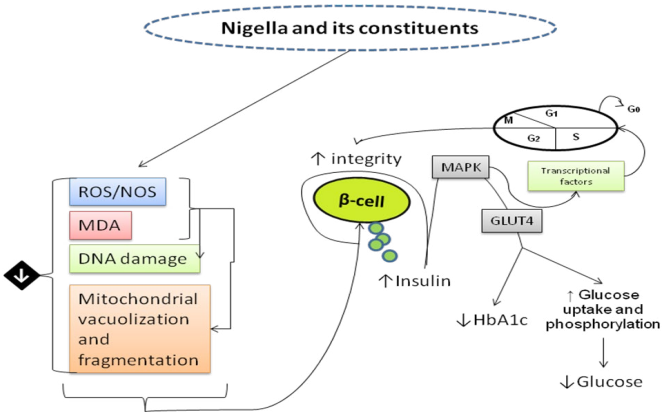

Figure 5. Possible antidiabetic action pathways of Nigella and its constituents.

NS effects on immune system:

Along with NK antitumor activity, the NS is a demodulator of the secretion of a number of pro-inflammatory mediators with up-modulation of secretion of Th2/Th1, cytokines in splenocytes. The NS is also evident to restore the resistance against granulocyte-dependent C. albicans. A study performed by the NS oil suggests decreasing antibody production in typhoid vaccination, which may be due to its immunosuppressive cytotoxic effect. It is also evident to correct the imbalance situation caused by oxytetracycline

(OXT) in leukocyte, lymphocyte counts, heterophil:lymphocyte ratio, lysosomal enzyme activity and reticuloendothelial system function. Moreover, the NS produced an immunoprotective effect in chronic antibiotic loaded pigeons. The NS oil was also exerted radioprotectivity, immune-stimulatory, reducing the effects of ionizing radiation-induced situations. In addition, an increased level of IFN-γ with a significant decreased in pathological changes of the guinea pigs' lung was reported by NS oil treatment. It is also effective in allergic diarrhea[3,4,14]. A recent evidence suggests that, the seed oil is protective against γ-radiation-induced damage in jejunal mucosa[21]. Moreover, EO from NS at 5-20 g/kg (oral feed for 6 weeks) in chickens improved FCR and plasma lipid profile and antibody-mediated immunity[22]. In a study, the NS oil also reduced thyroid stimulating hormone (TSH) and anti-thyroid peroxidase antibodies in patients with Hashimoto's thyroiditis[23].

NS on nervous system:

Methanolic extract of NS is reported as a potent analgesic and antidepressant. In addition, an anxiolytic activity via increasing serotonin (5-HT) and decreasing hydroxyindole acetic acid (5-HIAA) levels were also reported in rat brain. In another study, an increased 5-HT secretion along with improving learning and memory capacity in rats were detected with NS treatment. As NS may augment in tryptophan levels, it may be helpful in anxiety treatment. Otherwise, TQ produced GABA-mediated anxiolytic-like effect in mice with a decline of NO and MDA production[2]. The possible neuroprotective activity may be due to its antioxidant, free radical scavenging and anti-inflammatory capacities. Along with these, anti-acetylcholinesterase (anti-AChE) and anticonvulsant activities were also evident with NA and TQ treatments, respectively. There is a suggestion for GABAA-ergic anticonvulsant effect of TQ[2].

NS EO at 1 g/kg (i.g.)/day and TQ 30 at mg/kg/day (i.p.) in Wistar albino rats produced anti-nitrosative effects after a 10 days treatment[24]. The NS EO is also evident to prevent cerebral edema in the hippocampus tissue of the rat brain[25]. Fahmy et al[26] suggested that NS oil at a dose of 2.8 g/kg when treated orally (p.o.) in autoimmune encephalomyelitis rats for 4 weeks, significantly reduced the oxidative stress parameters in the cortex and hippocampus with the enhancing in remyelination in the hippocampus. Otherwise, oil at a dose of 4 mL/kg/day (p.o.) in tramadol treated male albino rats protected the cortical neurons and myelinated axons[27]. Furthermore, the NS EO at 500 mg (4 weeks treatment) in adolescent human males (n = 48) significantly stabilized mood, decreased anxiety and modulated cognition[28]. A possible neurological activity of NS is shown in Figure 6.

