The Black Seed *Nigella sativa* Linnaeus - A Mine for Multi Cures:
A Plea for Urgent Clinical Evaluation of its Volatile Oil

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Abstract: This review almost covers what is actually known to date about the black seed and its constituents. It is clear that most of the potent and fruitful activity resides in its volatile oil and a protein component. However, the volatile oil suffers the drawback of the bronhoconstricting effect of thymoquinone. However, the latter can be easily removed from the oil to obtain a dethymoquinoneated oil that has already been shown to possess the major characteristics of the whole oil. At this moment there are a lot of experimental data that hopefully, may stimulate the beginning of the era of pilot clinical studies to evaluate the clinical potential of the volatile oil, some of the protein fractions and the dethymoquinoneated volatile oil. It is hoped that this plea will have a rapid response.

Key words: Blackseed, Thymoquinone, P-Cymene, 1 -pinene –hederin, Nigellane

Introduction

The last two decades witnessed an enormous research rush to reveal the pharmacological actions of an annual spicy delicate and beautiful herb known by the Latin name *Nigella sativa* Linnaeus variety hispidula (brachyloba) that belongs to the botanical family *Ranunculaceae*. It was first identified and described by Linnaeus in 1753[1]. The plant is an erect profusely branched herb that can attain heights of 40 and up to 70cm. It bears alternate leaves, terminal white flowers and capsule like fruits. The latter are filled with black ovoid or obpyramidal seeds attaining lengths and widths ranging from 2.5 to 3.5mm and widths from 1.5 to 2mm. respectively. The detailed taxonomy of the plant was described by Muschler [2]. The plant is known to all Arabian and Islamic countries and carries various colloquial names. It is known generally by the names Habbat Albarakah, Alhabahat Alsawda and Alkamoun Aslaswad. In some countries it is known by the names Shuniz and Khodhira. Its English name is Black Cumin or Black Caraway. It should be noted that the latter two names bear no relation to the plants Cumin (*Cuminum cyminum*, Linne) and Caraway (*Caram carvi,* Linne) that belong to the botanical family *Umbelliferae*.

Of all the plant organs it is only the seeds which attracted most of the researchers starting from Egypt and the Sudan in Africa and extending to Saudi Arabia, India and Pakistan in Asia and most recently those in Japan, France, England, Canada and USA.

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During the last two decades, literature is replete with the subject of pharmacological actions of a pure component or an extract of the seeds. Most of the studies dealt with the volatile oil of the seeds and its major constituents. These studies revealed a multi-range of actions that covered almost all known ailments of man in the various body systems.This review is mainly intended to provide a comprehensive knowledge regarding our existing knowledge of the pharmacological and toxicological actions of this plant. It is hoped that the provided knowledge will generate a real clinical appraisal and evaluation of the effectiveness of at least the volatile oil of the seeds in the treatment of some cardiovascular diseases.

1.1 The Chemical Composition of the Seeds

Historically, the chemical investigations on the *N. sativa* seeds started on the year 1880 when Greenish [3] published the first report concerning the presence of 37% oil and 4.1% ash (calcium salts) in the seeds. The general chemical composition of the seeds as we know it today is depicted in Table 1.

Table 1: The general chemical composition of *N. sativa* seeds*

Constituent	% Range (w/w)
Oil	31-35.5
Protein	16-19.9
Carbohydrates	33-34
Fibre	4.5-6.5
Ash	3.7-7
Saponins	0.013
Moisture	5-7

* References [5-10]

1.1.1. Chemical Composition of N. sativa Oil

The chemical analysis of *N. sativa* total oil revealed the presence of both a fixed oil and a volatile oil. The major component was the fixed oil whereas the volatile oil ranged from 0.4-0.7% of the seeds' weight [5, 7, 9]. The fixed oil chemical composition is outlined in Table 2.

Table 2: The chemical composition of *N. sativa* fixed oil*

Constituent	% Range (w/w)
Linoleic Acid	44.7-56
Oleic Acid	20.7-24.6
Linolenic Acid	0.6-1.8
Arachidic Acid	2-3
Palmitoleic Acid	3
Eicosadienoic Acid	2-2.5
Palmitic Acid	12-14.3
Stearic Acid	2.7-3
Myristic Acid	0.16
Sterols	0.5

*References [6, 11-13]

Generally, there were no significant variations in the chemical composition of the fixed oils of seeds grown in Egypt, Sudan, Ethiopia, India, Turkey and Syria. However, Al-Jassir [7] noted that the seeds grown in Qassim, Saudi Arabia, contained, in addition to the fatty acids depicted in Table 2, two more acids which were lignoceric acid about (1%) and myristoleic acid (0.18%) without the presence of eicosadienoic acid (C20:2). Lignoceric acid is not found in many other edible vegetable oils.

