The medicinal potential of black seed 
(*Nigella sativa*) and its components

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**Abstract**

The seeds of *Nigella sativa* L., commonly known as black seed, have been used in traditional medicine by many Asian, Middle Eastern and Far Eastern Countries to treat headache, coughs, abdominal pain, diarrhea, asthma, rheumatism and other diseases. The seeds of this plant are the most extensively studied, both phytochemically and pharmacologically. The aqueous and oil extracts of the seeds have been shown to possess antioxidant, antiinflammatory, anticancer, analgesic and antimicrobial activities. Thymoquinone, the most abundant constituent of black seed essential oil, has been shown to be the active principle responsible for many of the seed’s beneficial effects. This review paper describes the seed, its chemical components and popular uses in traditional medicine. The paper also discusses the medicinal potential and therapeutic values of some of the individual components present in the extracts of the seeds.

**Keywords:** medicinal plants, *nigella sativa*, black seed, thymoquinone, *N. sativa* oil

**Abbreviations:** BP, benzo(a)pyrene; TQ₂, dithymoquinone; DOX, doxorubicin; GC, gas chromatography; HPLC, High-performance liquid chromatography; IL, interleukin; NSO, Nigella sativa oil; PGE₂, prostaglandin E₂; TLC, thin layer chromatography; THQ, thymohydroquinone; TOH, thymol; TQ, thymoquinone; TNF, tumor necrosis factor.

**I. Introduction**

Plants are natural factories for the production of chemical compounds, many of which are used to promote health and fight diseases and some of them are marketed as food or herbal medicines (*Dubick, 1986*). Herbal medicines have long been viewed as a source of curative remedy based on religious and cultural traditions (*Huxtable, 1992; Ghazanfer, 1994*). The use of indigenous plant medicines in developing countries became a World Health Organization policy since 1970. Of the 520 new drugs approved in the period 1983–1994 by either the US Food and Drug Administration or comparable entities in other countries, 30 drugs came directly from natural
product sources, 173 were either semi-synthetics or synthetics originally modeled on a natural parent product (De Smet, 1997).

*Nigella sativa* is an annual herb of the Ranunculaceae family, which grows in countries bordering the Mediterranean Sea, Pakistan and India. This widely distributed plant is native to Arab countries and other parts of the Mediterranean region (Jansen, 1981). For thousands of years, this plant has been used in many Asian, Middle Eastern and Far Eastern Countries as a spice and food preservative as well as a protective and health remedy in traditional folk medicine for the treatment of numerous disorders (Chopra et al., 1956; Nadkarni, 1976). The seed of this plant is commonly known as black seed and is referred to by the prophet Mohammed as having healing powers. The seeds are commonly eaten alone or in combination with honey and in many food preparations. The oil prepared by compressing the seeds of *N. sativa* is used for cooking. Black seed is also identified as the curative black cumin in the Holy Bible, and is described as the Melanthion of Hippocrates and Discroides and as the Gith of Pliny (Chopra et al., 1956; Nadkarni, 1976). Other names for the seed include black caraway seed, Habbatu Sawda and Habatul Baraka “the Blessed Seed”.

*N. sativa* plant is one of the most extensively studied, both phytochemically and pharmacologically. The extracts of *N. sativa* seeds have been used by patients to suppress coughs (Mahfouz et al., 1960), disintegrate renal calculi (Hashem and El-Kiey, 1982), retard the carcinogenic process (Hassan and El-Dakhakhny, 1992; Worthen et al., 1998), treat abdominal pain, diarrhea, flatulence and polio (Enomoto et al., 2001), exert choleretic and uricosuric activities (El-Dakhakhny, 1965), anti-inflammatory (Chakravarty, 1993; Houghton et al., 1995) and antioxidant effects (Mansour et al., 2002). Besides, the essential oil was shown to have antihelminthic (Agarwal et al., 1979), antinematodal (Akhtar and Riffat, 1991), antischistosomal (Mahmoud et al., 2002), antimicrobial (Hanafy and Hatem, 1991; Aboul-Ela et al., 1996) and antiviral (Salem and Hossain, 2000) effects. In addition, the crude oil prepared from the seeds produce a variety of pharmacological actions such as antihistaminic (El-Dakhakhny, 1965; Mahfouz et al., 1965; Chakravarty, 1993), diuretic and antihypertensive (El-Tahir et al., 1993b; Zaoui et al., 2000), hypoglycemic (Al-Hader et al., 1993), antioxytocic (Aqel and Shaheen, 1996), antinociceptive (Abdel-Fattah et al., 2000), respiratory stimulation (El-Tahir et al., 1993a), hematological (Enomoto et al., 2001) hepatoprotective (Daba and Abdel-Rahman, 1998) and immunopotentiating (Swamy and Tan, 2000) effects. The latter pharmacological properties appear to be involved in the beneficial effects of *N. sativa* oil on headache, flatulence, blood homeostasis abnormalities, rheumatism and related inflammatory diseases (Boulos, 1983). Moreover, the seeds are believed to have carminative, stimulatory and diaphoretic properties and are used in the treatment of bronchial asthma and eczema (Boulos, 1983). This chapter will review the medicinal potential of *N. sativa* seed extracts and emphasize the reasons for its long history of use in folklore medicine in Mediterranean countries.