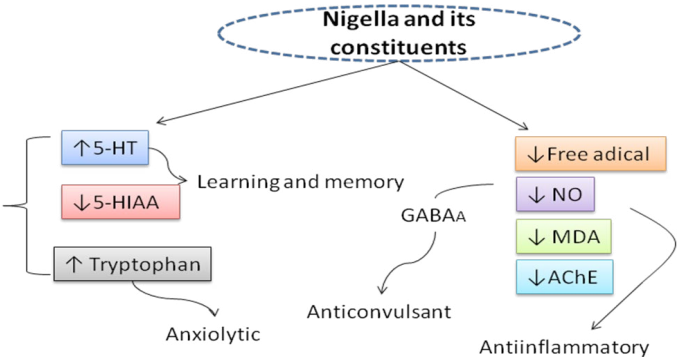

Figure 6. A possible neuroprotective trait of Nigella and its constituents.

Effects on gastrointestinal tract (GIT):

TQ is gastroprotective as it decreases gastric acid secretion, acid output (AO), pepsin, the mucosal content/activity of lipid peroxidase (LPO), proton (H+) pump, MPO and ulcer index (UI) while an increased in the content/activity of gastric mucin, GSH, total nitric oxide (TNO) and SOD. Decreased ulcer severity in rats was guessed via prostaglandin (PGD)-mediated and/or through antioxidant and anti-secretion pathways. A decreased in LPO and lactate dehydroginase (LDH), MPO, MDA and increased in GSH, SOD, GPx, GSH-ST without altering of gastric CAT was also reported in rats. TQ was found to exert significant effects in diarrhea, colitis, inflammatory bowel diseases, anti-Helicobacter pylori as well as in loss of body weight[2]. Possible GIT protective pathways of TQ are shown in Figure 7.

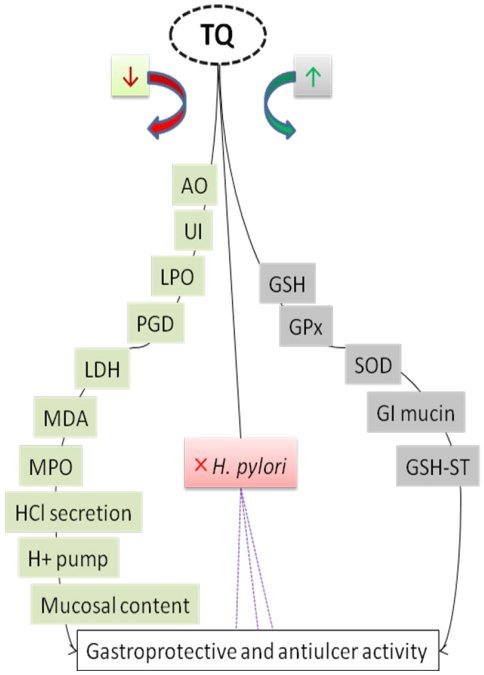

Figure 7. Possible GI-protective pathways of Nigella and its constituents

Effects on cardiovascular system (CVS)

TQ is evident to decrease motor fuel (diesel particle)-induced systolic blood pressure, leukocytes, IL-6 and plasma SOD activity. It is also prevented to decrease platelet counts and the prothrombin events rather than platelet aggregation[2]. The black seed oil reduced the total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), and thyroglobulin (TG) with an increased high-density lipoprotein-cholesterol (HDL-C) level[29].

Effects on hepatic system

The NS effect on alanine aminotransferase (ALT), aspartate aminotransferase (AST), LDH, total antioxidant capacity (TAC), CAT, MPO, total oxidative status (TOS) and oxidative stress index (OSI) tells that it has hepatoprotective activity. In addition GSH, TQ increased protein carbonyl content, thus the attenuation of protein oxidation and upgrading of the depleted antioxidant cellular fraction[2]. Moreover, the NS oil at a dose of 25-100 µg/mL protected hepatocytes from N-acetyl-p-aminophenol (APAP)-induced hepatotoxicity and metabolic disturbances in TIB-73 cells of mice[30]. A similar activity was also observed by Hamza and Al-Harbi[31] with aqueous extract of NS, where the activity was thought to be linked with an improved antioxidant potential and suppressed in lipid peroxidation and ROS generation[30]. In addition, the black seed oil at a dose of 2 mg/kg (p.o.) with cisplatin (CP)-treated rats are also evident for its hepatoprotective activity via improving energy metabolism and strengthening antioxidant defense pathways[32].