Specific chemical analyses of the volatile oil started during the years 1960-1963 by Mahfouz and El-Dakhakhny [13] and Canonica et al [14]. These studies were complemented by most recent ones which revealed various pharmacologically active constituents that included Thymoquinone (2-isopropyl-5-methyl-benzoquinone) that may attain up to 27.8% of the volatile oil (w/w) [12, 14-17], Carvacrol (2-methyl-5-(1-methyl ethyl) phenol which is also known as 2-hydroxy-p-cymene or isothymol) (5.8-11.6% (w/w)) [18], p-cymene (isopropyl toluene) in the range of 15.5-31.7% (w/w) [17, 18], α-pinene (2,6,6-trimethylbicyclo [3.1-1]-hepta-2-ene (9.3%) [18], 4-terpineol (or α-terpineol or α,α,4-trimethyl-3-cyclo-hexene-1-methanol or p-menth-1-en-8-ol) 2-6.6% [17]; longifolene (or Junipene or Kuromatsuene or decahyro-4,8,8-trimethyl-9-methylene-1,4-methanoazulene) 1-8% (w/w) [17], t-anethole (p-Propenyl anisole or 1-methoxy-4-(1-propenyl)benzene 0.25-2.3% w/w [17] and the reduction product of thymoquinone-thymohydroquinone [19] together with some esters about 16% [18].

1.1.2 Non-Oily Components of the Seeds
1.1.2a Minerals
Analysis of *N. sativa* seeds, ash revealed the presence of 0.5-1% calcium, 0.6% phosphorus, 0.6% potassium and 0.1% sodium of the total seeds weight [5].

1.1.2b Saponins
The major saponin in the defatted seeds of *N. sativa* is the glycoside α-hederin or Helixin or melanthin which on acid hydrolysis releases its sugar rhamnose / arabinose and gives the aglycone hederagenin (or melanthigenin) or caulosapogenin [9, 20-22].

1.1.2c Alkaloids
Three types of alkaloids were isolated from the defatted seeds of *N. sativa*. These were identified as the indazole nigelicine [23], the isoquinoline nigellimine [24] and its N-oxide [25] and the indazole alkaloid nigellidine [26].

1.2 Determination of *N. sativa* constituents
Quantitative determination of the individual fatty acids present in the fixed oil of *N. sativa* was achieved using GC/MS [5, 6, 12] whereas quantification of the volatile oil constituents was performed using thin layer chromatography [27] and high performance liquid chromatography [28].

2. Pharmacological Actions of *N. sativa*
Since 1960 to date various researchers throughout the world reported the pharmacological actions of the whole seeds of *N. sativa* as crushed powder or as an extract together with those of some of its constituents such as its total, fixed or volatile oils or the individual constituents of the latter. The most extensive studies concerned the volatile oil and its constituent thymoquinone. The results of these studies will be reviewed later. Some of the

studies dealt with the acute toxicity of the seeds and its components. In these studies the acute LD50 values of the fixed oil and volatile oil were found to be 11.9 ml/kg i.p. and 0.54 ml/kg i.p. in mice, respectively[29]. In another study mice tolerated the methanolic and the chloroform extracts for up to 21 g/kg orally [30]. The LD50 of the fixed oil in rats was found to be 12 ml/kg i.p. (unpublished observations). With regard to the whole powdered seeds, mice tolerated doses of 2g/kg orally/day for 5 days [31]. Humans also tolerated doses of 2 g of the crushed seeds per day for 28 days [32].

2.1 Pharmacological Actions of the Whole Crushed Seeds

Treatment of human volunteers with whole seeds' powder at doses of 1g twice daily for 4 weeks increased the ratio of T-lymphocytes helper cells to T-suppressor cells by 72% and enhanced T-killer cells function and number [32]. On the other hand, treatment of the human volunteers with the same dose for two weeks did not produce clear cut stable changes in the blood glucose, cholesterol or triglycerides levels [33]. Clinical studies in children revealed that administration of the powdered seeds orally at does of 40mg/kg to those infected with *Ascaris lumbricoides*, *Taenia saginata* or *Hymenolepsis nana* decreased the parasites' fecal eggs count by up to 93% [34].

Regarding the effects of the whole seeds in animals, significant enhancement of the phagocytic activity against *Candida albicans* was noted in mice at doses of 2g/kg/day for 5 days [31] and significant enhancement of milk production was observed in goats at doses of 0.1g/kg/day [35]. Treatment of rats with a suspension of the seeds at a dose level of 0.8 g/kg/day orally for 4 weeks protected against CCl_4-induced hepatotoxicity as reflected by the significant decreases in the plasma levels of Alanine transaminase (ALT) and Aspartate transaminase (AST) enzymes together with significant decreases in the plasma level of lipid peroxides measured as malondialdehyde and significant increases in the erythrocytes' content of glutathione peroxidase and superoxide dismutase [36].

