Research Article
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Nigellalogy: A review on *Nigella Sativa*

Abstract

Nigella sativa and its constitutions including some isolated compounds are the potential sources of remedies of varieties of ailments such as antioxidant, anti–inflammatory, antibacterial, antifungal, antiparasitic and antiprotozoal, antiviral, cytotoxic, anticancer, neuro–, gastro–, cardio–, hetapto– and nephroprotective activities. In addition, the *N. sativa* implies beneficiary effects on reproductive, pulmonary and immune systems along with diabetes mellitus (DM), fertility, breast cancer, dermatological complications, dehydration, dyspepsia, osmotic balance and so on. Among the other isolated chemical moieties, thymoquinone (TQ) is a good target for its potential antimicrobial, antimicrobial, anti–inflammatory, chemopreventive, antitumoral and other activities. The *N. sativa* is evident to promote health in some non–clinical and clinical studies. Otherwise, TQ in a number of animal test systems is evident to produce no negative alterations of the body biomarkers in contrary it improved health quality. This paper depicts a more mechanistic revision on *N. sativa* and its constitutions. In conclusion, findings on *Nigella* may be featured as a health jackpot.

Keywords: *nigella sativa* L., *nigella*–constitutions, shrub

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Muhammad Torequl Islam,[1,2,3] Bishwajit Guha,[3] Sajjad Hosen,[3] Thoufiqul Alam Riaz,[3] Sarrin Shahadat,[3] Leonardo da Rocha Sousa,[2] Jose Victor de Oliveira Santos,[2] Josemar José da Silva Júnior,[2] Rosália Maria Tôrres de Lima,[1,2] Antonio Lima Braga,[2] Antonielly Campinho dos Reis,[2] Marcus Vinicius Oliveira Barros de Alencar,[1,2] Ana Amélia de Carvalho Melo–Cavalcante[1,2]

[1]Northeast Biotechnology Network (RENORBIO), Federal University of Piauí, Brazil
[2]Laboratory of Toxicology and Genetics, Federal University of Piauí, Brazil
[3]Department of Pharmacy, Southern University Bangladesh, Bangladesh

Correspondence: Torequl Islam, Northeast Biotechnology Network (RENORBIO), Post–graduation Program in Biotechnology, Federal University of Piauí, Teresina (Piauí)–64.009–550, Brazil, Email mti031124@gmail.com

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Abbreviations: 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 b_1; bcl–2, cyclin b_2; bcl–xl, cyclin b xl; BUN, blood urea nitrogen; CAT, catalase; CDK–p^{16}, cyclin–dependent kinase p^{16}, CGD, conjugated diene; c–JUNK, c–jun–n–terminal kinase; CK, creatinine; COX–1, cyclooxygenase–1; COX–2, cyclooxygenase–2; CP, cisplatin; CVS, cardiovascular system; DM, diabetes mellitus; FABPs, fatty acid binding proteins; FAS, fatty acid synthase; GPx, glutathione peroxidase; GSH, reduced glutathione; GSH–ST, glutathione–s–transferase; HbA_1c, glycosylated haemoglobin; HDAC, histone deacetylase; HDL–C, high–density lipoprotein–c; HIV, human immunodeficiency virus; i.g., intragastric; i.p., intraperitoneal; INF–γ, interferon–gamma; IL–1, interleukin–1; IL–10, interleukin–10; IL–1β, interleukin –1beta; IL–6, interleukin–6; LDH, lactate dehydroginase; LDL–C, low–density lipoprotein–c; LPO, lipid peroxidase; LPO, LT_4, leukotriene–d_4; MDA, malonilealdehyde; MPO, myloperoxidase; NF–κB, nuclear factor–kappa–b; NK, natural killer; $NLRP_3$, nucleotide–binding oligomerization domain receptor 3; NO, nitric oxide; OSI, oxidative stress index; OXT, oxytetracycline; p.o., per oral; PET, pulmonary function test; PGD, prostaglandin; PGE_2, 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, cholesterol; TG, thyroglobulin; TNF–α, tumor necrosis factor–alpha; TOS, total oxidative status; TQ, thymoquinone; TSH, thyroid stimulating hormone; UI, ulcer index

Introduction

This revision is stimulated by the talks of the noble man, the last Prophet of the religion Islam, Hazrat Mohammad (Sm); 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. When we started, we found large amount of evidence (No. 1290) on this miraculous medicinal plant, belonging to its parts extracts (with aqueous/organic/aqueous–organic solvents), seed oil, essential oil, fatty acids, conjugations, and isolated compounds. A few of them covered co–treatments with other agents including biochemicals. We found a major revision on this plant done by Ahmad et al.[2] along with a dermatological revision of Aljabre et al.[3] an immunomodulatory and anti–inflammatory revision of Amin et al.[4] an anti–inflammatory, antioxidant, an immunomodulatory revision of Gholamnezhad et al.[5] male fertility revision of Mahdavi et al.[6] and metabolic parameters in diabetes mellitus revision of Heshmati et al.[7] These six articles inspired me to take them as a guide for previous evidences on *N. sativa*. Finally, we selected the potential publications on this plant from 2014 to March 15, 2016 and from the accumulated data we present here an activity–wise revision of this plant with an emphasized on mechanism of action way.