Effects on urinary system

The NS along with ascorbic acid (Vitamin C) produced a nephroprotective effect by lowering serum creatinine (CK), blood urea nitrogen (BUN) and antioxidant activity in rabbits. On the other hand, the TQ showed an effect on renal expression of organic ion transporters and multidrug resistance-associated proteins in rats. TQ-mediated increased in protein levels of the efflux transporters MRP2 and MRP4, and decreased expression of OAT1, OAT3, OCT1 and OCT2 was also observed in rats. Along with decreasing tubular necrosis score, NS significantly reduced the CK, urea, MDA, NO, ROS, OSI and TOS levels and augmenter of TAC, SOD, GPx in kidney tissue and blood. Furthermore, the TQ is also evident to antagonize the gentamicin (GM)-induced alteration of serum CK, BUN, thiobarbituric acid substances (TBARS), total nitrite/nitrate content, GSH, GPx, CAT and ATP values in rats[2]. Moreover, the ethanol extract of NS at 250-100 mg/kg (p.o.) in female Wistar Albino rats showed a significant nephroprotective activity against paracetamol-induced nephrotoxicity[33]. In another study, the NS exhibited a significant nephroprotective effect in Cd-induced nephrotoxicity in rats[34].

Effects on respiratory system

Both nigellone and TQ are evident to inhibit leukotriene-d4 (LT4) in the trachea, where the activity of the first one was suggested via mucociliary clearance. However, NS is evident to reduce the peribronchial inflammatory cell infiltration, alveolar septal infiltration, alveolar edema, alveolar exudates, alveolar macrophages, intestinal fibrosis, granuloma, necrosis formation, NOS and surfactant protein D in the pulmonary system. The NS is also evident to have beneficial effects against lung injury and hypoxia-induced lung damage. In a study, the NS puffs were proven to relieve asthma symptoms, frequency of asthma symptoms/weakness, chest wheezing and pulmonary function test (PFT) values with a bronchodilatory effect[2].

Effects on reproductive system

TQ decreased TAC and MPO levels in C57BL/6 male mice. In addition, TQ alerted the events produced by methotrexate such as intestinal space dilatation, edema, disruption in the somniferous epithelium and reduced diameter of the semniferous tubules. Moreover, infertile men (n = 34) when treated with 2.5 mL NS oil for 2 months, significantly improved abnormal semen quality without producing any adverse effect was observed[35]. According to Mahdavi et al[36] the NS oil is a good candidate for treating male infertility. Hexane and methanol extracts of NS produced significant anti-fertility in Sprague-Dawley male and female rats, respectively. Otherwise, NS is evident to inhibit the uterine smooth muscle contraction in rats and guinea pigs[2,36]. TQ when treated with olive oil caused a reduction in polycystic overy in rats possibly, via NF-κB signaling pathway[37]

NS in dyspepsia

Patients (n = 70) with functional dyspepsia when treated with NS oil of 5 mL (p.o.) for 8 weeks, a significant lowering of dyspepsia was observed[38].

NS in osmotic balance

The geriatric patients (n = 42) when treated with NS oil (22.6 µg/25 µL) for 2 weeks, it was demonstrated that NS may be an alternative therapy of the isotonic sodium chloride (0.9% NaCl) solution[39].

Topical applications

A TQ-induced skin darkening via chlonergic mechanisms of muscarinic receptor in the melanin dispersion is evident, whereas, NS oil for decreasing vitiligo area scoring index without seeing adverse effects. Moreover, TQ and nigellone inhibited histamine release, protected histamine-induced bronchospasm in guinea pigs, decreased lung ensiphilia, elevated Th2 cytokines and raised IgE and IgG1 antibodies in mice. To be mentioned that, the NS has a good recommendation in hand eczema. Linoleic acid from this plant is known for its percutaneous adsorption enhancing capability of drugs, while the oil emulsion for reducing skin irritation and improving moisturizing and epidermal barrier function. It has also anti-aging, mitigating, and protective effects[3]. Both NS and TQ can be used in oral health and hygiene[40].

