Review article

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OVERVIEW OF THE PRECLINICAL PHARMACOLOGICAL PROPERTIES OF *NIGELLA SATIVA* (BLACK SEEDS): A COMPLEMENTARY DRUG WITH HISTORICAL AND CLINICAL SIGNIFICANCE

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Nigella sativa (*N. sativa*, black seeds; or sometimes known by many other names such as the blessed seed by the Arabs, black cumin in the Holy Bible, black caraway and Kalonji in South Asia) has been traditionally used for many years not only as a food but also as complementary drug. It is the objective of this communication to review the evidence-based pre-clinical pharmacological actions of *N. sativa* as a basis of its existing and potential new human clinical uses. Primary PubMed literature searches and secondary Medline searches were conducted to define *N. sativa* pre-clinical pharmacological and toxicological actions using a retrospective narrative review of the published studies. The ground seeds, its oil and its various extracts exhibit very broad pharmacological actions in laboratory studies, which are predictive of human clinical efficacy. In laboratory studies, *N. sativa* possesses anti-inflammatory, analgesic, anti-diabetic, anti-hyperlipidemic, anti-convulsant, anti-microbial, anti-ulcer, anti-hypertensive, anti-asthmatic and anti-cancer activities. Its mode of action is mediated via several mechanisms, which include anti-oxidant, immunomodulating, cytoprotective and an inhibitory effect on some mediators of inflammation. Although the seeds contain many chemical components, thymoquinone and alpha-hederin are proven to be pharmacologically active. Despite *N. sativa* broad and worldwide pharmacological characterization, only limited non-clinical safety studies were reported. *N. sativa* has many potentially important therapeutic applications. The black seeds clearly warrant formal preclinical drug development consideration to investigate the pharmacology of its components, to standardize the contents of the dosage forms, to define the methods of the pharmaceutical preparation, to determine its pharmacokinetics characteristics and its safety profile. It is our opinion that *N. sativa* should be considered for clinical development initially for unmet therapeutic uses, especially in the fields of oncology, neurology, rheumatology, pulmonary medicine, infectious diseases and endocrinology.

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INTRODUCTION

For over two thousand years, *N. sativa* seeds, a plant from the Ranunculaceae (buttercup) family, has been used by various cultures in Asia, Africa, Europe, the Middle and Far East to flavor food and as a natural remedy for several diseases. The seeds have been traditionally used to treat variety of ailments related to respiratory health, digestive complaints, diabetes, infections, rheumatism, skin disorder, circulatory and immune system support (1).

The earliest written reference to *N. sativa* is thought to be in the book of Isaiah in the Old Testament, where the reaping of Nigella and wheat is contrasted (Isaiah 28: 25, 27; Wikipedia, the Free Encyclopedia). The Easton's Bible dictionary states that the Hebrew word ketsah refers to *N. sativa*. The many interesting uses of the black seeds have given it the Arabic approbation 'Habbatul Baraka', meaning the 'seed of blessing' (Wikipedia, the Free Encyclopedia). *Fig. 1* presents photos of the *Nigella sativa* plant, flower and seeds.

It is the objective of this communication is to provide a narrative review of the *N. sativa* seeds pharmaceutical, pharmacological and toxicological actions in order to define its intrinsic therapeutic actions and provide future research directions for identifying novel therapeutic drugs derived from this plant. Although other authors previously reviewed some aspects of the pharmacological studies included in this manuscript, our review provides a more comprehensive analysis of the relevant studies (1-3).

SEARCH METHODS

A primary and narrative search of published literatures was carried out using online PubMed literature searches, which

covered the period from January 1960 to April 2016. Secondary searches for some of the older studies identified in articles disclosed by PubMed literature was also performed using Medline. Only studies written in English language and with experiments that appeared to be properly designed and well controlled (before and after observations, with placebo or positive active comparator and with sufficient number of animals or observations) were considered for inclusion in this review. The key words in this searches included all the search items listed by PubMed under the heading of *Nigella sativa*. In addition sub-searches included the following additional key words: black seeds, black cumin seeds, pharmacology, chemistry, toxicology, pharmacokinetics, and pharmacodynamics were used both singly and in combination. Truncation and the use of adjacent searches were also conducted. In addition, some of the references of the articles uncovered from the primary searches were also obtained and examined because PubMed and Medline search engines do not index some foreign journals.

RESULTS

The PubMed online searches disclosed a total of 829 *N. sativa* available articles. The articles selected covered the pharmaceutical, pharmacological, biochemical and toxicological actions of *N. sativa* seeds, its oil and thymoquinone. Many articles were published in peer-reviewed international biomedical journals. Access to the complete manuscripts were either directly obtained from the authors or obtained from regional libraries, whenever possible. Fortunately, some of the abstracts uncovered by the Internet searches provided access to complete articles that were downloaded and printed. Therefore, the access to complete articles was of significant importance to this investigation.

All the published research specifically cited in this review adhered our selection criteria, especially with respect to the experimental design and the general conduct of the studies. All of the research investigations discussed in this review were concerned principally with the pharmacological characterization of *N. sativa* but not potential drug development considerations needed for regulatory submissions. Furthermore, all of the scientific investigations were financed either by the authors or their medical schools or by government grants, but not by any commercial sponsors or drug companies.

OVERVIEW OF CHEMICAL COMPOSITION AND DOSAGE FORMS

Two reviews of the chemical composition of the *N. sativa* seeds were published (4, 5). The *N. sativa* seeds contain numerous esters of unsaturated fatty acids with terpene alcohols. The *N. sativa* oil, also known by the name of essential oil, is cold-pressed from the seeds. The essential oil is a yellow to dark amber liquid and does not show fluorescence, not even when diluted with alcohol. A fixed oil is produced by the hydraulic expression of the seeds. Steam distillation of the seeds produces a yellowish brown volatile oil that has an unpleasant odor. The black seeds oil contains biologically active substances such thymol, thymoquinone, dithymoquinone (5). The reported oil content of the *N. sativa* seeds ranges from 0.1 – 1.5%. The essential oil contains linoleic acid (50-60%), oleic acid (20%), dihomolinoleic acid (10%), eicosadienoic acid (3%), and thymol related chemical compounds. Saturated fatty acids (palmitic and stearic acid) were present in an amount less than 30%. The seeds contain carbohydrates, proteins, alkaloids (nigellimin, nigellimin-N-oxide and pyrazole alkaloids include nigellidine and nigellicin) and saponins (4). Thymoquinone was identified as the main pharmacologically active component of the essential oil (up to 50%); p-cymene (40%), alpha-pinene (up to 15 %), dithymoquinone and thymohydroquinone were also present (4). In addition alpha-hederin, a triterpene saponin, was recently identified as another active component of *N. sativa*, however, not much was disclosed on its amount in the seeds or its chemical stability (6). Although both alpha-hederin and thymoquinone have cytotoxic action against many cancer cell lines and *in vivo* animal studies, alpha-hederin has not been as extensively studied as thymoquinone (7). Of interest, there are current attempts to prepare synthetic thymoquinone analogs in order to improve their anticancer pharmacology (5).

Of importance, the extent of thymoquinone concentration in the essential oil is dependent on the geographical source of the seeds since an Iranian sourced essential oil had a much lower concentration of thymoquinone than an Indian sourced essential oil. Hajhashemi *et al*. (8) analyzed the components of an Iranian sourced essential oil using gas chromatography and mass spectroscopy (GC/MS) methods. The analysis by GC/MS, identified some twenty compounds that were present in the oil with 0.4% (v/w) yield. Among the major compounds identified in the oil were para-cymene (37.3%) and thymoquinone (13.7%). Of

Fig. 1. Photographs of the *Nigella sativa* plant, flower and seeds. This figure was reproduced from Ahmad *et al*. (1) publication on the basis of noncommercial license.

interest, the concentration of thymoquinone present in the Iranian derived *N. sativa* essential oil (13.7%) was much lower than the concentration present in the Indian derived essential oil (up to 50%; 4). Such marked geographic variations point to the need for the standardization of active ingredient(s) by the use of acceptable chemical assay methods. *Fig. 2* presents the chemical structures of thymoquinone and related compounds isolated from the black seeds. *Fig. 3* presents the chemical structure of alpha-hederin.

Storage of the essential oil with a sunlight exposure results in chemical degradation of thymoquinone and higher oligocondensation products (4). Therefore, the essential oil needs to be protected from sunlight exposure due to the light-induced photo-isomerization of thymoquinone, which would lead to accumulation of dithymoquinone, a thymoquinone dimer (9). However, the degradation products have not been fully characterized with respect to their pharmacological and toxicological actions.