Nigella sativa

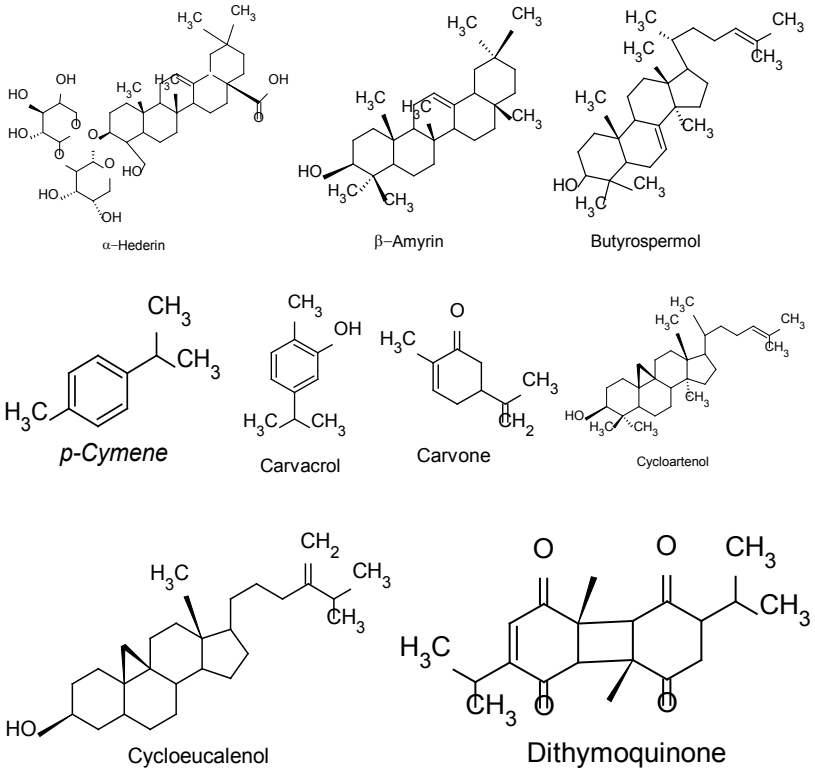
Nigella sativa L. (*N. sativa*) is a small shrub (20–90cm in tall) under the botanical family, *Ranunculaceae*. It is native to Southern Europe, North Africa and Southeast Asia; cultivated in many countries in the world like Middle Eastern, Mediterranean region, South Europe, India, Pakistan, Syria, Turkey, Saudi Arabia.[1] *N. sativa* 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 *N. sativa* was frequently used in ancient remedies (Unani, Ayurveda, Chinese and Arabic) in Asian countries and in the Middle–East. The use of *N. sativa* seeds had been mentioned by Ibne–Sina (980–1037) in his famous book Al–Qanoon fitt–Tibb.[2] Traditionally *N. sativa* is used as a medicament of a variety of disorders in the respiratory system, digestive tract, cardiovascular system (CVS), kidney, liver, and immune system. Its uses in fatigue and dispiritedness are 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 antiseptic and local anesthetic.[1]

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%),[1] cellulose (6.8–7.4%) and moisture (8.1–11.6%).[7] The seeds are also rich in various vitamins (e.g. – A, B_1, B_2, B_3 and C) and minerals (e.g., – Ca, K, Se, Cu, P, Zn, Fe). Carotene and vanillic acid are also found existing in seeds and roots, and shoots. 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.[1] 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.[8] in *N. sativa*.

The seed contains alkaloids that 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 in *N. sativa*. The other chemical components are carvone, nigellicine,[1] 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 ≥C_{15} 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 A_3, a_4, A_5, C, N. mines A_1, a_2, B_1, and B_2.[2] Chemical structures of some important chemical moieties are shown in Figure 1.