Studies *in vitro* using the crushed seeds against the different stages of *Schistosoma mansoni* namely the Miracidia and Cercariae revealed that exposure of the parasites to concentrations of 2-5 µg/ml resulted in 50-100% death of the parasites. Furthermore, exposure of the adult male and female worms to the seeds' powder in concentrations of 60-100 µg/ml induced 50-100% mortality [37]. At the same time, the seeds treatment decreased the activity of the antioxidant enzymes superoxide dismutase and glutathione peroxide and limited the energy production of the adult parasite via inhibition of the glycolytic enzyme hexokinase [37]. Furthermore, studies may characterize the active antischistosomal component of the seeds, a possible candidate being the isoquinoline alkaloids mentioned earlier.

Experiments *in vivo* in rats revealed that treatment of the animals with the whole seeds' powder mixed with the daily feed to provide doses of 250 mg/kg/day for 7 weeks induced significant protection against methionine-induced hyperhomocysteinemia suggesting a potential role of the seeds in prophylaxis against occlusive vascular diseases [38].

2.2 Pharmacological Actions of the Seeds' Aqueous Extract

Aqueous extracts of *N. sativa* seeds were studied both *in vitro* and *in vivo*. Administration of the aqueous extract in doses of 2 g/kg orally to rats induced significant protection against aspirin-induced increases in the volume of the gastric juice, the acid output and the gastric ulcers [39]. Studies in alloxan-diabetic rabbits revealed the ability of a 5% boiled aqueous extract administered at doses equivalent to 1g seed powder/kg/day orally for two months to significantly decrease the elevated blood glucose level and to increase the plasma level of tri-iodothyronine [40]. A similar treatment to the diabetic rabbits also suppressed the magnitude of lipids peroxidation and increased the plasma levels of reduced glutathione and ceruloplasmin [40].

Exposure of guinea-pigs to 5% aqueous extracts (boiled or unboiled) in the form of aerosols for 7 minutes protected the animals against 10% citric acid aerosols-induced cough in a manner equivalent to that exerted by 3% Codeine aerosols [41, 42].

Experiments in *Candida-albicans*-inoculated mice revealed the ability of an aqueous extract of the seeds at doses equivalent to 200 mg of the seeds protein/kg/day orally for 3 days to suppress the growth of the Candida by 5-11 folds in the kidney, liver and spleen of the animals [44].

Treatment of rats with an aqueous extract of the seeds resulted in significant inhibitions of experimentally-induced inflammations and pain but not fever [45].

Various *in vitro* studies were performed using the aqueous extract of the seeds or its protein fractions. In one of these studies exposure of human lymphocytes to the 10% aqueous extract in concentrations up to 5 μg/ml induced marked stimulation of the release of interleukin-3whereas exposure of the human macrophages resulted in increased release of Interleukin-1β [45]. In subsequent experiments exposure of the human leukocytes to the protein fraction of the seeds in concentrations of 1-2 μg/ml enhanced production of TNF-α in Pokweed nitrogen-activated and non-activated cells and significantly increased the production of IL-8 in the activated cells only [45].

In the isolated rat pancreatic islets of Langerhan, exposure of the cells to aqueous extracts or their basic aqueous subfractions in concentrations up to 5 mg/ml induced significant increases in insulin release [46]. Exposure of the isolated guinea-pig trachea to an aqueous extract of the seeds antagonized ACh- and histamine-induced contractions in a non-calcium antagonistic mechanism [47]. However, in the isolated heart, the extract produced negative inotropic and chronotropic actions via a seemingly calcium-channel blocking effect [48].

2.3 Pharmacological Actions of the Seeds' Ethanolic Extract

A few experiments were performed using the alcoholic extract of the seeds both for *in vivo* and in vitro studies. These studies revealed the successful effectiveness of the alcoholic extract of *N-sativa* seeds in doses of 150 mg/kg daily for 8 days in inducing significant

reductions of the volume of the gastric secretion, the free acid, the total acid content and the gastric ulcers induced by pyloric ligation in rats[49]. The extract in single doses equivalent to 40 mg powdered seeds/kg also significantly decreased the cestodal foecal egg count by 93% in children infected with some cestodes such as *Ascaris lumbricoidis, Taenia saginata* or *Hymenolepsis nana* [35]. Studies in rabbits also revealed the successful protection of the extract against CCl_4-induced liver fibrosis and cirrhosis [50]. In vitro studies using the isolated rabbit jejunum revealed the spasmolytic activity of the extract via a calcium channel blocking action [51].

2.4 Pharmacological Actions of the Methanolic Extract

In vitro exposure of oral and various other carcinomas such as Ehrlich ascites cells, Dalton's Lymphoma ascites cells and sarcoma – 180 cells to the methanolic extract of *N. sativa* seeds in concentration of 0.5-3µg resulted in significant cytotoxicity in the cells [52]. These results stimulated the researchers to investigate the influence of the extract in vivo in mice. The results revealed that treatment of mice with the extract at doses of 10 mg/kg/day (i.p) for 10 days significantly suppressed Ehrlich ascites carcinoma cells development via inhibition of DNA synthesis in the cells [52]. In other experiments, the extract succeeded in protecting mice against cisplatin-induced decreases in haemoglobin and leukocytes counts [53].