Agbaria *et al*. (9) had recently shown that controlled heating (roasting) of the black seeds for 10 minute at a temperature between 50 to 150 degrees centigrade significantly increased the content of thymoquinone in the essential oil and this increased concentration of thymoquinone was associated with an enhanced *in vitro* anti-proliferative action against mouse colon carcinoma
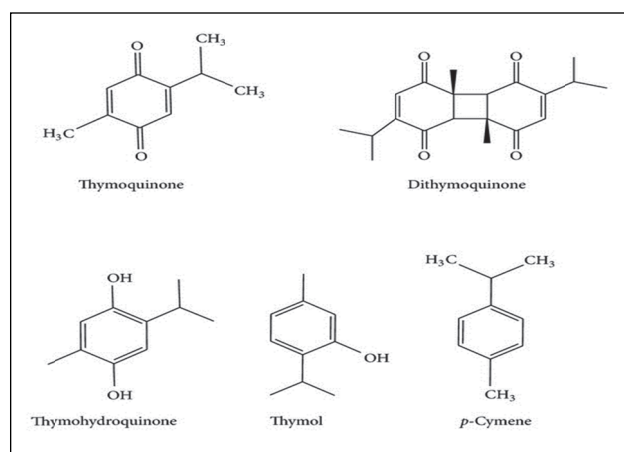
cells (MC38) growth rate (*Fig. 4*). However, roasting of the black seeds at temperature exceeding 200 degrees centigrade led to marked decrease of the essential oil anti-proliferative activity indicating a loss of its active ingredient (*Fig. 4*). Agbaria *et al*. (9) speculated that the controlled heating causes oxidation of thymol and converts it initially into thymohydroquinone and subsequently to thymoquinone. The Agbaria *et al*. (9) result is consistent with the work of Kiralan (10) who also observed that heating of the black seeds at 350 degrees centigrade decreased the content of thymoquinone and increased the contents of pyrazine and furan analogs, presumably considered as inactive compounds. Such lines of evidence strongly suggest that roasting of the seeds at 100 degree centigrade for 10 minutes is essential for increasing its anti-proliferative actions and that thymoquinone is probably the most active anti-proliferative component of the black seeds. Unfortunately, none of the pharmacological studies covered in this review disclosed whether the investigators had roasted the seeds prior to their milling to form a powder that was used in their laboratory studies. Clearly, it is most essential that a standardized pharmaceutical preparation of the dosage form must be prepared prior to embarking on formal drug development of the milled seeds, its oil, its extract(s) or an active principal.

OVERVIEW OF THE PHARMACOLOGICAL INVESTIGATIONS

N. sativa seeds had been found to possess diverse pharmacological activities involving many physiological systems. Despite the fact that many chemical components exist

Fig. 2. Chemical structures of thymoquinone and related compounds. This figure was reproduced from the Rahmani *et al*. (27) publication on the basis of noncommercial license.
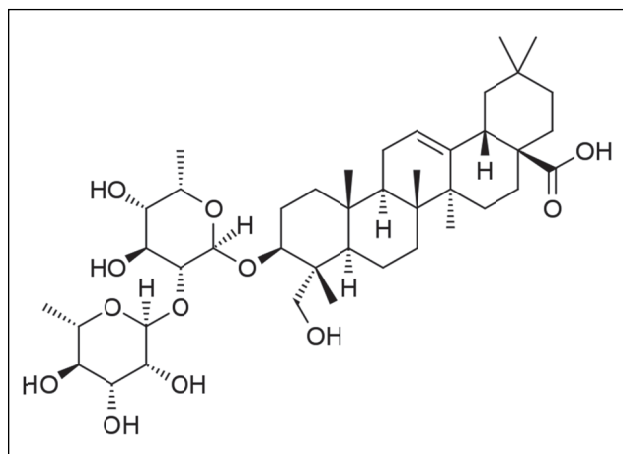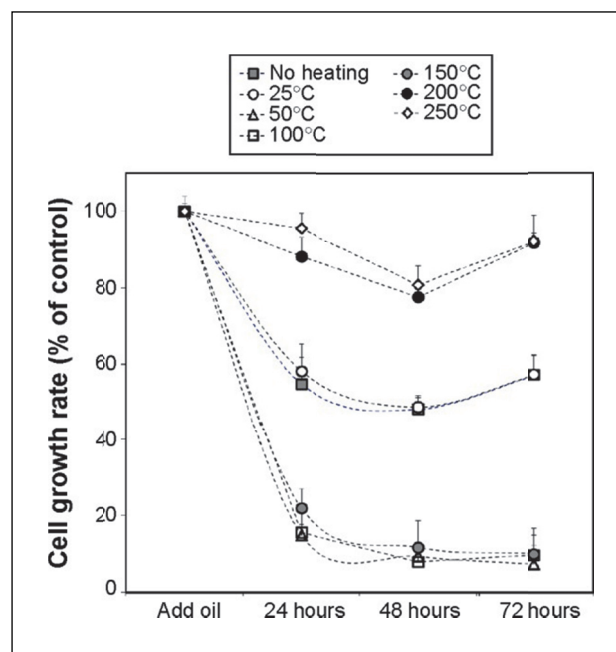

Fig. 3. Chemical structure of alpha-Hederin. This figure was reproduced from the Khan and Afzal (5) publication on the basis of a noncommercial license issued by Springer Press Limited.

Fig. 4. Effect of black seeds roasting on the anti-proliferative efficacy of the *Nigella sativa* oil which was extracted from seeds after different thermal processing protocols (no heating, 25°C, 50°C, 100°C, 150°C, 200°C, and 250°C for 10 minutes) on growth rate of mouse colon carcinoma (MC38) cells following 24, 48, or 72 hours of incubation. Data are presented as the mean ± standard deviation (n = 4). This figure was reproduced from the Agbaria *et al*. (9) study on the basis of a noncommercial license issued by Dove Medical Press Limited.

in the *N. sativa* seeds, no systematic attempts were made to pharmacologically characterize each individual component. Only thymoquinone was the most pharmacologically characterized active component of the seeds.

Despite the absence of formal standardization of active components in the pharmaceutical preparations of the dosage forms used in the laboratory studies, there were many important pharmacological findings reported with the *N. sativa* ground seeds, its essential oil and thymoquinone that were studied either *in vitro* or *in vivo*. In addition, the preclinical studies covered in this review employed well-characterized pharmacological models, which were shown to have good human predictive therapeutic effects. The major pharmacological findings are briefly discussed below.

ANALGESIC, ANTI-INFLAMMATORY AND IMMUNOMODULATORY ACTIONS

Amin and Hosseinzadeh (11) briefly reviewed some of the anti-inflammatory, analgesic and anti-rheumatic activities *N. sativa* administered, as pharmaceutical preparations comprised of either aqueous extracts or oil or thymoquinone. Hajhashemi *et al.* (8) and Ghannadi *et al.* (12) specifically investigated the analgesic actions of an Iranian derived *N. sativa* essential oil in the acetic acid-induced writhing, formalin and tail flick tests in rats. They also investigated the essential oil anti-inflammatory actions in the carrageenan-induced paw edema in rats and the croton oil-induced edema in the mice. As previously discussed in the Chemical Composition and Dosages Form section, the investigators also analyzed the active principal of the Iranian derived essential oil that they used in their study by the GC/MS method and determined that the concentration of thymoquinone was 13.7%.

Table 1 presents the summary of the analgesic and anti-inflammatory actions of the essential oil and thymoquinone in rodents. The *N. sativa* essential oil showed statistically significant analgesic effect in the acetic acid-induced writhing, formalin and the light tail-flick tests (8). The writhing test is primarily used for screening non-steroidal anti-inflammatory drugs for their analgesic actions; however this test is also non-specific since many none analgesic drugs (e.g., anti-cholinergics, anti-histamines, *etc.*) are also active (Esam Z. Dajani, personal observations). The investigators did not conduct sufficient dose-groups to permit the calculation of the ED_{50} values of the essential oil nor provided relative estimate of the potency of the oil versus reference standard analgesic drugs but the comparisons were always made with the control (placebo) groups. The fact that *N. sativa* essential oil was found effective in the light tail flick test could indicate that its analgesic effects could be partially mediated by opiate-like mechanism of action since only opiate analgesics are primarily effective in the tail flick test (13). However the fact that naloxone, a specific (µ) opioid receptor antagonist, did not reverse the essential oil analgesic effect in the formalin test does not support the concept that the mechanism of the analgesia induced by *N. sativa* is mediated by an opiate receptor interaction (14). Furthermore, the tail flick analgesic findings of Hajhashemi *et al.* (8) were not confirmed by another investigator (12) and the basis for this discrepancy between these two groups is unknown.

Intraperitoneal (i.p.) but not intragastric (i.g.) administration of the oil at the doses of 100, 200 and 400 microliter/kg doses produced significant anti-inflammatory action in the carrageenan-induced paw edema in rats. However, it was not active when applied topically in the croton oil-induced ear edema in mice. The fact that the oil was only effective *via* the

Table 1. Summary of the *in vivo* analgesic and anti-inflammatory actions of *Nigella sativa* in rodent[a].

Test	Species	Drug	Outcome	Reference
Analgesia-writhing test	Mouse	Essential oil	(+) analgesia at varied i.p. and i.g. doses	Hajhashemi *et al*. (8)
Analgesia-formalin test	Mouse	Essential oil	(+) analgesia at varied i.p. doses	Hajhashemi *et al*. (8) Ghannadi *et al*. (12)
Analgesia-tail flick test	Mouse	Essential oil	(+) analgesia at varied i.p. and i.g. doses	Hajhashemi *et al*. (8)
Analgesia-neuropathic pain	Rat	Thymoquinone	(+) analgesia at 100 – 400 mg/kg, i.g. doses	Celik *et al*. (15)
Anti-inflammatory carrageenan paw edema	Rat	Essential oil	(+) analgesia at 100 – 400 ug/kg, i.p. doses	Hajhashemi *et al*. (8)
Anti-inflammatory croton oil ear edema	Mouse	Essential oil	(+) analgesia at 100 – 400 ug/kg i.p. or topically doses	Hajhashemi *et al*. (8) Ghannadi *et al*. (12)
Adjuvant-induced arthritis	Rat	Thymoquinone	(+) analgesia at 2.5 and 5.0 mg/kg i.g. Similar efficacy to Methotrexate given at 0.3 mg/kg	Tekeoglu *et al*. (16)

[a]Groups of rats or mice received graded doses of *Nigella sativa* essential oil or thymoquinone (TQ) administered intragastrically (i.g.) or intraperitoneally (i.p.), prior to the application of the acute noxious pain stimulus. (+) denotes positive response in the drug groups in comparison with the placebo groups.

intraperitoneal route of administration, but not when given intragastrically, at the same doses as the those used for the intraperitoneal administration, could indicate decreased oral bioavailability of its active components(s) and this needs to be further investigated using standard pharmacokinetics evaluation (absorption, distribution, metabolism and excretion).