II. Chemical constituents and active principles in *N. sativa* seeds

Millions of people in the Mediterranean region and on the Indian subcontinent use the oil from the seed of *N. sativa* daily as a natural protective and curative remedy.
The seeds are very rich and diverse in chemical composition. They contain amino acids, proteins, carbohydrates, fixed and volatile oils (Khan, 1999). Many of the pharmacological activities mentioned above have been attributed to quinone constituents in the seed. As early as 1956, Chopra et al. found that thymoquinone (TQ) (Figure 1) is the main active constituent of the volatile oil of the black seed. Mahfouz and El-Dakhakhny (1960) were the first to report on the isolation of ‘nigellone’ from the oil of *N. sativa* seed, using Girard’s reagent. Nigellone was later found to possess antihistaminic properties in relatively low concentrations (Mahfouz et al., 1965). El-Dakhakhny (1963) was able to isolate the constitutive components of *N. sativa* seeds from its essential oil, among which TQ was later shown to be the main constituent of the volatile oil (Houghton et al., 1995). In addition, El-Dakhakhny determined that the ‘nigellone’ isolated earlier was a dimer of TQ, which was later named dithymoquinone (TQ₂) (Figure 1). The latter compound was shown to be formed via photodimerization of TQ as a consequence of exposure to sunlight during separation and extraction of the quinones from the seed. El-Fatatry (1975) reported the isolation of thymohydroquinone (THQ) from *N. sativa* seed volatile oil. In another study (Aboutabl et al., 1986), the chemical composition of the black seed of *N. sativa* was found to contain a fixed oil (30%) and a volatile oil (average 0.5%, maximum 1.5%). The volatile oil was found to contain 54% TQ and many monoterpenes such as p-cymene and *α*-pinene, TQ₂ and THQ.

In recent years, the seeds of *N. sativa* have been subjected to a range of phytochemical investigations. They have been shown to contain more than 30% (w/w) of a fixed oil with 85% of total unsaturated fatty acid (Houghton et al., 1995). The seeds also contain alkaloids of unknown pharmacological actions, such as nigellidine,
nigellimine and nigellicine (ur-Rahman et al., 1985), saponins and crude fiber as well as minerals such as calcium, iron, sodium and potassium. Other constituents of the volatile oil include thymol (TOH) (Figure 1) (Aboutabl et al., 1986). Recently, the presence of TQ, TQ₂ and TOH in N. sativa seed was confirmed using thin layer chromatography (TLC) and normal phase high-performance liquid chromatography (HPLC) methods (Abou-Basha et al., 1995; Aboul-Enein and Abou Basha, 1995). The content of TQ in N. sativa seed oil samples, obtained from different origins, was measured by gas chromatography (GC) analysis and found to be in the range of 0.13–0.17% w/v of the oil (Houghton et al., 1995). The seeds are also rich in proteins; when whole N. sativa seeds were fractionated using SDS-PAGE, they were found to contain a number of protein bands ranging from 10 to 94 kDa molecular mass (Haq et al., 1999). An HPLC method for quantifying the putative pharmacologically active constituents (TQ, TQ₂, THQ and TOH) in the oil of N. sativa seed was recently described by Ghosheh et al. (1999). In this procedure, the four compounds mentioned were separated and quantified in commercial N. sativa seed oil with good resolution, reproducibility and sensitivity. Both heat and light are known to affect the levels of the constituents in the oil. Since various storage and manufacturing conditions are expected to make a difference in the amounts of the quinone constituents of the oil, the analytical HPLC method described by Ghosheh et al. (1999) can be used to quantify the levels of the above constituents in the oil and seed extracts of N. sativa under different manufacturing conditions. The protocol is also useful as a quality control method for the determination of pharmacologically active quinones in N. sativa seed oil. Using TLC, the oil of black seed was found to contain TQ and the terpenoid components carvacrol, t-anethole and 4-terpineol (Burits and Bucar, 2000). GC-MS analysis of the essential oil obtained from six different samples of N. sativa seeds and from a commercial fixed oil showed that the qualitative composition of the volatile compounds was almost identical. Differences were mainly restricted to the quantitative composition (Burits and Bucar, 2000).

In conclusion, N. sativa seeds contain fixed oils and volatile oils, which are rich sources of quinones, unsaturated fatty acids, amino acids and proteins and contain traces of alkaloids and terpenoids. Most of the studies on the biological effects of N. sativa have dealt with its crude extracts in different solvents; however, some studies used its active principles. Among the components isolated from the volatile oil of N. sativa, TQ has been shown to be the principal active ingredient (Mahfouz and El-Dakhakhny, 1960) and thus is the most studied of all. In what follows, the physiological, antioxidant, antimicrobial, analgesic, antiinflammatory and chemopreventive effects of black seed with a special emphasis on TQ will be discussed.

III. Physiological effects of N. sativa and its component TQ

The oil extract of black seed has been shown to exert effects on various systems including the respiratory, cardiovascular, gastric and uterine and smooth muscle. The effects of intravenous administration of volatile oil and of TQ were investigated on the respiratory system of the guinea pig (El-Tahir et al., 1993a). The latter compounds were found to increase the intratracheal pressure in the dose range of 4–32 μl/kg and 1.6–6.4 mg/kg, respectively. Although N. sativa oil (NSO) significantly increased the
respiratory rate of guinea pigs, TQ was without any effect. The effects of NSO were significantly antagonized by treatment of the animals with antihistamines such as atropine and reserpine, suggesting that the oil-induced respiratory effects were mediated via the release of histamine and indirect activation of muscarinic and cholinergic mechanisms (El-Tahir et al., 1993a). This also suggested that the removal of TQ from black seed oil might provide a potential centrally acting respiratory stimulant (El-Tahir et al., 1993a). This same group demonstrated that the intravenous administration of NSO (4–32 μl/kg) or TQ (0.2–1.6 mg/kg) to rats decreased the arterial blood pressure and the heart rate in a dose-dependent manner (El Tahir et al., 1993b), suggesting that the oil may possess antihypertensive effects. The cardiovascular depressant effects of the oil were significantly antagonized by atropine and cyproheptadine, suggesting that these effects were mediated mainly centrally via indirect and direct mechanisms that involved both 5-hydroxy tryptaminergic and muscarinic mechanisms (El-Tahir et al., 1993b). NSO has also been shown to increase bile secretion in dogs and uric acid in rats as well as protect guinea pigs against histamine-induced bronchospasm (El-Dakhakhny, 1982). The fatty and petroleum extracts shortened bleeding time and inhibited fibrinolytic activity in rabbits (Ghoneim et al., 1982). In a recent study, the crude extract of \textit{N. sativa} seeds was found to exhibit spasmolytic and bronchodilator activities mediated possibly through calcium channel blockade and this activity was concentrated in the organic fraction of the extract (Gilani et al., 2001).