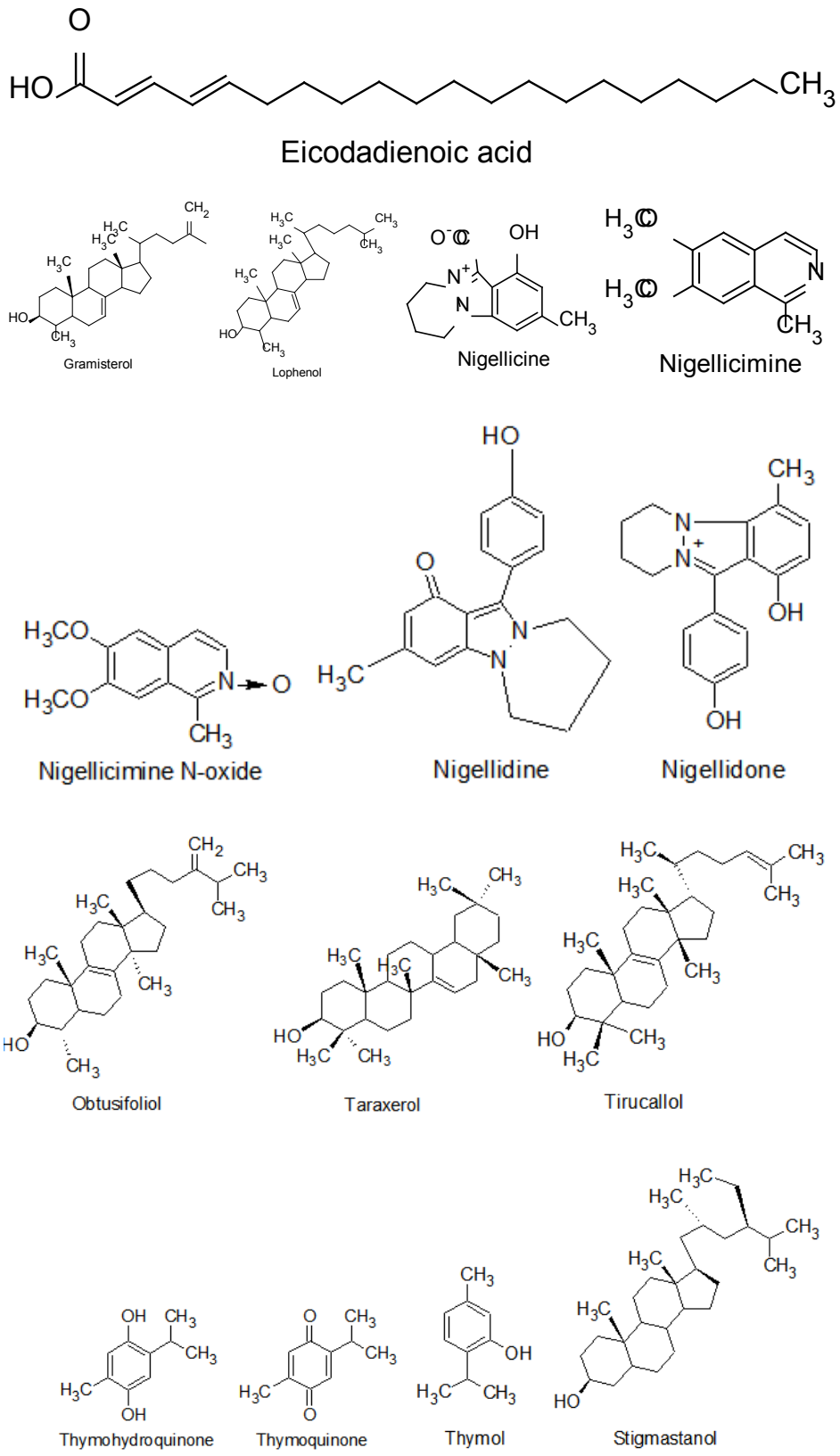


Figure 1 Some important chemical moieties isolated from *N. sativa*.

Potential activities

Nigella versus bacteria

N. sativa is reported to have strong antibacterial activity against gram positive (*Staphylococcus aureus*) and gram negative (*Pseudomonas aeruginosa* & *Escherichia coli*) species. It shows 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. It can fight against resistant microorganisms, including against many multi–drug–resistant gram positive and gram negative bacteria.[3] According to Manju et al.[9] the EO from *Nigella* is able to protect Artemia spp. from Vibrio parahaemolyticus Dahv$_2$ infection. According to Hariharan et al.[10] TQ has shown antimethicillin–resistant activity in *S. aureus*.

Nigella versus fungi

N. sativa acts against Candida albicans and Madurella mycetomatis and its isolated compound, TQ against *Aspergillus niger*, *Fusarium solani* and *Scopulariopsis brevicaulis*, where the activity was reported more effective than amphotericin–B and griseofulvin. The TQ also effective against *Trichophyton spp.*, *Epidermophyton spp*., and *Microsporum spp*. In addition TQ, thymohydroquinone and thymol are also demonstrated an antifungal effect against many clinical isolates, including dermatophytes, molds and yeasts.[3] Black seed oil (10–200µg/mL) is also evident to act against *Saccharomyces cerevisiae* and *C. utilis*.[11]

Nigella as antiviral agent

N. sativa was shown to enhance helper–T–cell (T_4) and suppressor–T–cell (T_8) ratio and increased natural killer (NK) cell activity in human. Otherwise, it is proven as a good inhibitor to the human immunodeficiency virus (HIV) protease and murine cytomegalovirus. In the latter case, it was found to increase in number and function of M–phi and CD_4+ve T cells with the production of interferon–gamma (INF–γ) was reported.[3]

Nigella versus parasites

N. sativa was shown to have anti–leishmaniasis, anti–miracidia, anti–cercariae and anti–*Schistosoma mansoni* potentials. In the latter case the oil of the black seed showed strong activity as compared to a well–known anti–schistosomal and anthelmintic drug for domestic animals, prazequantel; where it produced a potentiating effect with co–treatment.[3] Simalango[12] suggested that ethanol extract of *N. sativa* (0.5–8%) produced significant anti–Ascaris suum activity.

Nigella in wound infection

The wound healing capacity of *N. sativa* was evaluated in farm animals, mice and human gingival fibroblast. The accumulation result were indicated that there was a reduction in absolute differential WBC counts, local infection and inflammation, bacterial expansion and tissue impairment, and free radical production. An elevation of basic fibroblast growth factor and transforming growth factor beta were also reported.[3]

Antioxidant capacity of *Nigella*

A number of *in vitro* and *in vivo* antioxidant studies have been conducted with *N. sativa* extracts, seed oil and TQ. The finding is suggesting having potential radical scavenging and inhibitory effects of oxidative stress. TQ effectively 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). It also reduced the malonilealdehyde (MDA), conjugated diene (CGD) levels and pro–inflammatory mediators interleukin–1beta (IL–1β), interleukin–6 (IL–6), tumor necrosis factor–alpha (TNF–α), interferon–gamma (IFN–γ), and prostaglandin (PGE2) rather than interleukin–10 (IL–10).[1,4] Figure 2 tells the basic antioxidant pathways of *Nigella* and its constitution.

