Clinical and Therapeutic Trials of Nigella Sativa

INTRODUCTION

Despite all the marvelous advancements in modern medicine, traditional herbal medicine has always been practiced (1). Every culture and civilization, throughout history, has used a range of plant or plant derivatives for the prevention and treatment of diseases (2). The rapid increase in consumption of herbal remedies worldwide has been stimulated by several factors, that all herbal products are safe and effective, fear or disruption of physician, current interest of natural products, disappointment of prescribed drugs or traditional care, cultural influences and increase acceptance of alternative remedies (3).

Herbal medicine can be broadly classified into four basic systems: Traditional Chinese Herbalism, Ayurvedic Herbalism, Western Herbalism, which originally came from Greece and Rome to Europe and then spread to North and South America, and Arab traditional medicine, which forms the basis for alternative and herbal medicine in use today (3).

Nigella Sativa

Among the promising medicinal plants, N. sativa is an amazing herb with a rich historical and religious background. N. sativa is annual herbaceous plant belongs to dicotyledon of the Ranunculaceae family. It has been employed for thousands of years as a spice and food preservative. It commonly grows in Europe, Middle East, and Western Asia. The seeds of N. sativa are the source of the active ingredients of this plant (4). They are frequently used in folk medicine in the Middle East and some Asian countries for the promotion of good health, treatment of many ailments including fever, common cold, headache, asthma, rheumatic diseases, various microbial infections, and to expel worms from the...
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They also used for scorpion and spider stings and bites of snake, cat and dog. In addition, they used as a flavoring additive to bread and prickles (5).

Synonyms

Coequal names of its seed in Arab countries are Al–Habbah Al–Sawda, Habbet El–Baraka, Kamoun Aswad, Schuniz and Khodria. In Pakistan, India and Srilanka it is called Kalvanji, Kalaunji, Azmut, Gurat, Aof and Aosetta. In English language it is known as Black Seed, Black Cumin, Black Caraway, Cinnamon flower, Nutmeg flower and Love–In–a–Mist (6).

Historical background

N. sativa (Black Seed) was discovered in Tutankhamen’s tomb. It is known that Cleopatra had used it for its health and beauty giving qualities. Black seed is found in the book of Isaiah in the Old Testament 28: 25–27. Black seed is also identified as curative black cumin in the Holy Bible (7). The Greek physician Dioskorides used Black Seed to treat headaches, nasal congestion, toothache and intestinal parasites. Hippocrates regarded N. sativa as a valuable remedy in hepatic and digestive disorders (8). The Prophet Mohammed said “Hold on using the black seed, as it has a remedy for every illness except death” (4). Ibn Sina (428 H), recommended it to stimulate the metabolism and to recover from dispiritedness and lethargy. The Arabian authors (Ibn–El–Bitar (646 H), Dawood El Antaki (1932)) reported that the seeds are useful in expelling calculi, lactogouge, emmenagogue and diuretics (9).

Chemistry

N. sativa oil has been shown to possess 67 constituents, many of which are capable of inducing beneficial pharmacological effects in human (10). By HPLC analysis of N. sativa oil, thymoquinone (TQ), dithymquinone (DTQ), thymohydroquinone, and thymol are considered the main active ingredients. N. sativa seeds contain other ingredients, including nutritional components such as carbohydrates, fats, vitamins, minerals and proteins, including eight of essential amino acids (11). Fractionation of whole N. sativa seeds using SDS–PAGE shows a number of protein bands ranging from 10 to 94 kDa molecular mass (12). Monosaccharides in the form of glucose, rhamnose, xylose, and arabinose are also found. N. sativa seeds are rich in the unsaturated and essential fatty acids. Chemical characteristics, as well as fatty acid profile of the total lipids, revealed that the major unsaturated fatty acid is linoleic acid, followed by oleic acid (11,13). The major phospholipid is phosphatidylcholine, followed by phosphatidyl-ethanolamine, phosphatidylerine, and phosphatidyl- inistiot, respectively. The seeds contain carotene which is converted by the liver to vitamin A (13). The N. sativa seeds are also a source of calcium, iron, and potassium (14).