Traditionally \textit{N. sativa} plant has been in use in many Middle Eastern countries as a natural remedy for diabetes. Significant reduction in blood glucose and cholesterol levels in humans following the use of the plant was reported by Bamosa et al. (1997). The oil of this plant has a great potential in the treatment of diabetic animals because of its combined hypoglycemic (Al-Hader et al., 1993; Zaoui et al., 2002a) and immunopotentiating properties (Haq et al., 1999). A plant extract mixture comprising \textit{N. sativa}, myrrh, gum Olibanum, gum asafetida and aloe was found to lower blood glucose in streptozotocin diabetic rats (Al-Awadi et al., 1991). In an attempt to elucidate the mechanism of this antidiabetic action, the rate of gluconeogenesis in isolated hepatocytes as well as the activity of pyruvate carboxylase and phosphoenolpyruvate carboxykinase in rat liver homogenates was examined. It was found that the plant extracts significantly decreased hepatic gluconeogenesis, suggesting that it may prove to be a useful therapeutic agent in the treatment of non-insulin-dependent diabetes mellitus. Similar insulinotropic effects of NSO were recently observed in streptozotocin plus nicotinamide-induced diabetes mellitus in hamsters (a model of type 2 diabetes) orally fed with the oil (Fararh et al., 2002). In this study, positive immunoreactivity for the presence of insulin was observed in the pancreases from oil-treated vs. non-treated hamsters using immunohistochemical staining, suggesting that the hypoglycemic effect of NSO resulted, partly, from a stimulatory effect on beta cell function with consequent increase in serum insulin level. The ability of NSO to lower blood glucose concentrations was later confirmed in streptozotocin diabetic rats following 2, 4 or 6 weeks of treatment (El-Dakhakhny et al., 2002b). In addition, the effects of NSO, nigellone and TQ were studied on insulin secretion of isolated rat pancreatic islets. The blood glucose-lowering effect of NSO was not paralleled by a stimulation of insulin release. The data indicated that the hypoglycemic effect of NSO might be mediated by extrapancreatic actions, to be elucidated, rather than by stimulated insulin release (El-Dakhakhny et al., 2002b).
In many Arab countries *N. sativa* and its derived products are consumed abusively for traditional treatment of blood homeostasis abnormalities and as a treatment for dyslipidemia (Zaoui et al., 2002a). Several studies support the use of NSO extract for the treatment of thrombosis and dyslipidemia (Labhal et al., 1997; Enomoto et al., 2001; Zaoui et al., 2002a). The purified components (2-(2-methoxypropyl)-5-methyl-1,4-benzenediol, thymol and carvacrol) obtained from the methanol-soluble portion of NSO showed inhibitory effects on arachidonic acid-induced platelet aggregation and blood coagulation. Interestingly, some aromatic compounds present in the extract were found to be more potent than aspirin, which is well known as a remedy for thrombosis (Enomoto et al., 2001). In addition, an aqueous suspension of *N. sativa* seeds was found to decrease the serum total lipids and body weight in Psammomys obesus sand rat (Labhal et al., 1997). Analogous results, accompanied by decreases in serum lipid levels have also been observed in rats chronically treated with *N. sativa* fixed oil (Zaoui et al., 2002b). Animals were treated daily with an oral dose of 1 ml/kg body weight of the *N. sativa* seed fixed oil for 12 weeks. The serum cholesterol, triglycerides and the count of leukocytes and platelets decreased significantly by 15.5%, 22%, 35% and 32%, respectively, compared to the control values. Hematocrit and hemoglobin levels increased significantly by 6.4% and 17.4%, respectively (Zaoui et al., 2002a), suggesting that the oil influences blood homeostasis.

*N. sativa* is also used in Arab folk medicine as a diuretic and hypotensive plant. In an attempt to experimentally support the above traditional uses of the plant, a study was conducted on the diuretic and hypotensive effects of the dichloromethane extract of *N. sativa* seeds in the spontaneously hypertensive rat (Zaoui et al., 2000). An oral dose of either *N. sativa* extract (0.6 mL/kg/day) or furosemide (5 mg/kg/day) significantly increased diuresis by 16% and 30%, respectively, after 15 days of treatment. The urinary excretions of Cl⁻, Na⁺, K⁺ and urea were also increased after 15 days of treatment. In the same rat study, a comparison between *N. sativa* and nifedipine found mean arterial pressure to be decreased by 22% and 18% in the *N. sativa*- and nifedipine-treated rats, respectively, suggesting that *N. sativa* extract may play a role in decreasing blood pressure.

Evidence indicates that NSO has a protective role against gastric ulcers (El-Dakhakhny et al., 2000b). Oral administration of NSO for 2 weeks in rats produced a significant increase in gastric mucin content and glutathione level and a significant decrease in gastric mucosal histamine content without significant changes in free acidity and peptic activity of the gastric juice (El-Dakhakhny et al., 2000b). Ethanol administration, however, produced 100% ulcer induction accompanied by a reduction in free acidity, mucin content and glutathione level without any significant changes in peptic activity. When animals were pretreated with NSO before ulcer induction by ethanol, a protection ratio of 53.56% was noted as compared to the ethanol group (El-Dakhakhny et al., 2000b). The protective action of NSO was believed to be through the increase of the cytoprotective mucin content and/or decrease of histamine.