Studies *in vitro* demonstrated the potent inhibitory effect of the methanolic extract in suppressing the growth of *Bacillus subtilis, Escherichia coli*, *Streptococcus foecalis*, *Staphylococcus aureus, Pseudomonas aeroginosa,* and *Candida albicans* [54]. The effective *in vitro* concentrations ranged from 62.5 µg – 1000 µg/ml.

2.5 Pharmacological Actions of Petroleum Ether Extract

Administration of the dried petroleum ether extract of *N. sativa* seeds to rats by gavage orally for four weeks resulted in significant decreases in blood triglycerides, increased high density lipoprotein and potentiated insulin-induced activation of protein kinase enzyme [55].

Studies *in vitro* using the isolated rabbit jejunum and the guinea-pig trachea revealed a spasmolytic and a bronchodilatory actions, respectively that involved a calcium channel blocking mechanism [56].

2.6 Pharmacological Actions of the Hexane Extract

Treatment of pregnant rats with the hexane extract of the seeds during the first ten days of pregnancy in doses equivalent to 2g seeds/kg/day orally inhibited the implantation process [57].

2.7 Pharmacological Actions of the Ethereal Extract

Treatment of lactating rats with the ethereal extract significantly enhanced the milk production [58]. Studies in vitro revealed that exposure of the gram-positive bacteria *Staphylococcus aureus*, the gram –ve *Pseudomonas aerugenosa* and *Escherichia coli* and the yeast *Candida albicans* in concentrations of 250-400 µg/disc eradicated the microorganisms [59].

2.8   Pharmacological Actions of the Volatile Oil
2.8.1   Effect on the Cardiovascular System
        Cardiovascular pharmacological investigations using the volatile oil of *N. sativa* seeds were touched for the first time in 1962 when Mahfouz et al [60] reported broadly that administration of the volatile oil in a dose of 200 µl/kg I.M decreased the arterial blood pressure. However, in 1993/1994 El Tahir et al started a series of experiments to follow the detailed cardiovascular actions of the volatile oil and to elucidate its mechanism of action in both rats [61] and guinea-pigs [62]. These studies revealed the ability of the oil in doses of 4-32 µl/kg (IV) to decrease the arterial pressure and the heart rate in a dose-dependent manner.
Using various receptor blockers, ganglionic blockers, amine depletions and the technique of spinal pithing, the authors came to the conclusion that the cardiovascular depressant actions of the oil are mediated centrally in the brain via serotoninergic mechanisms that involved activation of $5-HT_1A$ receptors located on the nucleus tractus solitarius and the dorsal vagal motor nucleus in the brain stem [61].

        Besides the actions of the volatile oil on the cardiovascular system, its effects were studied in various other systems and tissues as follows:

2.8.2   Effect on the Respiratory System
        Influenced by the folklore claim that the black seed can treat asthma, Mahfouz and his collaborators investigated the effect of the volatile oil in guinea pigs and dogs. The results revealed that (i.m) or (i.p) injection of the volatile oil in doses of 200 µl/kg antagonized histamine-induced bronchoconstriction and induced bronchodilation [61, 63]. However, the oil was found to be ineffective in blocking histamine H_1 receptors in the trachea both *in vitro* [63] and *in vivo* [64]. To clarify the effect of the volatile oil on the respiratory system and to elucidate its mechanism of action, El Tahir et al [65] performed several experiments in guinea-pigs. The results revealed that intravenous administration of the oil in doses of 4-32 µl/kg induced dose-dependent increases in the respiratory rate and increased the intra-tracheal pressure pointing to a strong evidence of bronchoconstriction [65]. The oil did not contract the isolated trachea. With the help of various receptor blockers, mast cell stabilizers and amine depletors, the authors concluded that the volatile oil-induced bronchoconstriction and tachypnea were due to the release of histamine from pulmonary non-tracheal mast cells and circulating basophils with the consequent activation of the H_1 receptors located on the pulmonary irritant receptors and the pulmonary C sensory afferent nerves resulting in activation of the afferent sensory vagal nerves reaching the vagal nuclei in the brain stem. The final effect will be the activation of the efferent vagal activity to the pulmonary system [65].

2.8.3   Effect on Body Temperature
        The preliminary reports regarding the influence of the volatile oil on the body temperature appeared in 1997 [66] followed by a detailed review with elucidation of the mechanism of action in 2006[67]. These studies investigated the influence of the oil in the rectal temperature of mice. Administration of the oil in doses of 100-400 µl/kg (i.p) into mice induced dose-dependent decreases in the body temperature with a 4°C fall in 30 minutes following the injection of the oil and the effect diminished to non-significant levels 3 hours later [67]. Using various receptor blockers, the authors revealed the mechanism of action of the induced hypothermia as release and/or activation of central serotoninergic receptors located on the temperature regulating center in the anterior part of the hypothalamus [67].