Toxicological data of NS/TQ

In mice, the dose causing death of fifty percent experimental animals (LD50) values of fixed oil of NS was found 26.2-31.6 mg/kg and 1.86-2.26 mg/kg with single oral (p.o.) and intraperitoneal (i.p.) doses, respectively. In another study, calculated LD50 for TQ was 89.7-119.7 mg/kg and 647.1-1094.8 mg/kg after i.p. and p.o. administrations, respectively. In rat it was found to be 45.6-69.4 mg/kg and 469.8-1118.8 mg/kg after i.p. and p.o. administration, respectively. Data suggest TQ is more tolerated than the extract from NS[2]. Some important biological activities of NS have been given in Table 1.

Table 1. Some recent research evidences found on *Nigella*-constituentss.

Form/chemicals	Dose (route)/test systems	Activity	References
Essential oil	5-50 g/L for antioxidant assyas, 0.2-2.0 µg/mL for antimicrobial	Produced antioxidant activity and protected the Artemia spp. after experimental infection of Vibrio parahaemolyticus Dahv2.	Manju et al. 2016
Oil	p.o. administration in 22-50 yrs old patients	Reduced thyroid stimulating hormone (TSH) and anti-TPO antibodies in patients with Hashimoto's thyroiditis.	Tajmiri et al. 2016
Essential oil nanoemulsion (20–50 nm diameter)	20-80 µL/mL in MCF-7 cells	Produced cell membrane blebbing, cytoplasmic vacuolation, marginalization of chromatin, and fragmentation of the nucleus.	Periasamy et al. 2016
Oil	-	Reduced total cholesterol, LDL-C, and TG levels and increased HDL-C.	Sahebkar et al. 2016
Oil	400 mg/kg (i.g.) in Wistar albino rats	Lower malondialdehyde (MDA) levels, raised reduced glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activity in intestinal tissues samples.	Orhon et al. 2016
Oil	25-100 µg/mL in TIB-73 cells in mice	Protective effects against N-acetyl-p-aminophenol (APAP)-induced hepatotoxicity and metabolic disturbances by improving antioxidant activities and suppressing both lipid peroxidation and ROS generation.	Adam et al. 2016
Oil	-	Antioxidant and antimicrobial activities.	Ramadan 2016
Phenolic–protein complexes	100 µL in *in vitro* test.	Antioxidant and ACE inhibitory properties	Alu'datt et al. 2016
n-hexane and ethanol fractions	50-2000 µg/mL in ACHN (human renal adenocarcinoma) and GP-293 (normal renal epithelial) cell lines	Cytotoxic activity.	Shahraki et al. 2016
Oil	2 mg/kg (p.o.) in cisplatin (CP) treated rats	Induced hepatoprotectivity by improving energy metabolism and strengthening antioxidant defense mechanism.	Farooqui et al. 2016
Gold coated nanoparticles	Nano-particles (15.6- 28.4 nm) in A549 lung cancer cell line and Staphylococcus aureus 10 µg/mL	Inhibited A549 lung cancer cells and S. aureus.	Manju et al. 2016
TQ	1 µM/mL and 2 mg/200 µL (s.c.) with olive oil in rats	Remedy for polycystic overy via NF-κB signaling pathway.	Arif et al. 2016
TQ	In neutrophils	Strongly inhibited fMLF-induced superoxide production and granules exocytosis in neutrophils.	Boudiaf et al. 2016
Seeds ethanol extract	250-100 mg/kg (p.o.) in female Wistar Albino rats	Significant nephroprotective activity on paracetamol-induced nephrotoxicity.	Canayakin et al. 2016
TQ	50 mg/kg in male Wistar albino rats for 30 days	Significant nephroprotective potential against Cd-induced toxicity.	Erboga et al. 2016
TQ	In *Staphylococcus aureus*	Antimethicillin-resistant activity.	Hariharan et al. 2016
TQ	40 µM TQ and/or 0.6 µM topotecan in human colon cancer cells	Induced apoptosis through p53-independent pathway with an expression of p21 and arrested cell-cycle S phase.	Khalife et al. 2016
Oil	600 mg (topical) in woman (n=52) for 2 months	Clinical effectiveness comparable to topical diclofenac in the treatment of cyclic mastalgia.	Huseini et al. 2016