A final physiological effect of NSO includes its potential as an antioxytocic agent. Aqel and Shaheen (1996) tested the effects of NSO on the uterine smooth muscle of rats and guinea pigs *in vitro* using isolated uterine horns. The volatile oil was found to inhibit the spontaneous movements of rat and guinea pig uterine smooth muscle and also the contractions induced by oxytocin stimulation (Aqel and Shaheen, 1996). These effects were concentration dependent and reversible by tissue washing.
IV. Antimicrobial and antiparasitic effects of *N. sativa* oil

Extracts of *N. sativa* have shown promising effects against bacteria, fungi, viruses, parasites and worms. In 1975, the purified compound THQ from NSO was found to have high antimicrobial effect against Gram positive microorganisms (El-Fatatry, 1975). In later studies, seed extracts of *N. sativa* were found to inhibit the growth of *Escherichia coli*, *Bacillus subtilis* and *Streptococcus faecalis* (Saxena and Vyas, 1986). The antimicrobial activity of *N. sativa* was further established against several species of pathogenic bacteria and yeast (Topozada et al., 1965; Hanafy and Hatem, 1991). In the latter study, filter paper discs impregnated with the diethyl ether extract of *N. sativa* seeds caused concentration-dependent inhibition of Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa* and *E. coli* and a pathogenic yeast *Candida albicans*. The extract showed antibacterial synergism with streptomycin and gentamicin and showed additive antibacterial action with spectinomycin, erythromycin, tobramycin, doxycycline, chloramphenicol, nalidixic acid, ampicillin, lincomycin and sulfamethoxazole-trimethoprim combination. Interestingly, the extract successfully eradicated a non-fatal subcutaneous staphylococcal infection in mice when injected at the site of infection (Hanafy and Hatem, 1991). Recently, crude extracts of *N. sativa* showed promising antimicrobial effects against bacterial isolates with multiple resistances against antibiotics (Morsi, 2000). The most effective extracts were the crude alkaloid and water extracts.

The antiparasitic actions of NSO have been well documented by several researchers (Agarwal et al., 1979; Akhtar and Riffat, 1991; Abdel-Salam et al., 1993; Mahmoud et al., 2002). The antihelminthic activities of NSO were studied by Agarwal et al. (1979) who reported that the essential oil from the seeds of *N. sativa* showed pronounced activity even in 1:100 dilutions against tapeworms and earthworms. Anticestodal effects of *N. sativa* seeds were studied in children infected naturally with the respective worms. A single oral administration of 40 mg/kg of *N. sativa* ethanolic extract reduced the percentage of the fecal eggs without producing any adverse side effects in the doses tested (Akhtar and Riffat, 1991). When given orally to *Schistosoma mansoni*-infected mice, a 2-week treatment with NSO reduced the number of *S. mansoni* worms in the liver and decreased the total number of ova deposited in both the liver and the intestine (Mahmoud et al., 2002). Furthermore, it increased the number of dead ova in the intestinal wall and reduced the granuloma diameters markedly (Mahmoud et al., 2002). When NSO was administered in combination with praziquantel, the drug of choice for the treatment of schistosomiasis, the most prominent effect was a further lowering of the dead ova number over that produced by praziquantel alone. These changes were correlated mainly with the ability of NSO to improve liver function and the immunological system of infected mice and partly to its antioxidant effects (Mahmoud et al., 2002). The protection is also due to the ability of NSO and TQ to reduce the cytogenetic damage induced by schistosomiasis infection (Aboul-Ela, 2002). Karyotyping of bone marrow and spleen cells of infected mice showed that the main chromosomal abnormalities were gaps, fragments and deletions especially in chromosomes 2, 6 and some in chromosomes 13 and 14. Treatment with NSO or TQ for 7 days was found to reduce the percentage of chromosomal aberrations and the incidence of deletions and tetraploidy compared to the control level. Thus, NSO may be improving the therapeutic efficacy of *S. mansoni* infection by decreasing the induced chromosomal abnormalities.
The antiviral effect of NSO was only recently investigated using murine cytomegalovirus as a model (Salem and Hossain, 2000). The cytomegalovirus is a herpesvirus that causes disseminated and fatal disease in immunodeficient animals (Reynolds et al., 1993) similar to that caused by human cytomegalovirus in immunodeficient humans (Smith and Brennessel, 1994). Intraperitoneal administration of NSO to mice strikingly inhibited the virus titers in spleen and liver on day 3 of infection. The difference in the viral load in spleen and liver of the control and NSO-treated mice was very high, $45 \times 10^4$ vs. $7 \times 10^4$ and $23 \times 10^3$ vs. $3 \times 10^3$ for liver and spleen, respectively. This antiviral effect coincided with an increase in serum level of interferon-gamma and increased numbers of CD4$^+$ helper T cells and suppressor function and numbers of macrophages. On day 10 of infection, the virus titer was undetectable in spleen and liver of NSO-treated mice, while it was detectable in control mice (Salem and Hossain, 2000). The antiviral effects of NSO were more potent than the action of Chinese traditional herbal medicine hochuekki – against murine cytomegalovirus (Hossain et al., 1999).

V. Anticancer effects of N. sativa and its components

The active principles in NSO have been found to exert antineoplastic effects both in vitro and in vivo using various models of carcinogenesis. In what follows, the anticancer effects of NSO and its components will be discussed.