2.8.4   Effects on Experimentally-induced Gastric Ulcers

Oral treatment of rats with *N. sativa* volatile oil in doses of 1 ml/kg one hour before induction of gastric ulcers with ethanol, significantly protected the animals against the alcohol-induced ulcers and significantly elevated the gastric glutathione content, the gastric superoxidase dismutase and the glutathione-S-transferase activities together with significant reductions in alcohol-induced lipids peroxidation in the stomach cells [68, 69].

2.8.5   Effect on Experimentally-induced Inflammation

Intraperitoneal treatment of rats with the volatile oil in doses of 0.6-1.5 ml/kg significantly suppressed carrageenan-induced paw oedemas in rats and cotton-pellet-induced granuloma in the abdomens [70].

2.8.6   Effect on Blood Glucose Level

Administration of the volatile oil to both alloxan diabetic rabbits [71] and rats [72] induced significant decreases (23-43%) in the blood glucose level without affecting insulin release. However, treatment of streptozotocin diabetic rats with the oil at a dose of 0.2 ml/kg/day (i.p) one day after streptozotocin and for 30 consecutive days resulted in significant decrease in the blood glucose level, an increase in the blood insulin level and partial regeneration of the pancreatic β-cells of the islets of Langerhan[69].

2.8.7   Effect on Experimental Cancers

Treatment of hamsters with *N. sativa* volatile oil did not induce any dysplastic alterations or carcinomas in the cheek pouches of the animals [74]. Furthermore, chemical treatment of rats with 1, 2-dimethyl-hydrazine-induced colon cancer in the postinitiation stage but daily treatment with *N. sativa* volatile oil for 14 weeks induced significant reductions in the colonic lesions via suppression of the cell proliferation in the colonic mucosa [74]. The treatment did not induce any harmful effects in the blood or urine parameters and no pathological changes in the various vital body organs. Furthermore, exposure of various human cancer cell lines to the volatile oil in concentrations of 120-380 µg/ml induced significant death of the cells showing clear cytotoxic effect [75].

2.8.8 Effect on the Gall Bladder

Intramuscular injection of the volatile oil in a dose of 0.2ml/kg into dogs increased the concentration of the bile salts and the flow of bile into the intestine [60].

2.8.9   Effect on the Immune System

Treatment of typhoid-antigen-challenged rat with the volatile oil revealed an immunosuppressant action as evidenced by the significant decreases in the antibody titer and the splenocytes and neutrophils counts [75].

2.8.10 Effect on Microorganisms

Exposure of various types of bacteria and fungi to various concentrations of the volatile oil revealed a broad spectrum of antibacterial and antifungal activities. The bacterial species that were more susceptible included *Salmonella, Shigella shigae*, *Bacillus cereus, Vibrio cholerae, Pseudomonas aerogenosa* [6, 76, 77]. Furthermore, the oil was also effective against multi-drug resistant strains of *Staphylococcus aureus* [78]. The antifungal spectrum included *Aspergillus niger, Aspergillus flavus, Candida albicans, Microsporum gypseum* and *Rhizoctonia solani* [79].

With regard to viruses, the oil significantly suppressed the growth of the cytomegalovirus following its intraperitoneal administration to infected mice [80].

2.8.11 Effect on Smooth Muscles

Studies using various isolated smooth muscles revealed a smooth-muscle relaxant activity of the oil. This has been shown in the isolated rabbit aorta, rabbit jejunum and the isolated guinea-pig trachea [51]. The suggested mechanism was the blockade of calcium channels. Furthermore, the oil relaxed the uterine muscle of both rats and guinea-pigs and antagonized oxytocin-induced uterine contraction [81].

2.9 Pharmacological Actions of Some Volatile Oil Constituents

2.9.1 Pharmacological Actions of Thymoquinone

Thymoquinone was considered as one of the major components of *N. sativa* volatile oil. For this reason, the influence of this substance (whether natural or synthetic) was explored in most of the systems investigated for the volatile oil. These systems included:

2.9.1.1 Effects on the Cardiovascular System

Administration of thymoquinone (TQ) i.v. into rats in the dose-range 0.2-1.6 mg/kg induced dose-dependent decreases in the arterial blood pressure and heart rate [61]. It thus induced cardiovascular depressant actions similar to those of the whole volatile oil. However, El Tahir et al [61] found that its mechanism of action was not similar to the central mechanism of the volatile oil since its effects were completely abolished by 5-HT and ACh receptor blockers. TQ also protected mice against doxorubicin-induced cardiotoxicity [82].