Respiratory system

In Saudi Arabia and neighboring countries N. sativa seeds and oil are commonly used for the treatment of asthma. Nigellone (a carbonyl polymer of thymoquinone) proved to be an excellent prophylactic agent for both bronchial asthma and asthmatic bronchitis and was more effective in children than adults (15). The curative and protective effects of N. sativa against asthma may be attributed to its anti–histaminic effect (16). N. sativa volatile oil induced dose dependent increase in the respiratory rate and the intra–tracheal pressure, which were antagonized by mepyramine, atropine and reserpine but not by indomethacin, diethyl–carbamazine or hydrocortisone. A central mechanism was suggested for these effects (17). In Kuwait the extract of N. sativa used with natural fat for epistaxis (18).

Cardiovascular system

In Arabian folk medicine whole seeds of N. sativa alone or in combination with honey or garlic are promoted for the treatment of hypertension (19). N. sativa extract lowered blood pressure in dog (20). The volatile oil and thymoquinone produced a dose dependent decrease in the arterial blood pressure and the heart rate. Atropine, cyproheptadine, and hexamethonium significantly antagonized these effects. However reserpine only antagonized the effects of low doses of volatile oil but not of thymoquinone (21). The antihypertensive effect may be due to diuretic action of N. sativa oil (22). Thymol lowered blood pressure through blockade of calcium channels. TQ decreased the blood cholesterol triglycerides, and LDL level. TQ and TQ–rich fraction regulated genes involved in cholesterol metabolism by two mechanisms, the uptake of low–density lipoprotein cholesterol via the upregulation of the low–density lipoprotein receptor and inhibition of cholesterol synthesis via the suppression of the 3–hydroxy–3–methylglutaryl–coenzyme A reductase genes (23).
Genital system

In Omani medicine, *N. sativa* promoted for treatment of oligomenorrhea, to induce menstruation and to treat infertility (24). The ethanolic extract of *N. sativa* seeds showed antifertility effect in male rats that is probably due to its inherent estrogenic nature (25). *N. sativa* crude oil induced uterine contractions both in vivo in pregnant rabbits and in vitro of non-pregnant rat uteri (26). However, volatile oil of *N. sativa* inhibited spontaneous contractions of rat and guinea pig uterine smooth muscle those induced by oxytocin (27). These differences may be due to the different doses, preparations and the animal species used. *N. sativa* oil has a spermicidal effect, if put intravaginally postcoitally in rats. So, *N. sativa* oil could be considered as a postcoital contraceptive (28).

Urinary system

*N. sativa* aqueous extract had a protective effect against gentamycin–induced nephrotoxicity in unilateral nephrectomized rats (29). *N. sativa* extract improved phosphaturia, glucosuria, serum creatinine, urea, renal glutathione depletion and lipid peroxide accumulation in doxorubicin induced nephropathy (30).

Gastrointestinal Tract

*N. sativa* used for stomachache, as a digestive, carminative, laxative and anti–jaundice remedy or agent (24). The alcoholic extract of *N. sativa* had antiulcer activity in pyloric ligation and aspirin–induced gastric ulcer models (31). The gastroprotective effect of *N. sativa* oil against gastric lesions may be related to the conservation of the gastric mucosal redox state (32). *N. sativa* administration attenuated the ulcerative effects of ethanol on gastric mucosa by decreasing the glutathione–S transferase levels in gastric mucosa (33). The anti–ulcer effect of *N. sativa* was possibly prostaglandin–mediated and/or through its antioxidant and anti–secretory activities (34).

The aqueous–methanolic extract of Nigella seeds showed spasmylytic effect mediated through calcium antagonist effect thus providing scientific basis for its traditional use in diarrhea (15). Oral *N. sativa* powder was reported to relieve flatulence (19). The smaller dose of thymoquinone (5 mg/kg) produced partial protection; whereas, higher dose (10 mg/kg) was found to give complete protection on acetic acid–induced colitis in rats. The possible mechanism of the protective effects might be partly due to its antioxidant action (35).