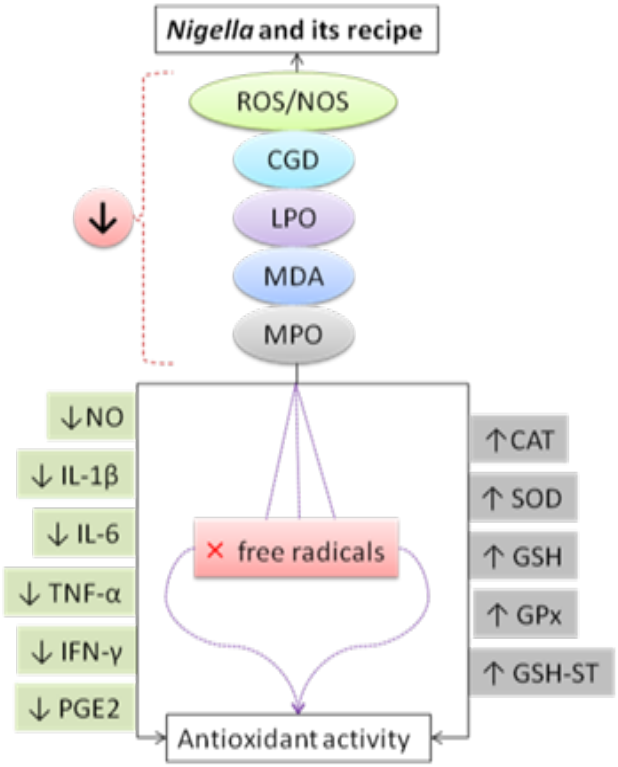


Figure 2 Anti–oxidative action pathways of *Nigella* and its recipe

Nigella in inflammation

Findings from different animal models suggest that *N. sativa* extracts, seed oil and TQ have anti–inflammatory potentials. This activity belongs to the reduction of NO production, interleukin–1 (IL–1), cyclooxygenase–1 (COX–1), cyclooxygenase–2 (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] Topical application of TQ induced the expression of hemeoxygenase–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 was also shown to diminish 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. TQ also attenuated the phosphorylation of Akt (protein kinase B), c–Jun–N–terminal kinase (c–JUNK) and p^{38} mitogen–activated protein kinase (MAPK–p^{38}). A decrease in expression of $NLRP_3$ (NACHT, LRR, and pyrin domain–containing protein 3) in $B_{16}F_{10}$ 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 $NLRP_3$ inflammasome.[3–5] Figure 3 tells the basic anti–inflammatory activity pathways of *Nigella* and its constitution.

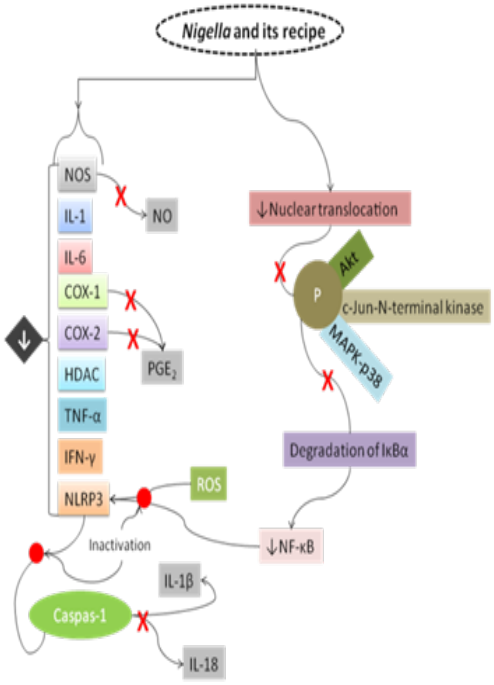


Figure 3 Anti–inflammatory capacity pathways of *Nigella* and its recipe

Nigella in cancer

The black seed oil is an enhancer of the NK cells, which is a potential applicability in immune therapy. However, the components in oil may induce antioxidative–induced prooxidant effects thus the carcinogenetic effect. In addition, 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 p^{16} (CDK–p^{16}) and a decrease in cyclin–d_1 (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 p^{53} with a decreased level of cyclin–b_1 (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), Fsa^R and murine tumor models of fibrosarcoma and SCC. TQ showed potent anticancer activity in A_{431} and Hep–2 cells via apoptosis by increasing the sub–G_1 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 were observed.[3] A research done by Khalife et al.[13] suggesting that TQ induced apoptosis through p^{53}–independent pathway with an expression of p^{21} and arrested cell–cycle S phase in human colon cancer cells. TQ is also anticancer agent to a number of cell lines including MCF–7/Topo breast carcinoma cells and is a significant down–regulator of 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.[1] Recent evidence suggests that the nanoemulsion of *Nigella* oil at a dose of 20–80μL/mL caused cell membrane blebbing, cytoplasmic vacuolation, marginalization of chromatin, and fragmentation of the nucleus in MCF–7 cells.[14] A recent evidence suggests that topical use of black seed oil (600mg) reduced cyclic mastalgia in woman (n=52) and the activity is significantly comparable to the painkiller, diclofenac.[15] A basic *Nigella*–anticancer potential has been sketched in Figure 4.

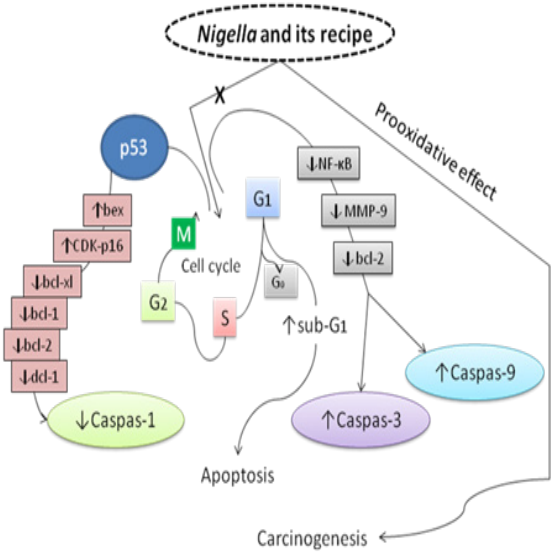


Figure 4 Basic anticancer pathways of *Nigella* and its derivatives.