Thymoquinone also exhibited significant analgesic action in a model of neuropathic pain induced by an experimentally applied spinal cord injury in rats (15). Two weeks following the induction of the experimental spinal injury, the application of mechanical and heat-cold provocations induced allodynia in the animals was made. The rats were intragastrically given thymoquinone at doses of 100 mg/kg, 200 mg/kg or 400 mg/kg. The mechanical and heat-cold tests were then repeated at 30, 60, 120, and 180 minutes after receiving either thymoquinone or the placebo using withdrawal threshold and withdrawal latency values. The withdrawal threshold and withdrawal latency values recorded from the mechanical and heat-cold allodynia measurements for all three thymoquinone treated groups were much higher than the control groups at all time points examined (30, 60, 120, and 180 minutes after the treatment). Of interest, there were no differences in the neuropathic analgesic response between the three-thymoquinone dose-groups indicating a lack of a dose-response relationship. Clearly, further studies are needed to understand and resolve the lack of a dose-response with thymoquinone in neuropathic pain.

Tekeoglu et al. (16) tested N. sativa in the adjuvant-induced arthritis test in rats. Group of rats were given either thymoquinone (administered at the doses of 2.5 mg/kg/day and 5.0 mg/kg/day, i.g.) or the reference standard drug methotrexate (0.3 mg/kg/day). Both thymoquinone and methotrexate showed comparable clinical and radiological suppression of the arthritis. The fact that thymoquinone showed comparable results to those obtained with methotrexate, although given at a much higher dose than methotrexate, clearly support the potential clinical investigation of thymoquinone and N. sativa seeds in rheumatoid arthritis and other rheumatic diseases in man.

The basis of the anti-rheumatic actions of N. sativa was not investigated in the Tekeglu et al. (16) study, but many studies indicate that N. sativa has an immunomodulating action. As reviewed by Salem (17), N. sativa oil and thymoquinone possess potent anti-inflammatory effects including experimental encephalomyelitis, colitis, peritonitis, edema, and arthritis through the suppression of the inflammatory mediators such as prostaglandins and leukotrienes. Furthermore, the beneficial immunomodulatory properties of N. sativa are mediated by augmenting the T cell- and natural killer cell-mediated immune responses (17). In addition, the fixed oil, essential oil and thymoquinone possess reproducible anti-oxidant effects through enhancing the oxidant scavenger system, which as a consequence prevents the toxic effects induced by several insults (18). All such immunotherapeutic and analgesic properties of N. sativa oil and thymoquinone should be prospectively investigated in various clinical settings.

ANTI-TUMOR AND CHEMOTHERAPEUTIC ACTIONS

Nigella sativa, its extract and some of its components such as thymoquinone and dithymoquinone have been shown to possess antitumor activities against a broad spectrum of cancer cell cultures, including prostate, colon, ovarian, myloblastic leukemia and lymphoma (9, 19-27). In addition, it has recently been reported that alpha-hederin, another recently identified active component of the black seeds possess cytotoxic activity on many cancer cell lines similar to thymoquinone (7). Although the precise mechanism(s) for the anti-tumor action of

thymoquinone is not fully understood, it include the release of reactive oxygen species (ROS), the inhibition of nuclear factor-kappaB (NF-κB), the activation of tumor suppressor gene, the induction of the peroxisome proliferator-activated receptors, inactivation of angiogenesis and anti-inflammatory gene, and the induction of apoptosis (26, 27).

Two relevant studies concerning the action of thymoquinone on the release of ROS will be briefly discussed (22, 25). Firstly, Koka et al. (22) investigated the action of thymoquinone in androgen receptor-independent (C4-2B) and androgen receptor nad've (PC-3) prostate cancer cells, as models of aggressive prostate cancers. Cells incubation with thymoquinone for 24 – 48 hours, at varying concentrations (25 – 150 micromole/L), inhibited the growth of both C4-2B and PC-3 cells, with a median inhibitory concentration (IC_{50}) values of approximately 50 and 80 micromole/L, respectively. In addition, thymoquinone increased the ROS levels three-fold and decreased glutathione levels by 60% in both cell types. Furthermore thymoquinone dose-dependently inhibited both total and nuclear androgen receptor levels by 4 – 5 fold and androgen receptor-directed transcriptional activity by 10 – 12 fold. The major conclusion of the Koka et al. study indicates that the thymoquinone-induced cell death was primarily due to increased ROS generation and decreased glutathione levels, and was independent of androgen receptor activity. Secondly, Hussain et al. (25) reported that thymoquinone showed marked inhibitory activity on the activated B-cell lymphoma (ABC), one of the three subtypes of the diffuse large B-cell lymphoma (DLBCL). This DLBCL has the worst survival rate after chemotherapy treatment and is characterized by constitutively activated NF-κB. Thymoquinone was shown to release ROS, which inhibits NF-κB activity, which then leads to the inhibition of cell viability and the induction of mitochondrial dependent apoptosis in the DLBCL cell lines. In this study, thymoquinone also up-regulated the death receptor. With respect to the ROS release noted in the current study, this result was consistent with a previous finding reported by the same investigators (28).

Given the anti-oxidant property of thymoquinone, it is important to establish that thymoquinone would not negate the cytotoxic effects of chemotherapeutic drugs acting via the release of ROS. Generally oncology patients are cautioned to avoid the concomitant use of any anti-oxidant supplements while they are receiving their chemotherapeutic drugs for the fear of negating their beneficial cytotoxic actions. Therefore, we examined the literature for the interaction of thymoquinone with chemotherapeutic drugs, if any, both in vitro and in vivo settings. Effenberger-Neidnicht and Schobert (29) studied the combined effects of thymoquinone and doxorubicin tested in vitro on various cell cultures. Doxorubicin, thymoquinone and equimolar mixtures of both drugs were tested alone and in combination for their anti-proliferative effects on human cells of HL-60 leukemia, 518A2 melanoma, HT-29 colon, KB-V1 cervix, and MCF-7 breast carcinomas as well as multi-drug-resistant variants thereof and on non-malignant human fibroblasts. In this test, thymoquinone improved the cytotoxic properties of doxorubicin in a cell line-specific manner. The mode of action of thymoquinone and doxorubicin as well their mixture was mainly apoptotic. In HL-60 cells, the combined drug mixture caused potentiation of caspase-3, which was not observed for either of the individual drug when tested alone. The impact of the combined drug mixture on the mitochondria of HL-60 cells was also greater than those of the individual drugs alone. In addition, the combined drug mixture led to a higher concentration of ROS in HL-60 cells. Such in vitro finding clearly suggests that thymoquinone, although possessing an anti-oxidant action, does not interfere with the actions of doxorubicin; but rather it augmented its cytotoxic properties.

Table 2. Summary of the *in vivo* anti-tumor actions of thymoquinone (TQ) in mice[a].

Test	Drugs (mg/kg)	Outcome	Reference
Lung cancer Athymic mouse	TQ 10 mg/kg, i.p.	Significant reduction in tumor mass when TQ was given for 18 days.	Attoub *et al.* (23)
Lung cancer	TQ 5 + Cisplatin 2.5 mg/kg, TQ 20 + Cisplatin 2.5 mg/kg, i.p.	Significant and dose dependent potentiation of anti-tumor action of cisplatin	Jafri *et al.* (30)
Pancreatic cancer	TQ + Gemcitabine and Oxaliplatin	Variable dosages; TQ induced significantly potentiation of anti-tumor action of the reference drugs	Banerjee *et al.* (31)
Multiple myeloma	TQ 1 mg/kg, i.p. + Bortezomid 0.25 mg/kg i.p. for 4 weeks	TQ & Bortezomib inhibited the tumor. Combined drugs had highest tumor inhibition	Siveen *et al.* (32)

[a]Groups of mice received fixed doses of thymoquinone (TQ) alone or with standard chemotherapeutic drugs, administered intraperitoneally (i.p.), in established mouse cancer models.

In experimental *in vivo* animal models, thymoquinone alone or combined with chemotherapeutic drugs had shown significant potentiation of their anti-tumor actions (*Table 2*). For example, Attoub *et al.* (23) investigated its anticancer action of thymoquinone in athymic mice inoculated with the LNM35 lung cancer cells. The administration of thymoquinone (10 mg/kg/day, IP) alone for 18 days inhibited the LNM35 tumor growth by 39% when compared with placebo (P < 0.05). The tumor growth inhibition was associated with significant increase in the activated caspase-3. Similarly, Jafri *et al.* (30) investigated the combined effects of thymoquinone and cisplatin in a xenograft mouse model of lung cancer and demonstrated that a combined regimen of thymoquinone and cisplatin was well tolerated and significantly reduced the tumor volume and tumor weight without any additional toxicity to the mice. In this study, the investigators studied two dosages of thymoquinone: 5 mg/kg/day and 20 mg/kg/day given with a fixed daily dose of cisplatin (2.5 mg/kg/day). When thymoquinone (5 mg/kg) was concurrently administered with cisplatin, the tumor volume was reduced by 59%. At the higher 20 mg/kg dose of thymoquinone, the tumor volume was reduced by 79%. Likewise *in vitro* interaction studies performed by the same investigators also showed enhanced anti-tumor effect of cisplatin when concurrently administered with thymoquinone, indicating that the *in vitro* result was consistent with the *in vivo* finding. Of interest is the observation that thymoquinone showed dose-dependent cytotoxic activity of the cancer cells both *in vitro* and *in vivo*. It is conceivable that thymoquinone down regulated NF-κB expression, which may explain its various cellular activities and this activity may prove useful in overcoming cisplatin resistance from over expression of NF-κB.