The hepatoprotective effect of *Nigella* oil was investigated in some models of liver toxicity. In Schistosoma mansoni infected mice, the oil succeeded partially to correct the previous changes in alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT) and alkaline phosphatase (AP) activity as well as the albumin content in serum. Thus, *N. sativa* oil suggested playing a role against the alterations caused by Schistosoma mansoni infection, an effect which may be partly mediated via improving the host immune system and to some extent its antioxidant effect (36). In another study, thymol, one of the constituents of *Nigella* seeds, exhibited hepatoprotective effect in rodents (37). The protective effect of *N. sativa* oil against carbon tetrachloride and D–galactosamine induced hepatic toxicity in rats was established through significant decrease in serum activities of AP, lactate dehydrogenase, malate dehydrogenase, aspartate aminotransferase, and ALT, and a significant increase in glutathione reductase (38). Similarly, *N. sativa* administration protects hepatic tissue from deleterious effects of toxic metals such as lead and attenuated hepatic lipid peroxidation following exposure to chemicals such as carbon tetrachloride (33,39). Finally, *N. sativa* relieved the deleterious effects of ischemia reperfusion injury in the liver (40).

Central nervous system (CNS)

The aqueous and methanol extracts of *N. sativa* seeds produced an alteration in the general behavior patterns, significant reduction of spontaneous motility, reduction in normal body temperature and significant analgesic action, suggesting CNS depressant action (41).

Immune system

The administration of one gram *N. sativa* twice daily in human volunteers enhanced immune functions as manifested by 72% increase in T helper cell (T4) to T suppressor cell (T8) ratio and improved natural killer cell activity. However, there was a decrease in the immune globulin (IgA, IgG and IgM) levels (11). *N. sativa* enhanced the production of cytokines, interleukin–3 and tumor necrosis factor–alpha by human lymphocytes when cultured with pooled allogenic cells or without any added stimulator. They also observed an increase in interleukin–1 beta suggesting that *N. sativa* has an
effect on macrophages as well (42). On mixed lymphocyte culture, whole N. sativa seeds and its purified proteins demonstrated stimulatory as well as suppressive effects depending upon the donor and the concentration used (12). The ethyl–acetate chromatographic of N. sativa ethanol extract stimulated cellular immune responses (43).

Antioxidant properties: (N. Sativa as antioxidant)

Thymoquinone and fixed oil of N. sativa were reported to inhibit non–enzymatic peroxidation in ox brain phospholipids liposome (44). N. sativa extracts and thymoquinone had protective effect against hematological, hepatic, renal and other toxicities induced by anti–cancer drugs and some toxims through their antioxidant action (30). Thymol, thymoquinone and dithymoquinone had free radical scavenging effects on the reactions generating reactive oxygen species such as superoxide anion radical, hydroxyl radical and singlet oxygen (45). N. sativa oil prevented lipid peroxidation and increased the antioxidant defense system in diabetic rabbits (46).

Nigella grains produced about 80% protection against methylnitrosoureca–induced oxidative stress, inflammatory response and carcinogenesis in rats (47). It decreased the lipid peroxidation, liver enzymes, and increased the antioxidant defense system activity in the carbon tetrachloride treated rats (48). N. sativa and thymoquinone corrected streptozotocin–induced diabetes alterations in CK–MB and brain monoamines due to their antioxidant properties (49). N. sativa attenuated the nephrotoxic side effects of cyclosporine due to its antioxidant properties (50).

Analgesics, anti–inflammatory, and anti–pyretic properties

The analgesic effect of crude fixed oil of N. sativa and thymoquinone was proved by inhibition of cyclooxygenase and 5–lipooxygenase pathways (43). This effect was confirmed in experimental animal studies (51). Another possibility for the analgesic action could be the activation of supraspinal mu (1)– and kappa–opioid receptors subtypes as elicited by the antagonistic effect of naloxone, naloxonazine and nor–binaltorphimine (52).

The anti–inflammatory, analgesic and antipyretic effects of the aqueous extract of N. sativa in animal models were compared to aspirin. The extract has anti–inflammatory effect demonstrated by its inhibitory effects on Carragenan induced paw edema. It also produced significant increase in the hot plate reaction time in mice indicating analgesic effect. However, N. sativa crude suspension had no effect on yeast induced pyrexia. This study supported its use in folk medicine both as analgesic and anti–inflammatory agents (53). The aqueous extract of N. sativa inhibited the production of nitric oxide, thus its anti–inflammatory action might be mediated partly through this mechanism (54).