V.A. In vitro effects

Black seed preparations (TQ and TQ$_2$) have been demonstrated to have significant antineoplastic activity against various tumor cells in vitro (Salomi et al., 1991, 1992; Swamy and Tan, 2000). The active principles of N. sativa showed 50% cytotoxicity against Ehrlich ascites carcinoma, Dalton’s lymphoma ascites and Sarcoma-180 cells at a concentration of 1.5, 3 and 1.5 $\mu$g, respectively, with little activity against lymphocytes (Salomi et al., 1991). In vitro cytotoxicity was also demonstrated against human pancreatic adenocarcinoma, uterine sarcoma and leukemic cell lines (Salomi et al., 1992). The growth inhibitory activity was found to be related to the extract’s ability to inhibit DNA synthesis as measured by the incorporation of tritiated thymidine into cells. These findings were later confirmed by Worthen et al. (1998) who assayed the in vitro cytotoxicity of a crude gum, a fixed oil and two purified components of N. sativa seed, TQ and TQ$_2$, on several parental and multidrug resistant human tumor cell lines. Although as much as 1% w/v of the gum or oil was devoid of cytotoxicity, both TQ and TQ$_2$ were cytotoxic for all of the tested cell lines (IC$_{50}$: 78 to 393 $\mu$M). Interestingly, the multidrug resistant cell variants that are over 10-fold more resistant to the standard antineoplastic agents doxorubicin and etoposide were sensitive to TQ and TQ$_2$ (Worthen et al., 1998). The ethyl acetate fraction of N. sativa seeds (identified as CC-5) was later found to exhibit significant growth inhibition on a variety of cancer cell lines without inhibiting the growth of normal human endothelial cells (Swamy and Tan, 2000). The ED$_{50}$ values of the extract showed increased sensitivity towards Hep G2, LL/2 and Molt4 cell lines compared with SW620 and J82 cell lines. Badary and Gamal El-Din (2001) also showed that
TQ inhibited the survival of fibrosarcoma cells with IC$_{50}$ of 15 µM by inhibiting the incorporation of $^3$H thymidine into cells. The cellular mechanism of antineoplastic activity of TQ was only recently investigated (Shoieb et al., 2003). In this study, the cellular mechanisms of TQ-induced cytotoxicity in parental and cisplatin-resistant osteosarcoma human breast adenocarcinoma, human ovarian adenocarcinoma and Madin–Darby canine cell lines have been examined. The cisplatin-resistant cells were the most sensitive to TQ treatment, while the canine cell lines were the least sensitive. A dose of 25 µM of TQ induced apoptosis of osteosarcoma cells 6 h after treatment. This dose also decreased the number of cells in S-phase and increased cells in G$_1$-phase, indicating cell cycle arrest at G$_1$. These results suggest that TQ induces cell cycle arrest and apoptosis in cancer cells. Interestingly, non-cancerous cells are relatively resistant to the apoptotic effects of TQ (Shoieb et al., 2003). In our laboratories, we have recently investigated the effects of TQ on the proliferation and cytotoxicity of a panel of primary, benign and malignant mouse and human epidermal keratinocytes and colon cells. Although lower doses of TQ were found to exert no effects on the morphology or proliferation of normal cells, they inhibited cellular proliferation of benign and malignant cells, confirming the selectivity of this compound to cancer cells (Gali-Muhtasib et al., 2004). The growth-inhibitory effects of TQ against colon cancer cells were found to be mainly due to the ability of this compound to induce G1 cell cycle arrest and apoptosis. The apoptotic effects of TQ are modulated by Bel-2 protein and are linked to and dependent on p53 (Gali-Muhtasib et al., 2004). Our data support the strong potential for using the agent TQ in the prevention or therapy against colon cancer. We are presently testing the potency of TQ in the 1,2-dimethyl hydrazine mouse model of colon carcinogenesis by administering it in drinking water, intraperitoneally or by gavage.

V.B. In vivo effects

Several studies have shown that NSO and TQ retard the carcinogenic process in animals. The active principles of *N. sativa* seeds containing fatty acids were found to completely inhibit the Ehrlich ascites carcinoma in mice (Salomi et al., 1991, 1992). A dose of 100 mg/kg body weight (b.w.) of *N. sativa* extract delayed the onset of papilloma formation and reduced the mean number of papillomas per mouse (Salomi et al., 1991). Intraperitoneal administration of *N. sativa* (10 mg/kg b.w.) 30 days after subcutaneous administration of 20-methylcholanthrene-induced soft tissue sarcoma restricted tumor incidence to 33.3% compared to 100% in methylcholanthrene-treated controls (Salomi et al., 1991). In vivo Ehrlich ascites carcinoma tumor development was completely inhibited by the active principle at the dose of 2 mg per mouse per day for 10 days (Salomi et al., 1992). Furthermore, NSO was reported to possess a protective effect on chemical-induced carcinogenesis in hamster cheek pouch (Hassan and El-Dakhakhny, 1992). In another study, the administration of a dose of 1 mg of TQ twice weekly for 4 weeks demonstrated powerful chemopreventive effects against benzo(a)pyrene (BP)-induced forestomach tumors (Badary et al., 1999). TQ inhibited both BP-induced forestomach tumor incidence and multiplicity by 70% and 67%, respectively. More recently, this same group (Badary and Gamal El-Din, 2001) demonstrated that the administration of 0.01% of TQ in drinking water 1 week before and after 20-methylcholanthrene treatment
significantly inhibited fibrosarcoma tumor incidence and tumor burden by 43% and 34%, respectively. Moreover, TQ delayed the onset of methylcholanthrene-induced fibrosarcoma tumors that appeared at 12 weeks and produced less methylcholanthrene-induced mortality. The possible modes of anticarcinogenic actions of TQ in the above two studies were suggested to be through its antioxidant and antiinflammatory activities, coupled with enhancement of detoxification processes.

In a recent study, the effect of CC-5 (ethyl acetate fraction of NSO) was evaluated for its *in vivo* antitumor activity against intraperitoneally implanted murine P388 leukemia and subcutaneously implanted Lewis lung carcinoma cells in BDF1 mice (Kumara and Huat, 2001). At doses of 200 and 400 mg/kg b.w., the fraction prolonged the life span of these mice by 153% compared to DMSO-treated control mice. The antitumor activity of a 21-day treatment of CC-5 against subcutaneously implanted LL/2 was tested and found to produce a 60–70% tumor inhibition rate. A triterpene saponin was isolated from the CC-5 fraction and identified to be *α*-hederin. This compound was found to exert more potent anticancer effects compared to the commonly used anticancer drug, cyclophosphamide. When *α*-hederin was given i.p. at doses of 5 and 10 mg/kg b.w. to mice with formed tumors, it produced significant dose-dependent tumor inhibition rate values of 50% and 71%, respectively, on day 15, compared to 42% on day 15 in the cyclophosphamide (CP)-treated group. The underlying mechanism(s) of antitumor activity of *α*-hederin is not defined yet (Kumara and Huat, 2001). The protective effect of Nigella grains on carcinogenesis induced by methylnitrosourea in Sprague Dawley rats was recently investigated (Mabrouk et al., 2002). When given orally (0.2 g ground Nigella grains) alone or with honey, a 6-month treatment reduced MNU-induced inflammatory reaction in lung and skin and MNU-induced colon adenocarcinomas by 80% (Mabrouk et al., 2002). There was an associated elevation of malondialdehyde and nitric oxide in sera obtained from methylnitrosourea-treated animals, which was lowered by ingestion of *N. sativa* grains. Interestingly, combined oral treatment of honey and *N. sativa* grains protected 100% against methylnitrosourea-induced oxidative stress, carcinogenesis and abolished the nitric oxide and malondialdehyde elevations shown in sera of animals that did not receive these nutrients (Mabrouk et al., 2002).