Banerjee *el al.* (31) had shown that thymoquinone potentiated the anti-tumor effects of gemcitabine and oxaliplatin in experimental mouse model of pancreatic cancer indicating that thymoquinone could abrogate gemcitabine- or oxaliplatin-induced activation of NF-κB resulting in the chemo-sensitization of pancreatic tumors to the conventional chemotherapeutic drugs. Furthermore, Siveen *et al.* (32) had shown that thymoquinone potentiated the anti-tumor activity of bortezomib in xenograft mouse model of multiple myeloma. In this study, four groups of mice received either placebo (Group 1), or bortezomib given at the dose of 0.25 mg/kg, i.p. once weekly for 4 weeks (Group 2), thymoquinone 1 mg/kg/day, i.p. for 4 weeks (Group 3) and the combined drug treatment of thymoquinone and bortezomib (Group 4). Although both thymoquinone and bortezomib administered alone reduced the tumor volume, the combined drug treatment had shown a greater and significant potentiation of their anti-tumor actions.

All such lines of evidence derived from the cell culture studies and in whole animal cancer studies clearly demonstrated that thymoquinone not only has direct cytotoxic potential against the tumors but it also augmented the cytotoxic effect of some chemothera-peutic drugs. *Table 2* presents a summary of the *in vivo* anti-tumor actions of *N. sativa* in the experimental cancer studies conducted in rodents.

A more compelling reason for considering the adjuvant use of *N. sativa* with some chemotherapeutic drugs, such as doxorubicin, is the fact the *N. sativa* has been shown to protect the experimental animals from the unintended but the well-known adverse actions of doxorubicin, without altering its primary anti-proliferative action against the tumor cells. It is well recognized that the long-term patient outcomes following treatment of leukemia with anthracyline drugs such as doxorubicin can result in cardiac abnormalities including arrhythmias, congestive heart failure, myocardial infarction, hypertension and left ventricular failure. Thymoquinone has been shown to possess cardiac protective effects against doxorubicin in both *in vitro* and *in vivo* studies as detailed from the following four studies:

- Brown *et al.* (33) investigated the *in vitro* effect of thymoquinone and doxorubicin in both RAW leukemia cells and in cardiac myocytes using cell culture when given alone or in combination for 24, 48 and 72 hours cell incubation. Cellular viability and morphological changes were determined in the RAW leukemia cells and cardiomyocytes. The investigators observed that thymoquinone alone reduced in RAW leukemia cell numbers without altering their morphology, while doxorubicin reduced RAW leukemia cell number, induced spindle cell formation and increased cellular damage. The incubation of cardiomyocytes with thymoquinone alone did not alter their morphology over the time periods examined; whereas doxorubicin treated cells showed evidence of loss of connectivity and disruption of cell membranes. The combined treatment of doxorubicin and thymoquinone demonstrated significant cardiomyocytes survival while reducing the number of the leukemia cells. In addition, the combined drugs increased the leukemia cell apoptosis when compared to each drug alone. The basis for the protective effects of thymoquinone against the

doxorubicin-induced cardiac toxicity, while not interfering with its anti-proliferative actions on the leukemia cells, is not known.

- Al-Shabanah *et al.* (34) investigated the oral (p.o.) effect of thymoquinone on the cardiac toxicity and antitumor activity of doxorubicin in mice bearing Ehrlich ascites carcinoma tumor. Thymoquinone (8 mg/kg/day) administered in drinking water starting 5 days before a single injection of doxorubicin (20 mg/kg, i.p.) and continuing during the experimental period ameliorated the doxorubicin-induced cardiotoxicity in mice as evidenced by significant reductions in the elevated levels of serum lactate dehydrogenase and creatine kinase and on cardiac histopathological basis. In addition, thymoquinone did not alter the doxorubicin plasma and heart concentrations and did not interfere with its anti-tumor activity clearly indicating that its protective effects against doxorubicin is not related to possible interference with its pharmacokinetics in heart and in plasma.

- Badary *et al.* (35) investigated protective effects of *N. sativa* against doxorubicin-induced nephrotoxicity in rats. A single intravenous injection of doxorubicin (6 mg/kg) induced a severe nephrotic syndrome after 5 weeks, which was associated with hypoalbuminemia, hypoproteinemia, elevated serum urea, hyperlipidemia, and a high urinary excretion of protein, albumin and N-acetyl-beta-D-glucosaminidase. In the kidney, doxorubicin induced a significant increase in total triglycerides, total cholesterol, and lipid peroxides and a significant decrease in non-protein sulfhydryl content and catalase activity. The treatment of rats with thymoquinone (10 mg/kg per day, p.o.) supplemented with their drinking water five days before doxorubicin, and daily thereafter, significantly lowered serum urea, triglycerides, total cholesterol and nearly normalized the other biochemical markers related to free radicals formation. These results confirm the involvement of free radicals in the pathogenesis of nephropathy induced by doxorubicin. The data also suggest that thymoquinone might also be useful as a protective agent for proteinuria and hyperlipidemia associated with the nephrotic syndrome and this potential use should be further investigated.

- Elsherbiny and El-Sherbiny (36) confirmed the Badary *et al.* (35) study on the protective effects of thymoquinone against the doxorubicin-induced renal injury. In their study, rats were treated with doxorubicin (3.5 mg/kg twice weekly, i.p.) with or without thymoquinone (50 mg/kg/day, oral supplementation) for 3 weeks. Renal histological changes and elevations of serum urea, creatinine and increased urinary albumin, and the levels of various redox markers documented the doxorubicin-induced nephrotoxicity. The treatment with thymoquinone reversed much of the doxorubicin-induced renal injury and restored the biochemical markers of renal injury toward their normal values. These results suggest that the doxorubicin-induced nephrotoxicity involved a redox imbalance in renal tissue, which was reversed by thymoquinone.

The preceding analysis was focused only on the protective effects of thymoquinone against doxorubicin, as a representative of the anthracycline drugs, but not with other commonly used chemotherapeutic agents. Therefore there is a need for investigating of the host protective effects of *N. sativa* against broad classes of chemotherapeutic drugs for the purpose of reducing some of their adverse effects, while not interfering with

Table 3. Summary of the *in vivo* central nervous system (CNS) and neurological actions of *Nigella sativa* in rodents[a].

Test Species	Drugs (mg/kg)	Outcome	Reference
Pentylenetetrazole seizure; Mice	TQ; 40 & 80 mg/kg; i.p.	(+) anti-convulsant	Hosseinzadeh & Parvardeh (38).
Maximal electro-shock seizure; Mice	TQ; 40 & 80 mg/kg; i.p.	No seizure protection but reduced mortality	Hosseinzadeh & Parvardeh (38)
Pentylenetetrazole seizure; Mice	Essential oil varied doses	(+) More effective than valproate	Ilhan *et al.* (40)
Intracortical Penicillin seizure; Rats	TQ; 10, 50, 100 mg/kg; i.p.	(+) Active	Beyazcicek *et al.* (41)
Spatial memory Rats	Essential oil; 6.0 ul/kg, i.g.	(+) Active	Sahak *et al.* (42)
Learning & memory Rats	Alcoholic extract; 100, 200, 400 mg/kg, i.g.	(+) Active	Behashti *et al.* (43)
Experimental autoimmune encephalitis; Mice	TQ; varied doses, i.p.	(+) High activity	Mohamed *et al.* (45)
Experimental autoimmune encephalitis; Rats	Powdered seeds 2.8 g/kg, i.g.	(+) High activity	Noor *et al.* (46)

[a]Mice and rats were treated with either *N. sativa* powdered seeds or its alcoholic extract or thymoquinone (Q), administered intragastrically (i.g.) or intraperitoneally (i.p.), in rodents. Maximal electroshock seizure (MES), pentylenetetrazole (PTZ)-induced seizure and Intracortical Penicillin seizure were models used for the assessment of *N. sativa* anti-convulsant actions. *Nigella sativa* did not protect against MES-induced seizure but did reduce its the mortality. The experimental autoimmune encephalitis (EAE) is a model that mimics features of multiple sclerosis.

their anti-cancer properties. As discussed below in the Gastrointestinal Section, *N. sativa* had shown general cytoprotective and anti-ulcer effects in the stomach and could exert similar protection in patients who develop gastrointestinal mucosal injury (e.g., mucositis) in response to the cytotoxic drugs.

These interesting pharmacological finding warrants clinical investigation of *N. sativa* alone or combined with cytotoxic drugs not only for the treatment of cancers, in various clinical settings but also for the prevention of some of the adverse events associated with the chemotherapy treatment.

CENTRAL NERVOUS SYSTEM AND NEUROLOGICAL ACTIONS

Beheshti *et al*. (37) reviewed some of the major central nervous system pharmacological actions of *N. sativa*. In various animal models, *N. sativa* had been shown to improve memory, anxiety, depression, epilepsy, neurotoxicity, neurodegeneration and pain. *N. sativa* inhibits the acetylcholinestrase enzyme and interacts with the GABA, opioid and nitric oxide (NO) systems. The details of the major findings are briefly discussed below and the results of the *in vivo* animal studies are displayed in *Table 3*.

Anticonvulsant actions

Hosseinzadeh and Parvardeh (38) investigated the anticonvulsant effects of thymoquinone in the pentylenetetrazole (PTZ) - and maximal electroshock-induced seizure models. The intraperitoneal administration of thymoquinone at doses of 40 and 80 mg/kg, prolonged the onset of seizures and reduced the duration of myoclonic seizures. The protective effect of thymoquinone against mortality was 71.4% and 100% at these doses, respectively. In the maximal electroshock model, thymoquinone failed to reduce the duration of seizures, whereas it completely protected the animals against death. These results indicate that thymoquinone may have anti-seizure activity in some types of epilepsy, probably as a consequence of increase in GABAergic tone (39).