N. Sativa as an anti–neoplastic agent

The topical administration of N. sativa extract inhibited the two stages of initiation/promotion skin carcinogenesis. In mice, a dose of 100 mg/kg body weight of their extract delayed the onset of papilloma formation and reduced the mean number of papilloma per mouse (55). When Nigella extract incubated with cancer cells, these cells were unable to produce fibroblast growth factor and the protein collagenase, both necessary for blood vessel growth into the tumor. Without a blood supply, a tumor cannot grow (56). The thymoquinone improved the anti–tumor activity in rats and mice most probably through its antioxidant action (57). Thymoquinone inhibited tumor incidence and tumor burden significantly both, in–vivo and in–vitro in male Swiss albino rats on fibrosarcoma induced by 20–methylcholanthrene. The possible mode of action was its antioxidant activity and interference with DNA synthesis coupled with enhancement of detoxification processes (58). The antitumor principle α–Hedrin (saponin) from the seeds of N. sativa was extracted and isolated. The extraction caused dose dependent inhibition of tumor induction and tumor growth when given before tumor implantation. The characteristic morphological changes of apoptosis had been observed with the extraction so, apoptosis could be a major mechanism by which α–hedrin prevent tumor growth (43). Also, α–hedrin had stimulating effect on the release of nitric oxide by up regulation nitric oxide synthase gene expression in mouse macrophages. This may explain an additional mechanism responsible for its biological effects including its antitumor activities (59). It has a promising results in the field of prevention and treatment of cancer. N. sativa alone or in combination with oxidative stress were found to be effective in vitro in inactivating MCF–7 breast cancer cells. (60). Thymoquinone killed cancer cells by process that involved apoptosis and cell cycle arrest (61). The volatile oil of N. sativa had the ability to inhibit colon carcino genesis of rats in the post initiation stage. The inhibition associated, in part, with suppression of cell proliferation in the colonic
macosa (62). N. sativa decreased DNA damage and thereby prevents initiation of carcinogenesis in colonic tissue secondary to exposure to toxic agents such as azoxymethane (63). In fact, sustained delivery of thymoquinone (derived from N. sativa) is almost as effective in causing apoptosis of colon cancer cells as sustained delivery of 5-fluorouracil (64). Similarly, hepatic metastasis from tumors such as mastocytomas in mice is markedly decreased following administration of N. sativa (65). N. sativa, when used in combination with Hemidesmus indicus and Smilax glabra, decrease hepatic carcinogenesis secondary to exposure to agents such as diethylnitrosamine (66). These anti–carcinogenic effects are mediated in part by thymoquinone secondary to its inhibitory influence on the Nuclear Factor–kappaB (NF–B) activation pathway (67). Thymoquinone induced apoptosis of human colon cancer cells via a p53–dependent mechanism (68). Ethanolic extracts of N.sativa tested against N–methyl–N’–nitro–N–nitrosoguanidine (MNNG), a directly acting mutagen in pre–treatment, combined treatment and post–treatment modules, proved an inhibitory effect of the extract on mutagenicity. A direct antimutagenic activity and an increased recovery at the chromosomal level were detected (69). Thymoquinone may be effective in treating hormone–sensitive and hormone–refractory prostate cancer. It inhibited DNA synthesis, proliferation, and viability of cancerous but not noncancerous prostate epithelial cell lines exerting a selective effect on cancer cells, and down–regulating androgen receptor (70).

As an anti–microbial, anti–fungal and anti–helminthic

The anti–bacterial effect of the phenol fraction of N. sativa oil was first reported by Topozada (71). Thymohydroquinone had high activity against gram–positive microorganisms (72). A concentration dependent inhibition of gram–positive bacteria (represented by Staphylococcus aureus) and gram–negative bacteria (represented by Pseudomonas aerogenosa and Escherichia coli) was reported. It also showed synergistic effect with streptomycin and gentamycin and additive effect with spectinomycin, erythromycin, tobramycin, doxycycline, chloramphenicol, nalidixic acid, ampicillin, lincomycin and co–trimoxazole (73). In addition, the extract was found to have a concentration dependent inhibitory effect against pathogenic yeast, Candida albicans. Crude extracts of N. sativa had a promising effect on multi–antibiotic resistant organisms including gram–positive and gram–negative bacteria (74). The aqueous extract of the seeds possessed potent in–vivo antifungal activity against candidiasis in mice (75).