Oil and TQ	-	Oral health and hygiene.	Al-Attass et al. 2016
Oil	40 mg/kg/day (i.g.) in male albino rats	Ameliorated the toxic changes caused by formaldehyde on corneas.	Salem et al. 2016
Oil	400 mg/kg (p.o.) in rats	Protective effects against gamma-radiation-induced damage in jejunal mucosa.	Orhon et al. 2016
TQ	10 mg/kg (i.p.) in rats	Decreased levels of MDA, NO, TNF-α, IL-1, increased activities of SOD, GPx, CAT with reduction of motor neuron apoptosis.	Gökce et al. 2016
TQ	In clonal β-cells and rodent islets	Protective action associated with normalization of chronic accumulation of malonyl CoA, and elevation of acetyl CoA carboxylase (ACC), fatty acid synthase (FAS) and fatty acid binding proteins (FABPs) following chronic glucose overload. Thus the modulated β-cell redox circuitry, and enhancing sensitivity of β-cell metabolic pathways to glucose and glucose-stimulated insulin secretion (GSIS) under both normal conditions and hyperglycemia.	Gray et al. 2016
EO	10-200 µg/mL free daricanl scavenging and anti-Saccharomyces cerevisiae, anti-Candida utilis, anti-Candida albicans	Antioxidant and anti-yeast activities.	Nadaf et al. 2015
Oil	4 mL/kg/day (p.o.) in tramadol treated male albino rats	Protected the cortical neurons and myelinated axons.	Omar 2015
Methanol extract	100 and 500 mg/kg (p.o.) in hyperlipidemic rats	Antioxidative and hypolipidemic effects.	Ahmad and Beg 2015
Ethanol extract	100-1000 µg/mL in male Wistar rats	Increased cytokines balance in Th1/Th2.	Gholamnezhad et al. 2015
Oil	3 g/day (dietary) in obese women (25–50 yrs) for 8 weeks	Modulated systemic inflammatory biomarkers.	Mahdavi et al. 2015
Oil	5 ml (p.o.) in patients with functional dyspepsia (n=70) for 8 weeks	Lowered dyspepsia	Mohtashami et al. 2015
EO and TQ	-	Regulation of immune reactions implicated in various infectious and non-infectious conditions including different types of allergy, autoimmunity, and cancer.	Majdalawieh and Fayyad 2015
Oil	-	Anti-inflammatory, antioxidant, and immunomodulatory activities.	Gholamnezhad et al. 2015
Oil	-	Good candidate for male infertility treatment.	Mahdavi et al. 2015
Oil	100-400 mg/kg (i.p.) in rats	Prevented hippocampal neural damage which is accompanied with improving effects on memory.	Seghatoleslam et al. 2015
Aqueous extract	0.25 g/kg in mice for 30 days	Powerful reducing capacity of APAP-induced hepatotoxicity and antioxidant activity.	Hamza and Salem Al-Harbi 2015
Oil and its components	-	Anti-diabetes mellitus potential.	Heshmati and Namazi 2015
Oil	3 g/day (one three times a day) in T2DM patients (n=72)	Improved glycemic status and lipid profile.	Heshmati et al. 2015