Nigella in diabetes

N. sativa was found playing an important role in the reduction of blood glucose level with an augmenting insulin level and C–peptide in rats. 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 is evident to increase the levels of insulin, Hb with a significant decrease in glucose and glycosylated hemoglobin (HbA_1c) levels. *N. sativa* showed a synergistic activity with parathyroid hormone in improving bone mass, connectivity, biomechanical behavior and strength in T_2D rats. The black seeds are also evident an advantageous therapy in insulin resistance syndrome and dislipidemic patients. An insulin–sensitization action *via* enhancing ACC phosphorylation (mainly MAPK signaling pathway) and muscle $GLUT_4$ content as well as progressive normalization of glycaemia are also seen in *N. sativa* treated diabetic Meriones shawi.[2,6] Lipid (4%) and volatile (3%) fractions in streptozotocin–induced diabetes mellitus (DM) rats reduced toxicological and adverse consequences to the animals.[16] In addition, an improved glycemic status and lipid profile with oil treatment at 3g/3–times/day in DM patients (n=72) were suggested by Heshmati et al.[17] 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 chronic glucose overload, suggesting a 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.[18] Otherwise, MAPK regulates a number of transcriptional factors, altering of which interferes in cell–cycle. Thus, *N. sativa* and TQ may be a good remedy for both type 1 and 2DM patients, as in this consequence maintenance of beta–cell integrity and secretion of insulin sufficient for glycogenesis and phosphorylation of raised glucose in 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 *N. sativa* and TQ may connect with their antioxidant, antimicrobial, cytotoxic and anti–inflammatory activities. Otherwise, the decreasing level of HbA_1c is one of the remedy for cardiovascular disease, nephropathy, neuropathy, and retinopathy. Figure 5 tells the possible antidiabetic actiion pathways of *Nigella* and its constitution.

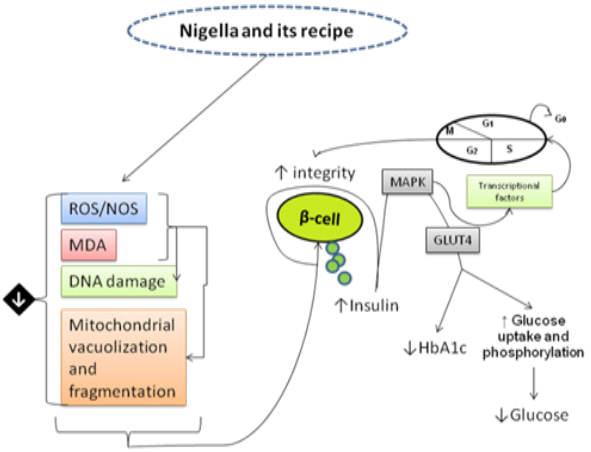


Figure 5 Possible anti–diabetic action pathways of *Nigella* and its recipe.

Nigella on immune system

Along with NK antitumor activity, *N. sativa* is a demodulator of secretion of a number of pro–inflammatory mediators with up–modulation of secretion of Th_2 versus Th_1, cytokines in splenocytes. The black seed extract also evident to restore the resistance against granulocyte–dependent *C. albicans*. A study performed by the 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. However, it produced immunoprotective effect when chronic administration of antibiotic occurred in pigeons. The black seed oil also acted as a radioprotective agent against immunesuppressive and oxidative effects of ionizing radiation. In addition, an increased level of IFN–γ with a significant decreased in pathological changes of the guinea pigs' lung was reported by *N. sativa* oil treatment. It is also effective in allergic diarrhea.[2,4,5] A recent evidence suggests that seed oil is protective against γ–radiation–induced damage in jejunal mucosa.[19] *Nigella* EO at a dose range of 5–20g/kg (oral feed) in chickens improved FCR and plasma lipid profile and antibody–mediated immunity in a 6 weeks treatment.[20] In addition, *Nigella* oil reduced thyroid stimulating hormone (TSH) and anti–thyroid peroxidase antibodies in patients with Hashimoto's thyroiditis.[21]

Nigella on nervous system (NS)

Methanolic extract of *N. sativa* is a potent analgesic and antidepressant. In addition, an anxiolytic activity via increasing serotonin (5–HT) and decreasing hydroxyindole acetic acid (5–HIAA) levels were noticed in rat brain. An increased 5–HT secretion along with improving learning and memory capacity were detected in rats. As it caused an 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 phenomena anti–acetylcholinesterase (anti–AChE) suggests *N. sativa* and TQ having anticonvulsant activity. There is a suggestion for GABAA–ergic anticonvulsant effect of TQ.[2] *Nigella* EO at 1g/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.[22] *Nigella* EO is also evident to prevent cerebral edema in the hippocampus tissue of the rat brain.[23] Fahmy et al.[24] suggested that oil at a dose of 2.8g/kg when treated orally (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. Otherwise, oil at a dose of 4mL/kg/day (p.o.) in tramadol treated male albino rats protected the cortical neurons and myelinated axons.[25] *Nigella* EO at 500mg in adolescent human males (n=48) stabilized mood, decrease anxiety and modulate cognition for a 4 weeks treatment.[26] A possible neuroprotectivity and activity on NS is shown in Figure 6.

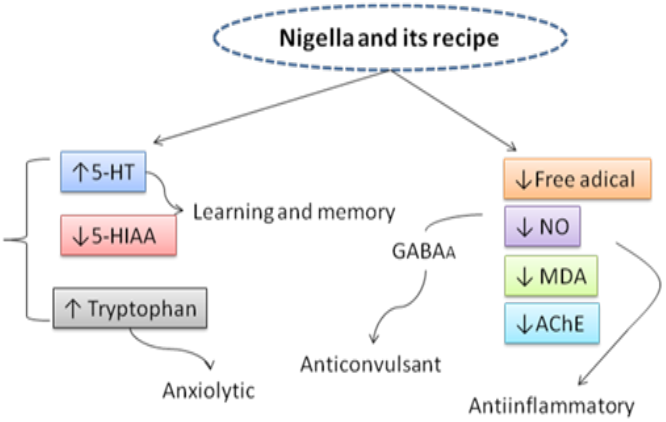


Figure 6 A possible neuroprotective trait of *Nigella* and its recipe.