TQ has also been shown to improve the therapeutic index of several anticancer agents and to protect non-tumor tissues from chemotherapy-induced damage. TQ protected against ifosfamide-induced Fanconi syndrome in rats and enhanced its antitumor activity in Ehrlich ascites carcinoma-bearing mice (Badary, 1999). The disease Fanconi syndrome is characterized by wasting off glucose, electrolytes and organic acids along with elevated serum creatinine and urea as well as decreased creatinine clearance rate (Brade et al., 1986). The changes in renal function observed in the rat model of Fanconi syndrome correlate well with the nephrotoxic effects of ifosfamide observed in man. Oral supplementation of TQ (5 mg/kg/day) with drinking water rendered rats significantly less susceptible to ifosfamide-induced renal abnormalities. It also corrected for the damage induced by ifosfamide on phosphorus, glucose, serum creatinine and urea levels and significantly normalized creatinine clearance rate. This effective dose of TQ was found to be very safe (Badary et al., 1998). TQ protected the kidney against ifosfamide-induced damage through an antioxidant mechanism, since it significantly prevented ifosfamide-induced renal glutathione depletion and lipid peroxide accumulation. In mice bearing Ehrlich
ascites carcinoma xenograft, TQ (10 mg/kg/day) administered in drinking water significantly enhanced the antitumor effect of ifosfamide. Furthermore, mice treated with ifosfamide in combination with TQ showed less body weight loss and mortality rate compared to ifosfamide single therapy. This finding is in full agreement with previous findings that TQ potentiates cisplatin antitumor activity and protects against cisplatin-induced nephrotoxicity in mice and rats (Badary et al., 1997; El-Daly, 1998), carbon tetrachloride-induced hepatotoxicity and lipid peroxidation (Al-Gharably et al., 1997; Nagi et al., 1999) in mice and doxorubicin (DOX)-induced cardiotoxicity (Al-Shabanah et al., 1998) in mice. In this context, *N. sativa* seed extract was shown to protect against cisplatin-induced myelosuppression in mice (Nair et al., 1991). Moreover, recent investigations by Nagi and Mansour (2000) showed that oral administration of TQ (10 mg/kg/day) with drinking water starting 5 days before a single i.p. injection of DOX (15 mg/kg) and continuing during the experimental period ameliorated the DOX-induced cardiotoxicity in rats. TQ also protected against the nephropathy and oxidative stress induced by DOX in rats (Badary et al., 2000). Although DOX is a potent cancer chemotherapeutic agent against several malignancies, its clinical efficacy is limited because of severe cytotoxic side effects, the most serious being cardiotoxicity (Cortes et al., 1975). Experimentally DOX induces hyperlipidemic nephropathy in rats associated with hypoalbuminemia, hypoproteinemina, elevated serum urea, hyperlipidemia and a high urinary excretion of protein and albumin. The nephropathy observed in this model resembles histologically and clinically the focal and segmental glomerulosclerosis that occurs in humans (Zima et al., 1997). There is increasing evidence that free radical generation by DOX is involved in the primary pathogenic mechanism of DOX-induced nephropathy in rats (Bertani et al., 1986). Treatment of rats with TQ (10 mg/kg per day) supplemented with the drinking water for 5 days before DOX, and daily thereafter significantly lowered serum urea and serum and kidney levels of triglycerides and total cholesterol. It also suppressed DOX-induced proteinuria and albuminuria (Badary et al., 2000). In both studies, TQ’s protective effects against DOX damage to the heart and kidney was found to be mainly due to its superoxide-scavenging and antilipid peroxidation effects.

In conclusion, the ability of TQ to enhance the therapeutic index of anticancer drugs and provide protection from cytotoxicity induced by these agents strengthens the potential use of this readily available drug as a cytoprotective agent. This protection documented by several investigators enforces its preclinical evaluation in combination with anticancer agents.

VI. Antiinflammatory and immunomodulatory effects of *N. sativa*

*N. sativa* and its derived products have been traditionally used as a treatment for rheumatism, liver diseases and related inflammatory disorders. The effect of black seed on the immune system has been investigated by several researchers (Houghton et al., 1995; El-Dakhakhny et al., 2000a; Haq et al., 1995; Al-Ghamdi, 2001). All studies have shown that the oil and its most abundant component, TQ, inhibit many inflammatory mediators, and, thus, may be useful in ameliorating inflammatory and autoimmune conditions. Chakravarty (1993) reported that the *N. sativa*-derived
nigellone, the carbonyl polymer of TQ, was very effective at low concentrations in inhibiting histamine release from rat peritoneal mast cells in vitro. He suggested that the mechanism of action is mainly due to the ability of TQ to decrease intracellular calcium by inhibiting protein kinase C and partly due to its ability to inhibit oxidative energy metabolism.

Several studies point to the effect of *N. sativa* on the human immune system (El-Kadi and Kandil, 1986; El-Kadi et al., 1987). The seeds were found to produce an increase in the ratio of helper to suppressor T cells and to enhance natural killer cell activity in normal volunteers (El-Kadi and Kandil, 1986). In vitro studies showed that the crude fixed oil and pure TQ were potent inhibitors of eicosanoid generation, namely thromboxane B2 and leukotriene B4, by inhibiting both cyclooxygenase and lipoxygenase, respectively (Houghton et al., 1995). Thromboxane B2 has been implicated in the mechanism of hepatocyte plasma membrane bleb formation, which is an early event in hepatocyte injury when exposed to oxidative stress (Horton and Wood, 1990). In another study, *N. sativa* enhanced the production of IL-3 by human lymphocytes and had a stimulatory effect on macrophages (Haq et al., 1995). Besides, the immunomodulatory effect of *N. sativa* purified proteins was found in mixed lymphocyte cultures and caused increased secretion in the levels of the cytokines IL-1β and IL-8 (Haq et al., 1999). Moreover, the fixed oil increased the release of PGE2, inhibited the release of leukotrienes and histamine from normal and sensitized guinea pig lungs. Other pieces of evidence include the inhibition of TNF-α production in murine septic peritonitis by TQ (El-Dakhakhny et al., 2000a) and the unique immunomodulatory properties of the ethyl acetate (CC-5) fraction of *N. sativa* at non-cytotoxic doses (Swamy and Tan, 2000). The ability of TQ to modulate cytokines and enhance the immune system has been implicated as the main reason for its protective effect against schistosome egg infection in the liver (Mahmoud et al., 2002).