2.9.1.2 Effects on the Respiratory System

Intravenous administration of TQ into guinea-pigs in the dose range 1.6-6.4 mg/kg induced bronchoconstriction as evidenced by the dose-dependent and significant increases in the intra-tracheal pressure and potentiation of histamine-induced bronchoconstriction. The effects were completely blocked by histamine H_1 receptor blocker e.g. Mepyramine maleate [65].

2.9.1.3 Effects on the Liver

Treatment of dogs with TQ in doses of 1 mg/kg (i.v.) stimulated the production of bile salts and enhanced bile flow [83]. Studies *in vitro*, using isolated rat hepatocytes, revealed that pretreatment of the cells with TQ protected the cells from the hepatotoxicity of t-butyl hydroperoxide [84]. Furthermore, TQ also protected mice against CCl_4-induced hepatotoxicity seemingly via an anti-oxidant mechanism [85, 86].

2.9.1.4 Pharmacological Actions on the Kidney

Intramuscular administration of TQ into rats in doses of 4 mg/kg/day for four days stimulated the excretion of uric acid in urine [83]. Furthermore, oral administration of TQ to both rats and mice in doses of 4-5 mg/kg for several days protected the animals against cisplatin-induced nephrotoxicity [87].

2.9.1.5 Effect on Chemically-induced Convulsions

Intraperitoneal administration of 40-80 mg/kg TQ in 60 minutes in mice or its intracebroventricular injection at doses of 200 and 400μM into rats protected significantly against pentylene tetrazole-induced tonic-clonic seizures and protected mice against maximal electroshock-induced seizures [88]. The protective effect seemed to involve an opioid receptor-mediated activation of $GABA_A$ receptors.

2.9.1.6 Effect on Experimental Pain

Although, Abdel-Fattah et al [89] found that both TQ and *N. sativa* volatile oil induced anti-nociceptive action in the formalin-induced nociception in mice via activation of kappa opioid receptors (OP_2) yet Ghannadi et al [90] reported that N. sativa volatile oil polyphenol (which include TQ), exhibited an antinociceptive effect in both rats and mice that did not involve opioid receptors. The compounds failed to show an anti-nociceptive effect in the tail flick test in mice.

2.9.1.7 Effect on Experimental Inflammations

Intraperitoneal administration of TQ into rats in doses of 0.5-5 mg/kg produced significant suppression of carrageenan-induced paw edema [71]. A similar anti-inflammatory activity was seen following the experimental intraperitoneal injection of the volatile oil polyphenols against carrageenan-induced paw oedema but not croton oil-induced ear oedema in mice [90]. The reported analgesic effect of TQ may be due to its ability to inhibit the prostaglandin cyclo-oxygenase enzyme (COX) [14] and the reported anti-inflammatory effect may be due to the inhibitory effect of TQ on both lipoxygenase and COX enzymes [14]. An inhibitory effect of TQ in lipid peroxides may also be involved [14].

2.9.1.8 Effect on Tumors

In 1998, Worthea and Ghosh [91] reported the general anti-tumor effect of thymoquinone. In 2005, Rooney and Ryan [92] extended these results and found that although TQ exerted cytotoxicity against lungs, larynx, colon and pancreas carcinomas yet it was more potent against the larynx ones. In a later investigation, the authors delineated the TQ-induced cytotoxicity being due to the depletion of cellular glutathione and the activation of caspase-3 enzyme [93].

2.9.1.9 Effect on Microorganisms

Studies in the 1940s and 1950s, before the discovery of TQ in *N. sativa* volatile oil, appeared for the antibacterial effect of TQ against *Staphylococcus aureus* [94, 95] and *Mycobacterium tuberculosis* [96] and against some fungi such as *Trichophyton rubrum* and *Trichophyton mentagrophytes* [97-99]. In a recent publication, Kahsai [100] re-investigated the effect of TQ in various bacteria, confirmed its antibacterial action and reported a minimal inhibitory concentration of (3.6 µg/ml) against *Staphylococcus aureus*. The inhibitory effect was due to the inhibition of RNA and protein synthesis.

2.9.1.10 Effect on the Stomach

Although Marozzi et al [66] reported a TQ-induced enhancement of histamine in induction of gastric ulcers, yet Kanter [68] in 2005 reported that oral administration of TQ at a dose of 10mg/kg orally one hour before oral absolute alcohol at a dose of 4 ml/kg to rats protected the animals against alcohol-induced ulcers by approximately 38% via antioxidant

mechanisms that involved inhibition of reactive oxygen radicals and an increase in superoxide dismutase availability.   In this respect the effect of TQ was less than that of the whole volatile oil.