Nigella on gastrointestinal tract (GIT) system

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 antisecretion pathways. A decreased LPO and lactate dehydroginase (LDH), MPO, MDA and increased GSH, SOD, GPx, GSH–ST without altering of gastric CAT was also reported in rats. TQ was found significant effects in diarrhea, colitis, inflammatory bowel diseases, anti–Helicobacter pylori and body weight loss.[1] Possible GIT protective pathways of *Nigella* and its constitution are shown in Figure 7.

Nigella 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–C (LDL–C), and

thyroglobulin (TG) with an increased high–density lipoprotein–C (HDL–C) level.[27]

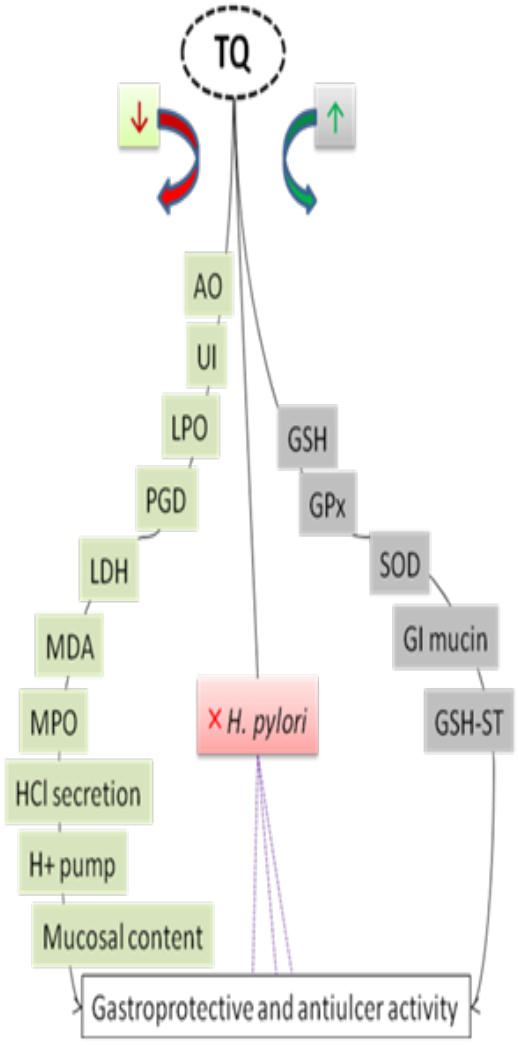


Figure 7 Possible GI-protective pathways of *Nigella* and its recipe.

Nigella in hepatic system

N. sativa 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] *N. sativa* oil at a dose of 25–100µg/mL protected hepatocytes from N–acetyl–p–aminophenol (APAP)–induced hepatotoxicity and metabolic disturbances in TIB–73 cells of mice.[28] A similar activity was also observed by Hamza & Salem Al–Harbi[29] with aqueous extract of *N. sativa*, where the activity was thought to be linked with improving antioxidant potential and suppressing both lipid peroxidation and ROS generation.[28] The black seed oil at a dose of 2mg/kg (p.o.) with cisplatin (CP)–treated rats are also evident for its hepatoprotective activity via improving energy metabolism and strengthening antioxidant defence pathways.[30]

Nigella in urinary system

N. sativa along with ascorbic acid (Vitamin C) produced a nephroprotective effect by lowering serum creatinine (CK), blood urea nitrogen (BUN) and antioxidant activity in rabbits. Otherwise, TQ showed an effect on renal expression of organic ion transporters and multidrug resistance–associated proteins in rats. An increased protein levels of the efflux transporters MRP_2 and MRP_4 and decreased expression of OAT_1, OAT_3, OCT_1 and OCT_2 was observed in rats. Along with decreasing tubular necrosis score, *N. sativa* is a good reducer of CK, urea, MDA, NO, ROS, OSI and TOS levels and augmenter of TAC, SOD, GPx in kidney tissue and blood. TQ is evident to have a complete reversal of 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] The black seed ethanol extract at 250–100mg/kg (p.o.) in female Wistar Albino rats showed a significant nephroprotective activity on paracetamol–induced nephrotoxicity.[31] Otherwise, Cd–induced nephroprotectivity is also evident in rats by Erboga et al.[32]

Nigella on pulmonary system

Both nigellone and TQ are evident to inhibit leukotriene–d_4 (LT_4) in the trachea, where the activity of the first one was concluded via mucociliary clearance. *N. sativa* reduced a significant peribronchial inflammatory cell infiltration, alveolar septal infiltration, alveolar edema, alveolar exudates, alveolar macrophages, intestinal fibrosis, granuloma, necrosis formation, NOS and a rise in surfactant protein D in the pulmonary system. *N. sativa* is also evident to have beneficial effects against lung injury and hypoxia–induced lung damage. Moreover, *N. sativa* puffs are proven to relieve asthma symptoms, frequency of asthma symptoms/weakness, chest wheezing and pulmonary function test (PFT) values with a bronchodilatory effect.[2]

Nigella on reproductive system

TQ decreased TAC and MPO levels in C_{57}BL/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. Infertile men (n=34) when treated with 2.5mL black seed oil for 2 months improved abnormal semen quality without producing any adverse effect was observed.[33] According to Mahdavi et al.[6] the black seed oil is a good candidate for treating male infertility. Hexane and methanol extracts of *N. sativa* produced significant anti–fertility in Sprague–Dawley male and female rats, respectively. Otherwise, *N. sativa* inhibited uterine smooth muscle contraction in rats and guinea pigs.[2,6] TQ when treated with olive oil caused reduction of polycystic overy in rats via NF–κB signaling pathway.[34]

Nigella in dyspepsia

Patients (n=70) with functional dyspepsia when treated with *Nigella* oil of 5mL (p.o.) for 8 weeks, a significant lowering of dyspepsia was observed.[35]

Nigella in osmotic balance

The geriatric patients (n=42) when treated with black seed oil (22.6µg/25µL) for 2 weeks, it was concluded that it should be an alternative therapy of the isotonic sodium chloride (0.9% NaCl) solution.[36] Table 1 bears some important activities found in 2014 to March 15, 2016.