In an attempt to determine the immunomodulatory role of TQ, the effect of this compound on the production of nitric oxide (NO) by rat peritoneal macrophages was investigated (El-Mahmoudy et al., 2002). It was found that it reduced production of NO in supernatants of lipopolysaccharide-stimulated macrophages without affecting the cell viability. The protein and mRNA levels of inducible nitric oxide synthase in peritoneal macrophages were also decreased by TQ. Immunofluorescence staining of inducible nitric oxide synthase in macrophages showed decreased immunoreactivity for inducible nitric oxide synthase after TQ treatment.

The antinflammatory effect of *N. sativa* has been found to be comparable to that of 100 mg/kg aspirin (Al-Ghamdi, 2001). In conclusion, the pharmacological activities of *N. sativa* documented by several researchers support its use in folk medicine to reduce inflammation.

**VII. Antioxidant and hepatoprotective effects of *N. sativa***

Health food stores sell *N. sativa* seeds as a natural remedy for a variety of complaints including liver diseases (Boulos, 1983). The hepatoprotective effects of TQ have been well documented and have been found to be related to its strong antioxidant potentials. In fact, the antioxidant and free radical scavenging properties of many
plants have been found to play an important role in their hepatoprotective activity (Kiso et al., 1984; Valenzuela et al., 1986; Navaro et al., 1993; Thabrew et al., 1995). Oxidant stress can increase the susceptibility to irreversible injury by oxidative intoxication and by free radicals that can result in lipid peroxidation, protein oxidation, protein inactivation, disturbance in calcium homeostasis and consequent loss of cell viability (Masaki et al., 1989; Shertzer et al., 1994). Most of the hepatoprotective drugs belong to the group of free radical scavengers and their mechanism of action involves membrane stabilization, neutralization of free radicals and immunomodulation. The flavanolignan mixture, silymarin and its most active constituent, silybin, obtained from the plant Silybum marianum have been studied intensively for their antihapatotoxic effects (Vogel, 1977; Miguez et al., 1994). They are now used clinically in the treatment of many liver diseases (Fernandez et al., 1995).

The oil of N. sativa and TQ are known to possess strong antioxidant activities (Aboul-Enein et al., 1999; Nagi and Mansour, 2000; Meral et al., 2001; El-Dakhakhny et al., 2002a; Mahmoud et al., 2002); TQ has been shown to inhibit non-enzymatic peroxidation in ox brain phospholipid liposomes (Houghton et al., 1995) with a potency that is 10 times higher than NSO. Using TLC screening methods, Burits and Bucar (2000) showed that TQ and NSO components, namely, carvacrol, t-anethole and 4-terpineol possess strong radical-scavenging properties. Moreover, TQ showed extremely high superoxide anion radical-scavenging abilities (as effective as superoxide dismutase against superoxide) in pure chemical systems (Nagi and Mansour, 2000). This high scavenging power of TQ was responsible for its protective effects against DOX-induced cardiotoxicity in rats (Nagi and Mansour, 2000). In a recent study, TQ was observed to be metabolized by liver DT diaphorase to dihydrothymoquinone, a phenolic metabolite that acts as a radical scavenger and inhibits lipid peroxidation in vitro (Mansour et al., 2002). TQ and dihydrothymoquinone acted not only as superoxide anion scavengers, but also as general free radical scavengers with IC$_{50}$ values in the nanomolar and micromolar ranges, respectively (Mansour et al., 2002). Treatment of mice with TQ orally for 5 successive days produced significant reductions in hepatic superoxide dismutase, catalase, and glutathione peroxidase activities (Mansour et al., 2002). Moreover, TQ significantly reduced hepatic and cardiac lipid peroxidation as compared with the respective control group. The most comprehensive evidence on the antioxidant effects of NSO and its components came from the studies conducted by Kruk et al. (2000) and El-Dakhakhny et al. (2002a). They showed that TOH, TQ and TQ$_2$ exhibit antioxidant properties and acted as scavengers of various reactive oxygen species. TOH, for example, acted as $^1$O$_2$ quencher, while TQ and TQ$_2$ showed superoxide dismutase-like activity removing $O_2^-$. The same group (El-Dakhakhny et al., 2002a) also showed that NSO as well as niggellone and TQ exert inhibitory actions on the production of leukotriene-type mediators of inflammation in vitro. Whereas TQ exerts a strong inhibitory activity (IC$_{50}$: 0.3 mg/ml), niggellone is far less active (IC$_{50}$: 12 mg/ml), possibly due to the loss of antioxidative activity through polymerization. The high antioxidative action of NSO and its components suggests their importance for the treatment of various diseases occurring with participation of reactive oxygen species.