N. sativa powder seeds were effective in treatment of cestodes in children (76). N. sativa seed extract when given orally in a single dose of 40 mg/kg to Giardia lamblia–infected rats showed 80% cure rate while the same dose of metronidazole showed the same cure rate in another group of animals. They also tried to give the same previous dose of N. sativa seed extract before the animals’ exposure to Giardia lamblia infection. The surprising result was 50% protection, i.e. 50% of the animals treated with N. sativa extract showed negative stool analysis for Giardia lamblia in spite of exposure to infection. On the other hand, the same dose of metronidazole had only 10% protection (77).

N. sativa seed extract and its main constituent, thymoquinone had protective effects on mouse cells infected with schistosomiasis and against chromosomal aberrations induced as a result of schistosomiasis (78). N. sativa had antiparasitic effects. Its administration decreased the number of eggs as well as worms in schistosomiasis, which affected hepatic and intestinal tissues (79).

N. Sativa as an hypoglycemic agent

The volatile oil of N. sativa produced a significant hypoglycemic effect on normal and alloxan–induced diabetic rabbits without changes in insulin levels (80). A significant decrease in blood sugar of healthy human volunteers treated with 1 gram of N. sativa capsules twice daily was detected (81). Another study was designed to investigate the possible insulinotropic properties of N. sativa oil in streptozotcin plus nicotinamide–induced diabetes mellitus in hamsters. After four weeks of treatment with N. sativa oil, significant decrease in blood glucose level together with significant increase in serum albumin level were observed. The results showed that the hypoglycemic effect of N. sativa oil was, at least partly, because of a stimulatory effect on beta cell function with consequent increase in serum insulin level and possess insulinotropic properties in type II–like animal model (82). Significant decrease in blood glucose level together with significant increase in serum insulin level were observed after treatment with N. sativa oil for 4 weeks. Big areas with positive immuno–reactivity for the presence of insulin were observed in the pancreatic tissue of N. sativa oil–treated group compared to non–treated one using anti–insulin monoclonal antibody immunohistochemical staining. N. sativa is of great
therapeutic benefits in diabetic individuals and those with glucose intolerance, as it accentuated glucose-induced secretion of insulin, besides having a negative impact on glucose absorption from the intestinal mucosa (83,84). In fact, N. sativa attenuated the damage to β–cells of the pancreas following exposure to toxic elements such as cadmium (85). N. sativa treatment caused a decrease in the elevated serum glucose, an increase in the lowered serum insulin concentrations and partial regeneration/proliferation of pancreatic β–cells in streptozotocin–induced diabetic rats. The hypoglycemic action of N. sativa could be partly due to amelioration in the β–cells of pancreatic islets causing an increase in insulin secretion (48). Several studies showed that extracts from the seeds of N. sativa had antidiabetic effects. N. sativa seed ethanol extract (NSE) induced an important insulin–like stimulation of glucose uptake in C2C12 skeletal muscle cells and 3T3–L1 adipocytes following an 18 h treatment. NSE increased activity of Akt, a key mediator of the effects of insulin, and activity of AMP–activated protein kinase (AMPK), a master metabolic regulating enzyme. It may be used as a treatment for diabetes, obesity and the metabolic syndrome (86). A plant mixture containing N. sativa used by diabetics in Kuwait (87).

**Toxicity**

The toxicity of the fixed oil of N. sativa seeds in mice and rats was investigated through the determination of LD50 values and examination of possible biochemical, hematological and histopathological changes. The low toxicity evidenced by high LD50 values, key hepatic enzyme stability and organ integrity suggested a wide margin of safety for therapeutic doses of fixed oil of the Nigella seeds (89).

The above examples clearly illustrate the massive clinical and therapeutic potentials of N. sativa. These promising results in prevention and treatment of many diseases will recommend the use of N. sativa in combination with different medical treatments in the near future.

**REFERENCES**