Table 1 Some recent research evidences found on Nigella recipes

Form/ chemicals	Dose/R.O./test systems	Activity	References
Essential oil	5–50g/L for antioxidant assyas, 0.2–2.0µg/mL for antimicrobial	Produced antioxidant activity and protected the Artemia spp. after experimental infection of Vibrio parahaemolyticus Dahv–2.	Manju et al.[41]
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.[21]
Essential Oil Nanoemulsion (20–50nm diameter)	20–80µL/mL in MCF–7 cells	Produced cell membrane blebbing, cytoplasmic vacuolation, marginalization of chromatin, and fragmentation of the nucleus.	Periasamy et al.[14]
Oil	–	Reduced total cholesterol, LDL–C, and TG levels and increased HDL–C.	Sahebkar et al.[27]
Oil	400mg/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.[42]
Oil	25–100µg/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.[28]
Oil	–	Antioxidant and antimicrobial activities.	Ramadan[43]
Phenolic–Protein complexes	100µL in *in vitro* test.	Antioxidant and ACE inhibitory properties.	Alu'datt et al.[44]
n–Hexane and ethanol fractions	50–2000µg/mL in ACHN (human renal adenocarcinoma) and GP–293 (normal renal epithelial) cell lines	Cytotoxic activity.	Shahraki et al.[45]
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.[30]
Gold coated nanoparticles	Nano–particles (15.6–28.4nm) in A_{549} lung cancer cell line and Staphylococcus aureus 10µg/mL	Inhibited A_{549} lung cancer cells and S. aureus.	Manju et al.[41]
TQ	1µM/mL and 2mg/200µL (s.c.) with olive oil in rats	Remedy for polycystic overy via NF–κB signaling pathway.	Arif et al.[34]
TQ	In neutrophils	Strongly inhibited fMLF–induced superoxide production and granules exocytosis in neutrophils.	Boudiaf et al.[46]
Seeds Ethanol extract	250–100mg/kg (p.o.) in female Wistar Albino rats	Significant nephroprotective activity on paracetamol–induced nephrotoxicity.	Canayakin et al.[31]
TQ	50mg/kg in male Wistar albino rats for 30 days	Significant nephroprotective potential against Cd–induced toxicity.	Erboga et al.[32]
TQ	In Staphylococcus aureus	Antimethicillin–resistant activity.	Hariharan et al.[10]
TQ	40µM TQ and/or 0.6µM 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.[13]

Table Continued...

Form/ chemicals	Dose/R.O./test systems	Activity	References
Oil	600mg (topical) in woman (n=52) for 2 months	Clinical effectiveness comparable to topical diclofenac in the treatment of cyclic mastalgia.	Huseini et al.[15]
Oil and TQ	–	Oral health and hygiene.	Al–Attass et al.[37]
Oil	40mg/kg/day (i.g.) in male albino rats	Ameliorated the toxic changes caused by formaldehyde on corneas.	Salem et al.[47]
Oil	400mg/kg (p.o.) in rats	Protective effects against gamma–radiation–induced damage in jejunal mucosa.	Orhon et al.[42]
TQ	10mg/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.[48]
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.[18]
EO	10–200µg/mL free daricanl scavenging and anti–Saccharomyces cerevisiae, anti–Candida utilis, anti–Candida albicans	Antioxidant and anti–yeast activities.	Nadaf et al.[11]
Oil	4mL/kg/day (p.o.) in tramadol treated male albino rats	Protected the cortical neurons and myelinated axons.	Omar[25]
Methanol extract	100 and 500mg/kg (p.o.) in hyperlipidemic rats	Antioxidative and hypolipidemic effects.	Ahmad & BEG[49]
Ethanol extract	100–1000µg/mL in male Wistar rats	Increased cytokines balance in Th1/Th2.	Gholamnezhad et al.[50]
Oil	3g/day (dietary) in obese women (25–50 yrs) for 8 weeks	Modulated systemic inflammatory biomarkers.	Mahdavi et al.[51]
Oil	5ml (p.o.) in patients with functional dyspepsia (n=70) for 8 weeks	Lowered dyspepsia	Mohtashami et al.[35]
EO and TQ	–	Regulation of immune reactions implicated in various infectious and non–infectious conditions including different types of allergy, autoimmunity, and cancer.	Majdalawieh & Fayyad[52]
Oil	–	Anti–inflammatory, antioxidant, and immunomodulatory activities.	Gholamnezhad et al.[4]
Oil	–	Good candidate for male infertility treatment.	Mahdavi et al.[5]
Oil	100–400mg/kg (i.p.) in rats	Prevented hippocampal neural damage which is accompanied with improving effects on memory.	Seghatoleslam et al.[53]
Aqueous extract	0.25g/kg in mice for 30 days	Powerful reducing capacity of APAP–induced hepatotoxicity and antioxidant activity.	Hamza & Salem Al–Harbi[29]

Table Continued..