2.9.2   Pharmacological Action of α-Pinene

No great attention is paid to the pharmacological actions of α-pinene, the unsaturated bicyclic monoterpene hydrocarbon present in *N. sativa* volatile oil.   However, its pharmacological spectrum included effects on:

2.9.2.1 Cardiovascular **System**

Intravenous administration of α-pinene into rats in doses of (2-16 µl/kg) decreased both the arterial blood pressure and the heart rate [101]. The effects seemed to be mediated centrally at the site of the nucleus tractus solitarius and the vasomotor center in the medulla [101].

2.9.2.2 Effect on Microorganisms

Studies *in vitro* revealed the antibacterial action of α-pinene against the acne vulgaris bacteria namely *Propionibacterium acnes* and *Staphylococcus epidermidis* and against *Staphylococcus aureus* [102, 103].

2.9.2.3 Effect on Tumors

Ruch and Silger[104] reported the effectiveness of α-pinene in inhibiting hepatic epithelial tumors in rats; however, it failed to inhibit 7,12-dimethyl [a] anthracene-induced mammary carcinomas in rats [105].

2.9.2.4 Other Actions

α-pinene has been shown to exert an anti-inflammatory action against PGE_2- and carrageenan-induced paw oedema in rats [106] and an increase in mucocillary clearance in patients with chronic pulmonary obstruction [107]. Administration of α-pinene (i.p.) into mice in doses of 5-200 µL/kg-induced dose-dependent decrease in body temperature for at least 3 hours by involving both serotoninergic and opioid mechanisms [67].

2.9.3 Pharmacological Actions of p-cymene

Here again p-cymene (or p-cymol) did not attract many pharmacologists as revealed by the paucity of data concerning its actions in various body systems. However, in 2003 El Tahir *et al*., [101] reported that administration of   p-cymene in doses of 2-32 µl/kg (i.v.) into rats induced dose-dependent decreases in the arterial blood pressure and the heart rate, the effect was potent as that of *N. sativa* volatile oil. The effects seemed to be mediated centrally at the level of the nucleus tracts solitarius and the vasomotor center [101]. Unlike α-pinene it did not affect body temperature in mice [67].   Its other actions included local anaesthetic effect [108] and a mild antibacterial action [109].

2.10 Pharmacological Actions of the De-thymoquinonated *N. sativa* **volatile oil**

Amazed by the potent cardiovascular depressant actions and disturbed by the potent bronchoconstrictor action of the volatile oil that is due to thymoquinone, El Tahir et al [101] managed to remove thymoquinone of the whole volatile oil of *N. sativa* and re-examined the

cardiovascular effects of the dethymoquinonated oil. The results revealed that intravenous administration of this oil into rats in doses of (2-16 µl/kg) produced dose-dependent decreases in both the arterial blood pressure and heart rate to an extent twice that of the whole volatile oil especially with regard to the induced bradycardia. At a dose of 16 µl/kg (i.v.) the induced decreases in the arterial blood pressure were 18.6 ± 4.6 and 33.5 ± 8.3 mmHg and the decreases in the heart rate were 27.6 ± 5.9 and $53.6 \pm 3\%$, following administration of the whole volatile oil and the de-thymoquinnated oil, respectively [61, 101]. These experiments also revealed the central mechanism of action of the dethymoquinnated volatile oil. The de-thymoquinnated oil retained its tachypnic effect and lost its bronchoconstricting action (unpublished observations). Thus, these experiments paved the way for the use of *N. sativa* volatile oil without any fear from thymoquinone-induced bronchoconstriction [65] or its potential ulcerogenic action, if any [64].

2.11 Pharmacological Actions of the Total Oil of *Nigella sativa*

The term total oil of *N. sativa* embraces the two purified oils present in the expressed or solvent-extracted oil, namely the fixed oil (>30% of the seeds' weight) and the volatile oil (0.5% of the seeds' weight). However, it should be noted that some investigators don't specify exactly the type of *N. sativa* oil used whether it is the total, fixed or volatile oil. This review did not consider any study in which the exact type of the oil used was not specified. With the above definition of the total oil, literature survey revealed only a few studies. These included the ability of the total oil to induce:

1. Suppression of pain in rats and mice at a dose level of 1 ml/kg orally [111].

2. Inhibition of thromboxane A_2 and LTB_4 in rat peritoneal leukocytes stimulated by the calcium ionophore A23187 [12].

3. Modulation of endothelial cells fibrinolytic potential by increasing the release of both tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-type 1 (PAI-1) in the endothelial cells of the human umbilical vein and the human uterine artery [111]. It modulates the balance between fibrinolysis and thrombus formation.

4. Inhibition of ADP- and arachidonic acid-induced platelets aggregation by 36-38% whereas the methanolic extract of the same total oil inhibited ADP- and arachidonic acid-induced aggregation by 51 and 93%, respectively. The anti-platelet effect was found to be due to presence of the compound 2-(2-methoxypropyl)-5-methyl-1, 4-henzene diol, thymol and carvacrol [112]. The methanolic fraction also inhibited blood coagulation without showing any fibrinolytic activity [112].