hydro-alcoholic extract	100-400 mg/kg (p.o.) in rats for 8 weeks	Decreased MDA concentration, improved learning and memory capacity through antioxidative ways.	Beheshti et al. 2015
Methanol extract	0.1 mg/disc in Trichophyton mentagrophytes, Microsporum canis and Microsporum gypseum	Antifungal activity.	Mahmoudvand et al. 2015
Oil	1 mg/kg in tramadol-induced male albino rats for 30 days	Hepato- and nephroprotective effects.	Elkhateeb et al. 2015
Oil	2.8 g/kg (p.o.) in autoimmune encephalomyelitis rats for 4 weeks	Reduced oxidative stress parameters in the cortex and hippocampus as well as enhanced remyelination in the hippocampus.	Fahmy et al., 2014
Lipid (4%) and volatile (3%) fractions	In streptozotocin induced diabetes mellitus Sprague Dawley rats for 56 days	Reduced toxicological and adverse consequences of diabetes mellitus.	Sultan et al. 2014
Oil	2.5 and 5.0 mL/kg (p.o.) in rats for 3 weeks	Increased plasma transaminase activities, hepatic triglyceride, malondialdehyde (MDA) levels and decreased hepatic glutathione (GSH) levels	Develi et al. 2014
Oil	2.5 mL in infertile men (n=34) for 2 months	Improved abnormal semen quality without producing any adverse effect.	Kolahdooz et al. 2014
Oil	22.6 µg/25 µL in geriatric patients (n=42) for 2 weeks	Can be used as an alternative to the isotonic sodium chloride solution.	Oysu et al. 2014
EO and TQ	EO 1 g/kg (i.g.)/day and TQ 30 mg/kg/day (i.p.) in Wistar albino rats for 10 days	Produced anti-nitrosative effects.	Ahlatci et al. 2014
EO	1-50 mg/kg (i.p.) in Wistar rats	Prevented cerebral edema in the hippocampus tissue of the brain.	Hobbenaghi et al. 2014
EO	5-20 g/kg (oral feed) in chickens for 6 weeks	Improved FCR of boilers and improved plasma lipid profile and antibody-mediated immunity.	Ghasemi et al. 2014
Methanol extract	200 mg/kg (p.o.) in male albino Wistar rats for 2 months	Anti-inflammatory activity by down-regulation of the expression of ASC protein of NLRP3 inflammasome in pancreas to minimize the activation of caspase-1.	Suguna et al. 2014
EO	500 mg in adolescent human males (n=48) for 4 weeks	Stabilized mood, decrease anxiety and modulate cognition.	Sayeed et al. 2014
Ethanol extract	0.5- 8% in Ascaris suum	Antihelminthic effect.	Simalango and Utami 2014

Drug interactions

Table 2 suggests NS interaction profiles with drugs/chemicals/biochemicals.

Table 2. *Nigella*-interactions with drugs/chemicals/biochemicals.

Drug/chemical/biochemical	***Nigella constituents***	**Observations**
Ampicillin	//	//
Amoxicillin	Methanol and hexane extract	Increased availability
Antibiotics	*Nigella*	Decreased resistance
Antiasthmatic drugs	//	Like/synergistic
Ascorbic acid (Vitamin C)	TQ	//
Ba/carbachol/leukotriene	TQ	//
Cadmium/$CdCl_2$	//	//
Chloramphenicol	//	//
Cisplatin	TQ	Antagonistic
Collagen	TQ	Antagonistic
Co-trimoxazole	//	//
Curcumin/valproate ameliorate	//	Agonistic
Cyclosporine A	Seed oil	//
1,2-dimethylhydrazine	Methanol extract/TQ	//
Diesel exhaust particle	//	//
Doxycycline	//	//
Doxorubicin	Seed extract/TQ	Synergistic
Ethinylestradiol	Seed oil	Like/synergistic
Ethanol/NaOH/NaCl/Indomethacin	//	Antagonistic
Erythromycin	//	//
Fe-NTA	//	//
5-flouorouracil	TQ	//
Formaldehyde	//	//
Garlic extract	//	//
Gentamycin	*Nigella* oil	Synergistic
Ionizing radiations	*Nigella* extract/TQ	//
L-carnitine/α-lipoic acid	*Nigella*	Synergistic
Lincomycin	//	//
L-N(G)-nitroarginine methyl ester/ N-acetylcysteine	Seed oil	//
Methicillin	//	Antagonistic
Methotrexate	//	//
Methylene blue/diazepam	//	//
Mupirocin	//	//
$NaNO_3$	Seed powder	//
Nalidixic acid	//	//
Nicotinamide	//	//
NO precursor/L-arginine	//	Antagonistic
Olive oil	//	//
Omeprazole	TQ	Agonistic