Ilhan *et al*. (40) subsequently confirmed the anti-convulsant effect of *N. sativa* oil in the PTZ-kindling seizures and its lethal effects in mice. They also investigated whether the *N. sativa* and the reference standard valproate attenuated the PTZ-induced oxidative injury in the brain tissue (antioxidant effect) when given as a pretreatment prior to each PTZ injection during kindling acquisition. Both substances studied significantly decreased oxidative injury in the mouse brain tissue. The *N. sativa* oil was found to be the most effective in preventing PTZ-induced seizures relative to valproate. The *N. sativa* oil showed anti-epileptogenic properties as it reduced the sensitivity of kindled mice to the convulsive and lethal effects of PTZ; valproate was ineffective in preventing these effects. The data suggests that *N. sativa* may have a better neuroprotective action than valproate.

Beyazcicek *et al*. (41) investigated the anticonvulsant actions of *N. sativa* in rats using the intracortical penicillin model. Thymoquinone, dissolved in dimethylsulfoxide, was administered intraperitoneally at doses of 10, 50 and 100 mg/kg. After the rats were anesthetized, the left part of the skull was removed. A pair of silver/silver chloride electrodes was placed on the somatomotor area, and electrocorticographic recording was started. After 5 minutes basal activity was recorded, and thymoquinone was given intraperitoneally at several doses. Thirty minute after thymoquinone administration, epileptiform activity was induced by the intracortical penicillin administration. The first spike latency, spike frequency, and the amplitude of epileptiform activity were statistically analyzed.

All doses of thymoquinone significantly increased the latency time to onset of first spike wave, and decreased the frequency, and amplitude of epileptiform activity in the first 20 minutes when compared with the control group.

Unlike the positive anti-convulsant effects shown with *N. sativa* in pentylenetetrazole- and penicillin-induced seizure, Noor *et al*. (39) did not find this drug to be effective against brain histopathological and amino acid changes in rats which was induced by intraperitoneal pilocarpine administration, whereas curcumin, and valproate were effective in reducing these neural changes. In control animals, pilocarpine induced a significant increase in hippocampal aspartate and a significant decrease in glycine and taurine levels. In the cortex, a significant increase in aspartate, glutamate, GABA, glycine, and taurine levels was obtained after pilocarpine injection. Treatment of pilocarpinized rats with curcumin and valproate but not *N. sativa* ameliorated most of the changes in amino acid concentrations and reduced the histopathological abnormalities induced by pilocarpine. Although there is no single available universal anti-seizure drug effective for all types of epilepsy, *N. sativa* should be clinically investigated in various types of epilepsy.

Memory and cognition

Sahak *et al*. (42) investigated the effect of *N. sativa* oil on the spatial memory performance of male adult rats using eight-arm radial arm maze. Sprague Dawley rats (7 – 9 weeks old) were forced-fed daily with 6.0 μL/100 g body weight of *N. sativa* oil or 0.1 mL/100 g body weight of corn oil (control) for a period of 20 consecutive weeks. For each weekly evaluation of spatial memory performance, a one-day food deprived rats were allowed a three-minute period to explore the radial arm maze for food as their rewards. Similar to the control group, the spatial memory performance of the *N. sativa* treated group was not hindered when compared to the control group. A statistically significant lesser mean numbers of error were observed for the drug-treated group when compared to the placebo treated group indicating that *N. sativa* enhanced the learning and memory abilities of the rats. Of interest, Beheshti *et al*. (43) recently reported that feeding rats a hydro-alcoholic extract of *N. sativa* in their drinking water during their neonatal and juvenile growth (100 mg/kg/day, 200 mg/kg/day and 400 mg/kg/day) had a positive and dose-dependent effect on learning and memory.

Azzubaidi *et al*. (44) investigated the possible memory enhancement with *N. sativa* in rats that underwent cerebrovascular hypoperfusion. Chronic cerebral hypoperfusion has been linked to neurodegenerative disorders including Alzheimer's disease and its subsequent cognitive impairment. Cerebrovascular hypoperfusion was experimentally induced by bilateral common carotid arteries occlusion. Morris water maze test was employed to assess the effects of *N. sativa* on spatial cognitive function before and after the carotid artery occlusion. Rats were divided into long-term memory and short-term memory groups, each was further subdivided into three subgroups: sham control, untreated occlusion group and *N. sativa* treated. All subgroups were tested with the Morris water maze on the tenth postoperative week. Working memory test results for both sham control and *N. sativa* treated groups showed significantly lower escape latency time and total distance travelled than untreated occluded group. Similarly, long-term memory and short-term memory in the Morris water maze tests for sham control and *N. sativa* treated groups revealed significantly better maze test performance as compared to untreated occluded group. Sham control and *N. sativa* treated occluded groups demonstrated superior memory test performance as compared to untreated occluded group. The *N. sativa* essential oil group showed noticeable spatial cognitive

preservation in rats challenged with chronic cerebral hypoperfusion, which indicates that *N. sativa* has a promising neuroprotective effect and this action should be further investigated.

Experimental autoimmune encephalomyelitis (EAE)

Experimental autoimmune encephalomyelitis (EAE) is an animal model that mimics multiple sclerosis (MS) in man. Axonal damage, demyelination and inflammation of the central nervous system are the major pathological features of the human MS. In addition, MS is believed to be due to abnormal T cell mediated immune response and oxidative stress plays an important role in the advancement of MS.

Mohamed *et al*. (45) investigated the effects of thymoquinone on experimental autoimmune encephalomyelitis (EAE) in mice. Thirty female mice of strain C57BL/6J and aged between 6 to 12 weeks were placed into 3 groups of 10 and were given myelin oligodendrocyte glycoprotein (MOG) subcutaneously to induce EAE. Group 1 was the control group. Group 2 received MOG subcutaneously and thymoquinone administered intraperitoneally from day 1 till day 50. Group 3 received MOG subcutaneously and thymoquinone intraperitoneally on the appearance of first sign and symptoms of chronic relapsing EAE. All Mice were examined daily for behavioral deficits and all were sacrificed on day 50. The result showed that thymoquinone was almost 90% effective in preventing and 50% effective in curing chronic relapsing EAE.

Noor *et al*. (46) confirmed the protective action of *N. sativa* in EAE of Mohamed *et al*. (45) observations. Experimental autoimmune encephalitis was induced in rats in a manner similar to the previous study and the protective effects of powdered *N. sativa* seeds (2.8 g/kg body weight given orally) were investigated. The investigators established that *N. sativa* suppressed the inflammation in the EAE-induced rats. In addition, *N. sativa* enhanced the remyelination of the cerebellum. Moreover, *N. sativa* reduced the expression of transforming growth factor beta-1 (TGF β1). Therefore, both set of EAE results obtained in mice and in rats indicate that either

thymoquinone or a powdered *N. sativa* seeds are effective for the prevention and treatment of EAE. Additional studies are needed to explore the optimum dosages, frequency of the treatment and specifically dose-response relationships, and potential safety concerns, if any, as well as its mechanism of protective action in chronic relapsing experimental autoimmune encephalitis.

Given the current unmet therapeutic need for the treatment of multiple sclerosis, the possibility of treating human chronic relapsing multiple sclerosis with *N. sativa* clearly warrants clinical investigation.

METABOLIC EFFECTS

Many laboratory studies disclosed that *N. sativa* powdered seeds, essential oil and aqueous seed extracts exhibited hypolipidemic actions in rats (47) and anti-diabetic actions in rats subjected to experimentally induced diabetes (48-50). In healthy rats, feeding powdered seeds in various daily doses (100 mg/kg, 200 mg/kg, 400 mg/kg and 600 mg/kg), for one to four week test duration, induced significant decreases in serum lipids at all dosage tested (*Table 4*). There were no apparent changes induced by the *N. sativa* treatment on the high density lipoprotein cholesterol (HDL-C), however the powdered seed induced significant decreases in the low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), total cholesterol (TC) and triglycerides (TG) levels (*Table 4*). However, there was no linear dose or time dependent effect of the *N. sativa* powdered seeds on the lipid parameters (*Table 5*).

In the streptozotocin-induced diabetes in rats, Kaleem *et al*. (51) showed that ethanol extracts of the *N. sativa* powdered seeds, administered orally at the dose of 300 mg/kg for 30 days, significantly reduced the elevated levels of blood glucose, lipids, plasma insulin and improved altered levels of lipid peroxidation products (hydroperoxides), the antioxidant enzymes catalase, superoxide dismutase, reduced glutathione and the glutathione peroxidase of the liver and kidney (*Table 4*). In the alloxan-induced diabetes in rats, *N. sativa* induced

Table 4. Summary of the *in vivo* metabolic properties of *Nigella sativa* in rats[a].

Test	Drugs (mg/kg)	Outcome	Reference
Serum lipids Healthy Rats i.g. for 4 weeks	Powdered seeds; 100 to 600 mg/kg (see *Table 5*)	Induced hypo-lipidemia	Kocyigit *et al*. (47)
Streptozotocin-induced diabetes	Powdered seeds; 300 mg/kg/day, i.g. for 30 days	Decreased glucose, lipids, and insulin	Kaleem *et al*. (51)
Metabolic syndrome (high fructose feeding)	TQ; 25, 50, 100 mg/kg, i.g. once daily for 6 weeks Pioglitazone; 10 mg/kg/day, i.g.	TQ prevented hyperglycemia, hyperlipidemia, hypertension similar to the reference. standard Pioglitazone	Prabhakar *et al*. (56)
Metabolic syndrome (high fructose feeding)	Powdered NS seeds and turmeric powder alone or combined in various dosages for 6 weeks i.g.	Each drug alone prevented the changes induced by the metabolic syndrome. Turmeric significantly enhanced the efficacy of NS	Amin *et al*. (57)

[a] Rats were treated with either *N. sativa* (NS) powdered seeds or thymoquinone (TQ), administered intragastrically (i.g.) at various dosages and for various durations. *Nigella sativa* significantly induced hypolipidemia in healthy rats and reduced serum glucose in experimental diabetes. *Nigella sativa* also prevented the metabolic and cardiovascular changes in an experimental model of metabolic syndrome that was induced by the high fructose feeding.