2.12 Pharmacological Actions of the Fixed Oil

Only limited pharmacological experiments were performed using the pure fixed oil of *N. sativa* that is devoid of any volatile fraction. Two of these studies showed (a) the ability of fixed oil to prolong the prothrombin time in rats leading to transient anticoagulant activity [113] and (b) El Tahir et al [114] showed that short-term treatment of normal and streptozotocin-diabetic rats

with the fixed oil in doses of 1 and 2 ml/kg per day revealed the ability of the oil.

1. To induce desensitization of α_1-adrenoceptors in the blood vessels.
2. Sensitization of β_2-adrenoceptors in the blood vessels.
3. Stimulation of conversion of arachidonic acid to vasodilatory PGE_2 and PGI_2 in normal animals.
4. Suppression of ADP-induced platelets aggregation in the normal and diabetic animals.
5. Decrease in the uterine sensitivity to the oxytocic actions of both oxytocin and PGE_2 [66, 114].

2.13 Pharmacological Actions of α-Hederin

Treatment of four types of human carcinomas namely lung, larynx, colon and pancreas with α-hederin *in vitro* resulted in reduction of cytotoxicity, necrosis and apoptosis [92, 93]. At doses of 5-10 mg/kg orally to mice, for 8 or 15 days it produced significant inhibition of murine P388 leukemia and Lewis lung carcinoma cells [22]. Its protective action seemed to be due to depletion of intracellular glutathione and production of reactive oxygen species together with activation of caspase-3 [115].

Treatment of Hep C_{12} cells line with α-hederin in doses of (1.5-3 µg/ml) protected the cells against H_2O_2-induced DNA damage via scavenging free radicals and enhancing catalase activity [116].

Studies in mice revealed that administration of α-hederin decreased the hepatic content of Cytochrome P450 and the activities of the subtypes CYP 1A1, 1A2 and 2E1. The treatment decreased the levels of mRNA except that of CYP 2E1 [117]. Treatment of mice with doses of 30 µmol/kg/day S.C for 3 days protected mice from paracetamol-, bromobenzene-, CCl_4-, furosemide- and thioacetamide-induced hepatotoxicity. The protective activity seemed to be due to inhibition of various types of CY P450 subtypes such as CYP 1A, CYP 2A and CYP 3A [118].

Exposure of *Leishmania medicana* in the promastigote and amastigote stages to low concentrations of α-hederin potentiated the leishmanicidal action of pentamidine [119]. However, α-hederin was highly toxic to human's monocytes cells [119].

2.14 Pharmacological Actions of Nigellone

The name Nigellone was coined by Drs. Mahfouz and El-Dakhakhny in 1960 to label the resinous compound they isolated from the volatile oil of *N. sativa*. They assigned it the chemical formula $C_{18}H_{22}O_4$. They believed that it was the active component of the volatile oil [13; 120]. However, in 1963, El Dakhakhny re-examined the extraction process of the volatile oil and managed to isolate a bright yellow crystalline compound with the chemical formula $C_{10}H_{12}O_2$ which was characterized as thymoquinone [15]. It was then realized that nigellone was actually a dimer of thymoquinone previously known as dithymoquinone synthesized in 1944[121]. Thymoquinone can be converted to dithymoquinone via exposure to air to facilitate dimerization.

Nigellone exerted some pharmacological actions that included protection of guinea-pigs against histamine-induced bronchoconstriction [83] and suppression of bronchial asthma in children [120, 121]. It should be pointed out that it is this report that gave the impression that the volatile oil of the black seed is effective in asthma! Furthermore, it inhibited histamine release from egg-albumin sensitized non peritoneal mast cells [123]. It was devoid of any hypotensive activity [83].

2.15 Pharmacological Actions of Melanin

Melanin is recently isolated and purified from the outer coats of the seeds of *Nigella sativa* and some of its pharmacological actions were revealed. El-Obeid et al [124] reported its ability to activate To11 like receptors type 4 to stimulate the release of IL-8 from PBMCs and other cell lines. While, ELTahir et al showed its ability to protect against alcohol- aspirin-indomethacin and stress- induced ulcers in presence or absence of commensal gastric bacteria (unpublished data).

Concluding Remarks

The above comprehensive review almost covered what is actually known to date about the black seed and its constituents. It is clear that most of the potent and fruitful activity resides in its volatile oil and a protein component. However, the volatile oil suffers the drawback of the bronhoconstricting effect of thymoquinone. However, the latter can be easily removed from the oil to obtain dethymoquinoneated oil that has already been shown to possess the major characteristics of the whole oil. At this moment we have a lot of experimental data that, I hope, may stimulate the beginning of the era of pilot clinical studies to evaluate the clinical potential of the volatile oil, some of the protein fractions and the dethymoquinoneated volatile oil. It is hoped that this plea will have a rapid response.

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