OVA-antigen	TQ	Antagonistic
Oxitocin	//	Antagonistic
Oxytetracycline	//	//
Paracetamol	TQ	Antagonistic
Parath-hormone	*Nigella*	Synergistic
p-cymene/α-pinene	TQ	//
Pilocarpine	//	//
Prazequental	//	Synergistic
Spectinomycin	//	Additive
Streptomycin	//	//
Streptozotocin	TQ	Antagonistic
Tobramycin	//	//
Topotecan	//	Additive
Typhoid vaccine	Seed extract	Antagonistic

Authors' view-points

At low levels and temporary spikes of ROS are beneficial for health[55] rather than high production and chronic effects as they may up-regulate pro-inflammatory cytokines, chemokines and pro-inflammatory transcription factors[56] and induces cell death by damaging macromolecules such as lipids, DNA, RNA, and other proteins. In extrinsic pathway, excessive ROS are generated by Fas ligand which in association with death domain and caspase-8 cause apoptosis[56]. Otherwise, in the caspase cascade pathway (intrinsic) ROS facilitate to release cytochrome C by activating bcl-2 and bcl-xl and bcl-2-associated X protein as well as bcl-2 homologous antagonist/killer[57]. ROS implicates a variety of detrimental responses, including CVS diseases (e.g. – stroke and heart attack), hearing impairment via cochlear damage, decline memory capability (degenerative diseases, e.g. – AD), ischaemic injury, and so on. Unlike apoptosis and necrosis, autophagy cell death occurs by self-digest of the damaging portion to take an attempt to minimize the damage and can no longer survive. However, it is possible to make available ROS to the other normal cells by this process as cellular programming is enough for a programmed cell death. Radiations form radiotherapy induces ROS-mediated cell death and mitotic failure[56]. However, an ideal ROS neutralizer (antioxidant/cytoprotective agent) is not enough in the cancer therapy, even if it has antioxidant-mediated pro-oxidant effect, as it may act like dual nature of ROS! Therefore, cell targeting, self-redox balancing; genotoxic, but non-mutagenic, exact concentrations of ROS at the targeted site along with action period are the major concerns in the chemo-/radio-therapeutic cancer treatments.

In the above discussion, TQ, the well-known NS-derived quinone and other NS constitutions are evident to have targeted effects in a range of cellular proteins. It is doubtless, that TQ is ready to go for a clinical trial, due to its numerous promising biological effects and therapeutic potentials[8]. Having strong antioxidant capacity through antiradical including ROS, direct reduction of oxidizable substrates and induction of cellular antioxidant molecules, they may be good sources as cytoprotective agents, especially, the TQ, although' its mutagenic effect is yet to be found out. The carcinogenic and immunosuppressive cytotoxic effects of NS oil can be overcome by the co-treatment with antibiotics or radiotherapy.

Being a spacious habitual world-wide and having good number already isolated chemical moieties, NS is a weapon to the drug scientists. A number of researches have been done on this plant and its isolated compounds, especially on TQ and its derivatives and nigellone telling that chemical modification may bring a fruitful outcome to the drug library. In addition, some clinical uses suggest that NS is a safe and health promoter, especially observed in anti-fertility test. Although, the exact mechanism of actions of the investigated pharmacological potentials is yet to be found out, but the toxicological and its interaction profiles suggesting beneficial rather than detrimental effects. Generally, substances having antioxidant, antimicrobial or cytotoxic other than genotoxic and mutagenic potentials are good for healthy consumption. NS falls in this category. Finally, for its wide variety of activities, NS may be called the 'marvelous shrub'.

CONCLUSION

Drugs from the shrubs are one of the potential plant derived sources. Interestingly, now a day herbal medicaments are in a great attention to the consumers world-wide. Doubtless, the traditional medicines are still occupying potential sources of phyto-based remedies. A potential and diverse activity of a scrupulous source is the stimulation to the drug researchers. The NS and few of its isolated compounds such as TQ (including its derivatives), nigellone, α-hederin and linoleic acid have been demonstrated for a number of important pharmacological activities. In addition, the clinical usages of NS, making the herb and its constituents potential phytotherapeutic tools. The NS is a health promoting herb.

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Conflict of interest

None declared.

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