Table 5. Hypolipidemic effects powdered *Nigella sativa* in rats[a].

Parameter/ Groups	Dose (mg/kg)	Treatment time (mean ± S.D.)	
		1 week (n = 5)	4 weeks (n = 5)
HDL-C (mg/dl)			
Control group	0	39 ± 4.18	47 ± 5.7
Treated group	100	41 ± 4.18	46 ± 4.18
	200	42 ± 2.73	50 ± 3.53
	400	47 ± 5.7*	45 ± 3.27
	600	42 ± 2.73	49 ± 6.51
LDL-C (mg/dl)			
Control group	0	44 ± 5.47	38 ± 13.03
Treated group	100	36 ± 15.16	23 ± 12.04
	200	31 ± 11.4	16 ± 5.47*
	400	22 ± 8.36*	22 ± 13.03
	600	16 ± 8.94**	14 ± 5.47
VLDL-C (mg/dl)			
Control group	0	14.24 ± 2.7	14.26 ± 0.96
Treated group	100	12.82 ± 1.49	8.62 ± 1.65**
	200	0.68 ± 0.90**	7.56 ± 1.65**
	400	8.30 ± 1.32**	6.16 ± 0.93**
	600	7.02 ± 0.80**	4.94 ± 1.19**
Triglycerides (mg/dl)			
Control group	0	60 ± 50	60 ± 50
Treated group	100	54 ± 4.18	47 ± 2.12**
	200	57 ± 5.7	44 ± 4.18**
	400	56 ± 2.94	44 ± 3.56**
	600	53 ± 8.31	45 ± 50**
Total cholesterol (mg/dl)			
Control group	0	82 ± 2.73	83 ± 4.47
Treated group	100	81 ± 4.18	74 ± 4.18**
	200	82 ± 2.73	74 ± 4.18**
	400	80 ± 6.12	74 ± 4.18**
	600	78 ± 5.70	74 ± 4.18**

Groups of Wistar rats received graded doses of powdered seeds orally for 4 weeks. Blood collection and analyses were performed on week 1, week 2 and week 4 using 5 animals per group. Data are presented only for week 1 and week 4.
*Significantly different from corresponding control at $P < 0.05$; **Significantly different from corresponding control at $P < 0.001$; [a]adapted from Kocyigit *et al*. (47).

significant amelioration of monocytes and granulocytes, with significant increases in the concentrations of lymphocytes and the TNF-α, IL-4 and IL-8 levels (52). However, none of these studies consistently investigated whether the anti-diabetic or the hypolipidemic actions of *N. sativa* was dose-dependent and this should be prospectively investigated in additional studies.

Streptozotocin was shown to produce oxygen free radicals by stimulating H_2O_2 generation in pancreatic β-cells that can damage the β-cell membranes and results in depletion of intracellular nicotinamide adenine dinucleotide (NAD), thus leading to onset and progression of diabetes mellitus (53). More recently, Zywert *et al*. (54) and Szkudelski *et al*. (55) demonstrated that cyclic adenosine monophosphate (cAMP) levels in the pancreatic islets of the streptozotocin-nicontinamide-induced diabetes in rats exposed to glucose alone are significantly lower than in islets of non-diabetic animals indicating a defect in insulin secretion. In addition, the increased free radical production results in diabetic complications including cardiac hypertrophy, myocardial infraction, and liver and kidney damage. Most of these complications are due to excessive free radical production and immuno-suppression. Clearly, the anti-oxidant, cytoprotective and immune-modulating actions of *N. sativa* may therefore be pharmacologically relevant to the treatment of diabetes and its complications.

Of interest is the observation that thymoquinone attenuated the experimentally induced metabolic syndrome in rats. This experimental metabolic syndrome has some features that are similar to those observed in man. Prabhakar *et al*. (56) had demonstrated that thymoquinone prevented the development of metabolic syndrome in male rats fed a high fructose diet (60% of diet). Thymoquinone (25, 50 and 100 mg/kg, PO) co-administered once daily with the high fructose diet (60% of the diet) for 42 days. Pioglitazone (10 mg/kg, orally once daily) was used as a reference standard drug. Plasma glucose, triglycerides,

Table 6. In vivo cardiovascular effects of *Nigella sativa* in rats[a].

Test	Drugs (mg/kg)	Outcome	Reference
Anesthetized rats blood pressure (BP) **heart rate**	volatile oil @ 4 – 32 uL/kg; i.v.	Dose dependent decreases in arterial BP and heart rate	El-Tahir *et al.* (58)
	TQ @ 0.2 – 1.6 mg/kg; i.v.	Dose dependent decreases in arterial BP and heart rate	El-Tahir *et al.* (58)
Nitric oxide Deficient rats (L-NAME) Hypertension[a]	TQ @ 0.5 – 1.0 mg/kg/day; i.g.	Dose dependent decreases in systolic BP and renal protection from L-NAME injury	Khattab & Nagi (60)
	NS oil @ 2.5 mg/kg; i.g. Nicardipine 30 mg/kg; i.g.	The oil showed identical hypotensive effects to Nicardipine. The oil also inhibited angiotensin converting enzyme (ACE)	Jaarin *et al.* (65)
Hyperhomocysteinemia (HHcy)[b]	0.5 – 1.0 mg/kg/day; i.g.	TQ completely blocked the biochemical changes induced by HHcy	El Saleh *et al.* (66)

[a]Rats were treated with either *Nigella sativa* oil or thymoquinone (TQ) administered either intravenously (i.v.) or intragastrically (i.g.) at various doses. [b]Rats were pretreated with 100 mg/kg of TQ, given i.g., 30 min before the methionine load (100 mg/kg). TQ completely protected the animals against the induced hyperhomocysteinemia.

total cholesterol and HDL-cholesterol were determined on days 0 and 42. Change in blood pressure, oral glucose tolerance and insulin resistance were measured. Hepatic thiobarbituric acid reactive substances (TBARS), reduced glutathione, superoxide dismutase and catalase levels were estimated as measures of hepatic oxidative stress. Hepatic mRNA of PPAR-α and PPAR-γ were also investigated.

The Prabhakar *et al.* (56) clearly established that thymoquinone prevented some of the characteristic features of the metabolic syndrome such as hyperglycemia, hypertriglyceridemia, hyper-cholesterolemia and hypertension. Thymoquinone also prevented the impaired glucose tolerance and insulin resistance. It also ameliorated the high fructose diet-induced increase in hepatic TBARS and depletion of superoxide dismutase, catalase and glutathione. In addition, thymoquinone prevented reduction in hepatic mRNA of PPAR-α and PPAR-γ associated with the feeding of this high fructose diet. All of the protective effects noted with thymoquinone were comparable to the reference standard drug pioglitazone.

Amin *et al.* (57) confirmed of the protective effects of *N. sativa* against the development of the high fructose diet-induced metabolic syndrome. These investigators showed that feeding rats either powdered *N. sativa* seeds or turmeric powder prevented the development of metabolic syndrome in this 6 weeks study. At the 3-week treatment interval, either the black seeds or the turmeric administered alone, significantly lowered high blood pressure and low-density lipoprotein cholesterol, respectively, when compared with placebo (P < 0.01). At 6 weeks, the co-administration of both drugs, at half of their individually effective doses, was most effective (P < 0.001) in preventing hypertension, hyperglycemia, dyslipidemia, hyperinsulinemia, and endothelial dysfunction than each of the individual drug alone. This study also demonstrates the therapeutic superiority of the combined treatment of black seeds and turmeric, given at low doses over individually tested drugs, in improving the metabolic syndrome. The basis for the enhanced pharmacological action of *N. sativa* seeds by turmeric is unknown.

ANTI-HYPERTENSIVE AND CARDIOVASCULAR EFFECTS

Nigella sativa has been shown to possess blood pressure-lowering effects in both animals and humans (58-64). The *N. sativa* hypotensive effect was investigated in the nitric oxide deficient rat, induced by (omega)-nitro-l-arginine methyl esters (L-NAME) treatment (60). The L-NAME-induced hypertension is an interesting model that was employed in several studies with *N. sativa*; however, *N. sativa* was not investigated in broad hypertension models commonly used by industrial pharmacologists such as the spontaneous hypertension (SHR) rat.

The hypotensive effect of *N. sativa* seeds and its oil appear be mediated by calcium channel blockade and diuretic properties (63, 64). In fact, *N. sativa* oil given at an oral dose of 2.5 mg/kg showed identical antihypertensive activity in rats when compared with the calcium channel blocker nicardipine, given at the dose of 3.0 mg/kg, in attenuating the L-NAME-induced increases in blood pressure (65). In addition, the essential oil inhibited the angiotensin-converting enzyme (ACE) activity and prevented plasma nitric oxide loss (63).

Of interest is the observation that *N. sativa* has the capacity of reducing the blood concentration of homocysteine since hyperhomocysteinemia (HHcy) is believed to be associated with higher risks of coronary, cerebral and peripheral vascular disease and could induce a pathogenic state of oxidative stress (66). El-Saleh *et al.* (66) demonstrated that the pretreatment of rats with an intragastric (100 mg/kg) dose of thymoquinone, given either 30 min before the methionine load (100 mg/kg) completely protected the rats against the induced hyperhomocysteinemia. Likewise, under similar conditions, the pretreatment with the essential oil (100 microliter/kg, i.g.) for 1 week also produced significant reduction of hyperhomocysteinemia. In addition, El-Saleh *et al.* showed that hyperhomocysteinemia was also associated with significant increases in the plasma levels of triglycerides, lipid peroxidation and cholesterol. Hyperhomocysteinemia also increased the activities of glutathione peroxidase and superoxide dismutase. In addition,

Table 7. In vivo intragastric pulmonary effects of *Nigella sativa* essential oil in animals[a].