In an attempt to evaluate the hepatoprotective effects of TQ, Daba and Abdel-Rahman (1998) studied its ability to protect against oxidative stress caused by tert-butyl hydroperoxide in isolated rat hepatocytes and compared it to the effects of the known hepatoprotective agent silybin. The toxicity of tert-butyl hydroperoxide was manifested
by the loss of cell viability and the progressive depletion of intracellular glutathione and leakage of cytosolic enzymes, alanine transaminase and aspartic transaminase in isolated rat hepatocytes treated with this compound. Preincubation of cells with 1 mM of either TQ or silybin resulted in protection against tert-butyl hydroperoxide-induced toxicity as evidenced by decreased leakage of alanine transaminase and aspartic transaminase and increased cell viability. Silybin was slightly more potent in preventing loss of cell viability and enzyme leakage, but both compounds prevented tert-butyl hydroperoxide-induced depletion of glutathione to the same extent (Daba and Abdel-Rahman, 1998). Hepatoprotective effects of TQ were also documented against carbon tetrachloride-induced toxicity (Mansour, 2000). In this study, oral administration of TQ in drinking water, starting 5 days before carbon tetrachloride injection and continuing during the experimental period, ameliorated the hepatotoxicity induced by carbon tetrachloride, as evidenced by a significant reduction in the elevated levels of serum enzymes as well as a significant decrease in the hepatic malonaldehyde content and a significant increase in the total sulfhydryl content. While oral administration of TQ in a single dose (100 mg/kg) resulted in significant hepatoprotection against carbon tetrachloride-induced toxicity in male Swiss albino mice, dihydrothymoquinone (IC50: 0.34), the reduction metabolite of TQ, was found to be more potent than TQ (IC50: 0.87) in protecting against carbon tetrachloride-induced hepatotoxicity (Nagi et al., 1999). Similar hepatoprotective effects in the same system (carbon tetrachloride-induced hepatotoxicity) were obtained following a 4-weeks’ oral intake of NSO in male albino rats (El-Dakhakhny et al., 2000c). Recently, it was shown that *N. sativa* seeds given orally every day for 2 months decreased the lipid peroxidation, increased the antioxidant defense system and prevented the lipid peroxidation-induced liver damage in experimentally induced diabetic rabbits (Meral et al., 2001), suggesting that the seed may be used in diabetic patients to prevent lipid peroxidation.

VIII. Analgesic and antinociceptive effects of *N. sativa*

The analgesic and antinociceptive effects of *N. sativa* were only recently reported (Abdel-Fattah et al., 2000; Al-Ghamdi, 2001) and the mechanisms by which they occur are not fully understood. Evidence, however, points to the potential of using the aqueous extract of *N. sativa* as an analgesic agent. *N. sativa* crude aqueous suspension was found to produce significant increase in the hot plate reaction time in mice (indicating analgesic effects); however, it had no effect on yeast-induced pyrexia. The absence of antipyretic effect suggests that the constituents of these seeds may not inhibit the synthesis of prostaglandins (Al-Ghamdi, 2001). This was in agreement with the findings of Abdel-Fattah et al. (2000) who showed that the oral administration of NSO (50–400 mg/kg) dose-dependently suppressed the nociceptive responses caused by thermal, mechanical and chemical nociceptive stimuli in mice. In this study, the systemic administration and the i.c.v. injection of NSO attenuated the response in not only the early phase, but also the late phase of the chemical test. In another study, upon using several opioid receptor antagonists, it was demonstrated that NSO and TQ produce antinociceptive effects through indirect activation of the supraspinal opioid systems (Abdel-Fattah et al., 2000). It remains unclear if TQ antinociception in the chemical test is due to its direct interaction with opioid
receptors, since no information is available regarding the *in vitro* opioid receptor binding of TQ. However, the difference in receptor antagonist sensitivity of TQ antinociception between the early and late phases raises the possibility that the antinociceptive action of TQ in the chemical test is mediated by mechanisms other than direct stimulation of opioid receptors located in the central nervous system. Other mechanisms that could explain the antinociceptive effects of NSO include its inhibitory effect on the inflammatory mediators. Further experiments are needed to clarify the mechanisms underlying the antinociceptive action of NSO and TQ.

IX. Are *N. sativa* seeds or its components safe to consume?

The toxicity properties of TQ and THQ were investigated in male rats whereby the drugs were dissolved in propylene glycol, injected i.p. into 30 male rats and LD$_{50}$ determined (El-Dakhakhny, 1965). Using this protocol, TQ (LD$_{50}$: 10 mg/kg b.w.) was found to be more toxic than THQ (LD$_{50}$: 25 mg/kg b.w.).

In more recent studies, the oral administration of aqueous extracts of the seeds of *N. sativa* for 14 days has been shown to cause no toxicity symptoms in male Sprague-Dawley rats (Tennekoon et al., 1991). The safety of consuming *N. sativa* seeds was also recently reported by Al-Homidan et al. (2002) whereby the seeds did not affect the growth of 7-day-old Hibro broiler chicks when fed to them at 20 and 100 g/kg of the diet for 7 weeks.

Although several studies have reported the safety of consuming *N. sativa* seeds, a recent comprehensive investigation has shown that the plant is relatively unsafe if consumed for prolonged periods of time (Zaoui et al., 2002b). LD$_{50}$ values obtained by single doses, orally and intraperitoneally administered in mice were 28.8 and 2.06 ml/kg b.w., respectively. Treatment of animals with a daily oral dose of 1 ml/kg b.w. of NSO for 12 weeks resulted in significant slowdown of the body weight in *N. sativa*-treated animals compared to untreated control animals. Changes in key hepatic enzymes levels and histopathological modifications (heart, liver, kidneys and pancreas) were not observed in rats treated with *N. sativa* after 12 weeks. However, the serum cholesterol, triglyceride and glucose levels and the count of leukocytes and platelets decreased significantly, compared to control values, while hematocrit and hemoglobin levels increased significantly. The decrease in body weight in *N. sativa*-treated rats was thought to be related to the decrease in serum lipids and glucose levels as a consequence of a possible reduction in food intake by NSO administration (Zaoui et al., 2002b). Interestingly, no evidence of toxicity was noted in 10 times this dose in mice, suggesting only a seeming margin of safety for the used therapeutic doses of *N. sativa*.

In this regard, it is worth mentioning that TQ is both an irritant and a potent elicitor of allergic contact dermatitis (Steinmann et al., 1997; Zedlitz et al., 2002).

X. Conclusions

The use of ethnobotanical drugs among Asians as complementary medicine is prevalent and is also gaining increasing popularity in the West. More than 25% of
currently used drugs are derived directly from plants; while the other 25% are chemically altered natural products. Evidence indicates that *N. sativa* seeds have a potential medicinal value and are relatively safe to consume. Future research should focus on the mechanisms by which *N. sativa* seeds exert their medicinal effects. With the increased understanding of its mechanism of bioactivity, the incorporation of this medicinal herb as complementary medicine into mainstream medical science can be achieved in the future.

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