Form/ chemicals	Dose/R.O./test systems	Activity	References
Oil and its components	–	Anti–diabetes mellitus potential.	Heshmati & Namazi[6]
Oil	3g/day (one three times a day) in T2 DM patients (n=72)	Improved glycemic status and lipid profile.	Heshmati et al.[17]
Hydro–alcoholic extract	100–400mg/kg (p.o.) in rats for 8 weeks	Decreased MDA concentration, improved learning and memory capacity through antioxidative ways.	Beheshti et al.[54]
Methanol extract	0.1mg/disc in Trichophyton mentagrophytes, Microsporum canis and Microsporum gypseum	Antifungal activity.	Mahmoudvand et al.[55]
Oil	1mg/kg in tramadol–induced male albino rats for 30 days	Hepato– and nephroprotective effects.	Elkhateeb et al.[56]
Oil	2.8g/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.[24]
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.[16]
Oil	2.5 and 5.0mL/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.[57]
Oil	2.5mL in infertile men (n=34) for 2 months	Improved abnormal semen quality without producing any adverse effect.	Kolahdooz et al.[33]
Oil	22.6µg/25µL in geriatric patients (n=42) for 2 weeks	Can be used as an alternative to the isotonic sodium chloride solution.	Oysu et al.[36]
EO and TQ	EO 1g/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.[22]
EO	1–50mg/kg (i.p.) in Wistar rats	Prevented cerebral edema in the hippocampus tissue of the brain.	Hobbenaghi et al.[23]
EO	5–20g/kg (oral feed) in chickens for 6 weeks	Improved FCR of boilers and improved plasma lipid profile and antibody–mediated immunity.	Ghasemi et al.[20]

Table Continued..

Form/ chemicals	Dose/R.O./test systems	Activity	References
Methanol extract	200mg/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.[58]
EO	500mg in adolescent human males (n=48) for 4 weeks	Stabilized mood, decrease anxiety and modulate cognition.	Sayeed et al.[26]
Ethanol extract	0.5– 8% in Ascaris suum	Anti–helminthic effect.	Simalango & Utami[12]

Topical applications

TQ–induced skin darkening via chlonergic mechanisms of muscarinic receptor in the melanin dispersion is evident, whereas, *N. sativa* oil for decreasing vitiligo area scoring index without seeing adverse effects. However, TQ and nigellone inhibited histamine release, protected histamine–induced bronchospasm in guinea pigs, decreased lung ensiphilia, elevated Th_2 cytokines and raised Ig_E and IgG_1 antibodies in mice. Otherwise, *N. sativa* is 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 potentials.[3] There is evidence on oral health and hygiene of black seed oil and TQ.[37]

Nigella lethal dose (LD)

In mice, the dose causing death of fifty percent experimental animals (LD_{50}) values of fixed oil of black seed was reported to be 26.2–31.6mg/kg and 1.86–2.26mg/kg with single oral (p.o.) and intraperitoneal (i.p.) doses, respectively. In another study, calculated LD_{50} for TQ was 89.7–119.7mg/kg and 647.1–1094.8mg/kg after i.p. and p.o. administrations, respectively. In rat it was found to be 45.6–69.4mg/kg and 469.8–1118.8mg/kg after i.p. and p.o. administration, respectively. Data, suggesting TQ is more tolerated than the extract from *N. sativa*.[2]

Drug interactions

Table 2 tells that *N. sativa* has a good number of beneficial drug/chemical/biochemical interactions.

Table 2 Some important *Nigella* interactions observed with investigational cases in literatures

Drug/chemical/biochemical (induced activity)	*Nigella* recipe	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/CdC_{l2}	//	//
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

Table Continued..

Drug/chemical/biochemical (induced activity)	*Nigella* recipe	Observations
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

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. Otherwise, traditional medicines are still occupying a remedy–kingdom in particular areas. A potential and diverse activity of a scrupulous source is the stimulation to the drug researchers. *N. sativa*, in the previous literatures showing the shot, particularly TQ and its derivatives, nigellone, α–hederin and linoleic acid produced remarkable pharmacological activities. In addition, few clinical uses of human, suggesting that *N. sativa* and its constitution have safety profile.

Featuring

At low levels and temporary spikes of ROS are beneficial for health[38] rather than high production and chronic effects as they cause induction of pro–inflammatory cytokines, chemokines and pro–inflammatory transcription factors (NF–κB)[39] as well as induction of 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.[39] 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.[40] 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.[39] However, an ideal ROS neutralizer (antioxidant/cytoprotective agent) is not enough in the cancer therapy, even if it has antioxidant–mediated prooxidant capacity, 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 *Nigella* derived quinone and other *N. sativa* constitutions are evident to have target for a range of cellular proteins in their activity pathways. 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 the whose mutagenic effect is yet to be found out. The carcinogenic and immunosuppressive cytotoxic effects of *N. sativa* oil can be overcome by co–treatment with antibiotics or radiotherapy. Being a spacious habitual world–wide and having a good number already isolated chemical moieties of *N. sativa* is a weapon to the drug scientists. A number research has 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 *N. sativa* is safe and health promoter, especially observed in anti–fertility test. Although, the exact mechanism of action of the investigated pharmacological potentials yet to be found out, but the toxicological and its interaction profiles suggesting beneficial rather than detrimental effects. Generally, substances having antioxidant, antimicrobial cytotoxic other than genotoxic and mutagenic potentials are good for healthy consumption. TQ falls in this category, although the genotoxic and mutagenic potentials are still to be investigated. Finally, for its wide variety of activities, *Nigella* may be called the 'marvelous shrub'.

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Conflict of interest

The author declares no conflict of interest.

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