Test	Drugs (mg/kg)	Outcome	Reference
Histamine bronchosopasm Guinea pigs	variable doses	Inhibition of bronchospasm	Mahfouze & El-Dakhakny (74)
Ovalbumin bronchial asthma Guinea pigs	variable doses	Decreased airway hyper response	El-Tahir *et al*. (58)
Ovalbumin bronchial asthma Mice	variable doses	Decreased airway hyper response	Balaha *et al*. (76)
Cough Guinea pigs	variable doses	Cough supression	Boskabady *et al*. (75)

the total antioxidant status was significantly depressed. All of these pathological alterations were almost totally blocked by prior treatment with either thymoquinone or the *N. sativa* essential oil. Although, the protective effects of thymoquinone and *N. sativa* essential oil against experimentally-induced hyperhomocysteinemia were demonstrated at a much higher dose (thymoquinone 100 mg/kg, i.g.) than doses shown to be effective in reducing blood pressure in rats (*N. sativa* oil given at 2.5 mg/kg, i.g.) indicates the need for performing dose-response studies to determine whether the typical anti-hypertensive dose of *N. sativa* would also be associated with decreased homocysteine concentrations in the blood.

These preclinical anti-hypertensive studies would suggest a potentially important role of *N. sativa* in the management of hypertension in man, and this action should be prospectively investigated in comparison with standard anti-hypertensive drugs in the treatment of essential hypertension.

PULMONARY EFFECTS

Many *in vitro* studies disclosed that *N. sativa* essential oil and its aqueous extracts exhibited a relaxant effect on various smooth muscle preparations such as the aorta, jejunum and guinea pig isolated tracheal smooth muscle (67-70). Keyhanmanesh *et al*. (70) provided an updated review of many of the smooth muscle relaxant effects induced by *N. sativa*. Boskabady *et al*. (71-73) published several important *in vitro* pulmonary pharmacology studies that were conducted with *N. sativa*. Briefly, the essential oil exhibited functional antagonistic effects on the muscarinic receptor (71), an inhibitory effect on histamine (H$_1$) receptor (72), an inhibitory effect on calcium channel (73), opening effects on potassium channel, and stimulatory effects on beta-adreno-receptor (69).

There was also several interesting *in vivo* pulmonary pharmacology studies conducted with *N. sativa* and will be briefly reviewed. *N. sativa* oil had been shown to protect the guinea pigs against histamine-induced bronchospasm (58, 74). *N. sativa* exhibited an antitussive action in the guinea pigs (75). In the ovalbumin (OVA)-induced bronchial asthma in mice, the oral treatment with *N. sativa* oil significantly (P < 0.05) decreased the airway hyper-responsiveness as measured by: the number of total leukocytes, macrophages and eosinophils, levels of IL-4, IL-5 and IL-13, serum levels of total IgE, ovalbumin-specific IgE and IgG1. In addition, *N. sativa* significantly increased the level of IFN-γ and serum level of OVA-specific IgG2a (76). Furthermore, the Balaha *et al*. study (76) showed that the *N. sativa* oil significantly

abrogated the histopathological changes of the lungs, as the images were nearly normal. Consistent with the Balaha *et al*. results, thymoquinone was shown to inhibit the ovalbumin-induced bronchial asthma in the guinea pigs by inhibiting the adenosine receptors, which suggest that some of the pulmonary anti-inflammatory effects of thymoquinone may be mediated by these receptors (77). Thymoquinone was also shown to inhibit other mediators of the pulmonary inflammation such as the 5-lipoxygenase and leukotriene C4 synthase in human blood cells (78).

Nigella sativa has shown anti-asthmatic action in humans (79, 80). Given *N. sativa* combined smooth muscle relaxant effects, the immunomodulating and inhibitory effects on inflammatory mediators clearly suggest a potentially important role for the treatment of bronchial asthma, emphysema and chronic obstructive pulmonary disease; these potential therapeutic uses should be prospectively investigated.

ANTI-MICROBIAL ACTIONS

Forouzanfar *et al*. (81) published a review, which explored the antimicrobial role of *N. sativa*. The review disclosed that aqueous extracts of *N. sativa* oil and thymoquinone exhibit broad antimicrobiological spectrum including Gram-negative, Gram-positive bacteria, viruses, parasites, schistosoma and fungi. However, the effectiveness of *N. sativa* seeds and thymoquinone was variable and depended on species of the target microorganisms (82). Thymoquinone showed greater antibacterial actions than aqueous extracts of *N. sativa* seeds. For Gram-positive bacteria, the minimum inhibitory concentrations (MICs) of thymoquinone were 3-6 µg/ml, however, Gram-negative bacteria had much higher MIC of 400 µg/ml indicating that Gram-negative bacteria are less susceptible to thymoquinone. Aqueous extract of *N. sativa* seed was effective against the methicillin resistant *Staphylococcus aureus* (MRSA) with MIC of 0.2 – 0.5 mg/ml (81). Furthermore, when thymoquinone was combined with several antibiotics (ampicillin, cephalexin, tetracycline, chloroamphenicol, gentamycin and ciprofloxacin), it showed synergistic properties especially against *S. aureus* (79).

In addition, aqueous extracts of *N. sativa* exhibited anti-dermatophyte activity both *in vitro* and *in vivo* animal studies (83). In addition, *N. sativa* showed antiviral activities in several studies (84). Such results warrant clinical investigations of *N. sativa* aqueous extracts, oil and thymoquinone not only for their antibacterial actions but also more importantly for their antiviral, antidermatophyte and

Table 8. Summary of the acute toxicity studies with *Nigella sativa* in rodents[a].

Substance Tested	Species	LD$_{50}$ value (95% CI) mg/kg or ml/kg	Route	References
Thymoquinone	Mice	104.7 mg (89.7 – 119.7)	i.p.	Al-Ali *et al*. (92)
Thymoquinone	Mice	870.9 mg (647.1 – 1094.8)	i.g.	Al-Ali *et al*. (92)
Thymoquinone	Rats	57.5 mg (45.6 – 69.4)	i.p.	Al-Ali *et al*. (92)
Thymoquinone	Rats	797.3 mg (469.8 – 1118.8)	i.g.	Al-Ali *et al*. (92)
Fixed oil	Mice	2.06 ml (1.86 – 2.26)	i.p.	Zaoui et al. (93)
Fixed oil	Mice	28.8 ml (26.2 – 31.6)	i.g.	Zaoui et al. (93)

[a] Groups of mice and rats received graded doses of thymoquinone or the fixed oil either intragastrically (i.g.) or intraperitoneally (i.p.). The median lethal dose in 50% of the animals (LD$_{50}$) and its 95% confidence intervals were then calculated.

antiparasitic activities. Additional studies are needed to explore the specific cellular and molecular mechanisms of the antimicrobial effects of *N. sativa* given alone or in combination with other drugs.

GASTROINTESTINAL PHARMACOLOGY

N. sativa powder and thymoquinone have shown anti-ulcer and cytoprotective actions in several laboratory animal studies. For example, Kanter *et al*. (85) investigated the cytoprotective effects of *N. sativa* powdered seeds and thymoquinone on the ethanol-induced gastric mucosal damage in rats using the Robert *et al*. (86) method. Four groups of rats were orally pretreated with either *N. sativa* powdered seeds (500 mg/kg) or thymoquinone (10 mg/kg) and two control groups one hour prior to the intragastric administration of absolute ethanol (1.0 ml/rat). The animals were subsequently sacrificed after one hour and their stomachs were histo-morphometrically examined for the extent of mucosal injury, mast cells, histamine and myeloperoxidase activity. The control group receiving ethanol alone showed significant gastric mucosal erosions, increased mast cells number and increased myeloperoxidase activity when compared to another control group not given ethanol. Both the *N. sativa* powder seeds and thymoquinone significantly decreased the extent of gastric mucosal injury, the number of mast cells, histamine concentrations and myeloperoxidase activity. Of interest was the observation that the *N. sativa* powdered seeds showed greater protective activity than thymoquinone. The mechanism of the gastric cytoprotective effect of *N. sativa* against ethanol-induced injury is not fully understood but appears to be consequence of multiple mechanisms that may include the stabilization of the mast cells, antihistamine, antioxidant and antiperoxidase effects (83). The findings of this study were consistent with a similar study previously performed by the same investigators which showed that *N. sativa* also increased gastric glutathione content, enzymatic activities of gastric superoxide dismutase and glutathione-S-transferase in response to the ethanol injury indicating that the gastroprotective effects of *N. sativa* may be partly explained by its free radical scavenging activity (85).

The *N. sativa* powdered seeds also showed interesting anti-ulcer actions in several standard experimental rat ulcer models (88, 89). In addition, *N. sativa* has also shown hepato-renal protective activity against the bromobenzene induced toxicity in rats (90) clearly suggesting that its cytoprotective activity is broad and covers many organs. However, the mechanisms of the anti-ulcer actions of *N. sativa* in these studies were not systematically explored.

SAFETY CONSIDERATIONS

Only limited toxicological evaluation of *N. sativa* has been reported to date. Some of these safety studies were briefly reviewed by Rahmani *et al*. (27). The cytogenic and genotoxic effects of thymoquinone were tested *in vitro* using primary rat hepatocyte culture (91). Mitotic indices and the rates of apoptoses and necroses were determined as endpoints of cytotoxicity, while chromosomal aberrations and micronucleated cells served as endpoints of this genotoxicity. In this approach, thymoquinone demonstrated cytotoxic and genotoxic effects in a concentration dependent manner: it induced significant anti-proliferative effects at 20 microM and acute cytotoxicity at much higher concentrations.

Al-Ali *et al*. (92) conducted acute toxicity study of thymoquinone in rodents and calculated its median lethal dose in 50% of the animals (LD$_{50}$) following intragastric and intraperitoneal administration. As is evident from *Table 8*, the mouse intraperitoneal LD$_{50}$ value (95% confidence interval) was 104.7 mg/kg (89.7 – 119.7) and the intragastric LD$_{50}$ value was 870.9 mg/kg (647.1 – 1094.8). Whereas in rats, the intraperitoneal LD$_{50}$ of value of thymoquinone was determined to be 57.5 mg/kg (45.6 – 69.4, 95%) and the intragastric LD$_{50}$ was 794.3 mg/kg (469.8 – 1118.8). In both rats and mice, the LD$_{50}$ values were essentially similar thus indicating no apparent species-dependent toxicity concerns. Of interest is the observation that the ratio of intragastric LD$_{50}$ value to the intraperitoneal LD$_{50}$ value appears to be greater than ten indicating decreased oral bioavailability of thymoquinone and this needs to be further investigated in prospective pharmacokinetics studies.

Zaoui *et al*. (93) also evaluated *N. sativa* fixed oil was for its acute and chronic toxicity in rodents. The intragastric and intraperitoneal LD$_{50}$ values (95% confidence limits) in mice were 28.8 ml/kg (26.2 – 31.6) and 2.06 ml/kg (1.86 – 2.26), respectively (*Table 8*). The Zaoui *et al*. (93) acute toxicity results are consistent with the results obtained by other investigators (92, 94). The findings of these published acute toxicity studies indicate a good margin of safety exists of some uses of *N. sativa* (either the oil or thymoquinone) and its potential acute toxicity in rodents. Therefore, *N. sativa* appears to be a relatively safe drug, particularly when given orally to rodents.

AbuKhader (95) established that the minimum toxic dose (MTD) of thymoquinone following its acute oral and intraperitoneal administration in both male and female rats. The results showed that the maximum tolerated dose following intraperitoneal injection was 22.5 mg/kg in the male rats and 15 mg/kg in the female rats, whereas after oral administration it was 250 mg/kg in both male and female rats. Furthermore examination of the heart, lung, kidney, liver and the peritoneum

performed on day 5 following the acute administration did not disclose any apparent signs of toxicity to these organs. However, when thymoquinone was administered at the higher acute intragastric dose of 500 mg/kg, it resulted in the death of 2 out of 8 treated animals and there was evidence of bowel obstruction in the dead animals. Although the dose intervals chosen by this investigator were not logarithmically spaced to permit proper quantal LD_{50} estimation for thymoquinone, the determination of the minimum toxic doses supports a good margin between its efficacy and potential safety concerns.

Zaoui *et al*. (93) also carried out a 12-week oral chronic toxicity study in rats. The rats were treated daily with either an oral dose of 2.0 ml/kg of *N. sativa* oil or its matching placebo for 12 weeks. The 2.0 ml/kg dose of the oil chosen for this chronic toxicity study represented 1/15th of its acute LD_{50} value by these investigators. At the end of 12 weeks of treatment, the investigators evaluated body weight changes and performed complete blood count and blood chemistry analyses. In addition, detailed histopathological examinations of heart, liver, kidneys and pancreas were also performed at the end of the 12-week treatment period. The investigators noted a decreased body weight gain in the *N. sativa* treated rats when compared with the control animals. Blood chemistry analyses did not disclose any apparent organ specific toxicity. Furthermore, no apparent histopathological toxicity differences were noted in any of the examined organs between the placebo and drug treated groups. In addition, serum cholesterol, triglycerides and glucose levels were significantly decreased in the drug treated group when compared with the placebo group. However, leukocytes and platelets counts were significantly decreased in the drug treated group when compared to the control values. In addition, hematocrit and hemoglobin levels were also significantly increased in the drug treated group when compared with the control group.

The decreased serum levels of cholesterol, triglycerides and glucose noted in this chronic toxicity study of the *N. sativa* oil were consistent with its pharmacological action as had been previously discussed. However, the changes in hemoglobin metabolism, the decreased leukocyte and platelet counts were surprising and need additional investigations. Therefore, the finding from the acute and chronic toxicity investigations, suggests a good margin of safety for therapeutic doses of *N. sativa* oil and thymoquinone, but the changes in hemoglobin metabolism and the decreased leukocyte and platelet counts require further investigation.

DISCUSSION

This retrospective literature review is an attempt to summarize the evidence-based preclinical pharmacological characterization of *Nigella sativa* seeds, its oil, its extracts and its active components by diverse multinational authors. We have attempted to include studies that appeared to be well-conducted in order to provide pharmacological inferences about *N. sativa*. Although none of the authors cited in this review indicated whether their studies adhered to the provisions of Good Laboratory Practices (GLP), which are mandatory by many regulatory authorities for preclinical safety studies, but not for pharmacology studies, we believe that the quality of the studies reviewed and included in this communication is good.

The pharmacological characterization described in this review was conducted on many pharmaceutical dosage forms of *N. sativa*. It included the ground seeds, the aqueous extracts, its essential oil or thymoquinone, which is considered to be one its active component of the seeds. Of the many reviewed pharmacological studies performed with *N. sativa,* only one study specifically measured the concentrations of its active components and the geographical source of the seed (8). However, the fact that one of the active component of *N. sativa* is thymoquinone, which is potentially unstable, no efforts were made to stabilize pharmaceutical formulations of thymoquinone prior to its evaluation probably due to the absence of highly sophisticated analytical methods such as gas chromatography and mass spectroscopy (GC/MS) assay essential for the measurement of its components, especially in many older studies. Clearly, a standardized concentration of active ingredients is needed for formal drug development especially in toxicology, pharmaceutical development of the dosage forms prior to the clinical studies.

The chemical analyses of the pharmaceutical dosage forms need to adhere to United States and European regulations concerning Chemistry and Manufacturing Control (CMC). This CMC document is typically filed with the regulatory submission in the United States Federal Food and Drug Administration (FDA) at the start of formal drug development. However, in the United States, complementary drugs, such as the black seeds, do not require 'Investigation of New Drug (IND)' filing. Furthermore, in the United States, all complementary drugs are only promoted on the basis of structure and function but not on the basis of a formal medical therapeutic claim to treat a specific disease entity. In complementary medicine, the lack of standardization of active ingredients by acceptable analytical methods is a major drawback for many plants derived complementary drugs. Formal drug development essential for establishing a therapeutic claim requires this IND filing with the FDA.

The acute and the limited chronic toxicity studies demonstrated that *N. sativa* exhibits toxicity only at a higher multiple of its effective doses. The findings of these published acute toxicity studies indicate a good margin of safety exists of some uses of *N. sativa* (either the oil or thymoquinone) and its potential acute toxicity in rodents. Therefore, *N. sativa* appears to be a relatively safe drug, particularly when given orally to rodents.

Given the lack of standardization of the active chemical contents of the various pharmaceutical dosage forms of *N. sativa* used in almost all of the published preclinical pharmacological and toxicological studies and the fact that *N. sativa* has many diverse pharmacological actions, it is difficult to establish a precise degree of specificity for a desired pharmacological actions vs. undesired adverse reactions or toxicological effects until there is a defined pharmaceutical dosage form that has optimum bioavailability. For the estimation of the specificity for a therapeutic compound, pharmacologists usually calculate the therapeutic ratio, which is derived from dividing the median intolerated or toxic dose (LD_{50}) over the median effective dose (ED_{50}) for a desired pharmacological action and the higher the ratio indicates a higher margin of its specificity and safety (13). However, both pharmacology and safety studies must be performed on a well defined pharmaceutical preparation with known contents and similar bioavailability. Therefore at this time, it is difficult to infer quantitative information about the specificity for each potential therapeutic use. Although *N. sativa* has been used as food for many years without any reported toxicity concerns, some potential therapeutic uses may require high dosage and hence formal laboratory safety studies with a defined pharmaceutical preparation must be conducted to support the safety and efficacy for a specific prospective clinical application.

Although some of the pharmacological studies discussed in this review are based on a single dose acute administration, there are several chronic studies in which *N. sativa* was administered for 30 to 40 days to animals without obvious toxicity specifically linked to the drug treatment by the investigators.

The specific pharmacological mechanism of action for *N. sativa* for the many of its therapeutic properties is very broad and we have attempted to list the known disclosed mechanisms. Overall, the known mechanisms include: anti-oxidant, immunomodulating, cytoprotective, neuroprotective and inhibitory effect on many mediators of inflammation known to exist with some of the animal models corresponding to a specific disease entity (17, 18, 26-28, 37, 39, 46, 51, 52, 63, 64, 69, 71-73, 76, 78, 87).

There are three obvious limitations to our investigation that needs to be pointed out: firstly, we only examined published literature dating from 1960 and did not examine in details older literature. Secondly, we had rejected few older studies due to their preliminary nature or poor conduct or interpretation. Thirdly, we only examined studies written in the English language.

It is hoped that this review would provide a stimulus for basic and clinical scientists to investigate the full potential of black seeds and/or its potential analogs for human therapeutics. Despite the reservations expressed about the pharmaceutical dosage forms and the stability of the drug, there are several potential and promising clinical uses, especially for unmet therapeutic indications in the fields of oncology, neurology rheumatology, pulmonary medicine, infectious diseases and endocrinology.

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