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Clinical and Therapeutic Trials of Nigella Sativa

[Nigella Sativa'nın Klinik ve Terapötik Özellikleri]

SUMMARY

Seeds of Nigella sativa (N. sativa) have been used for thousands of years as a spice and food preservative. The oil and seed constituents have shown potential medicinal properties in traditional medicine. This review lists and discusses different therapeutic trials of N. sativa seeds and its active ingredients in many diseases affecting body systems. It has anti–oxidant effects through enhancing the oxidant scavenger system that leads to antitoxic effects induced by several insults. Its anti–inflammatory effects conduct through suppression of the inflammatory mediators' prostaglandins and leukotriens. Its immunomodulatory properties were proved by augmenting the T cell and natural killer cell–mediated immune responses. It expresses antimicrobial and anti–tumor properties toward different microbes and cancers. It decreases DNA damage and thereby prevents initiation of carcinogenesis in colonic tissue secondary to exposure to toxic agents. N. sativa is of immense therapeutic benefit in DM. It stimulates glucose–induced secretion of insulin besides having a negative impact on glucose absorption from the intestinal mucosa. N. sativa administration protects hepatic tissue from deleterious effects of toxic substances and attenuates hepatic lipid peroxidation. N. sativa provides a promising strategy that combines anti–inflammatory, antioxidants, and antineoplastics modes of action.

ÖZET

Nigella sativa (N. Sativa) tohumları binlerce yıldır baharat ve gıda koruma maddesi olarak kullanılmaktadır. Bitkinin tohumları ve yağı geleneksel tıp alanında kullanılabilecek potansiyel ilaç özellikleri göstermektedir. Bu derlemede N. Sativa'nın terapötik kullanım alanları ve bu konudaki tartışmalar ile vücut sistemlerini etkileyen hastalıkların tedavisindeki rolü incelenmiştir. N. Sativa, oksidan ajanları temizleyen ati–oksidan sistemi harekete geçirme özelliğine de sahiptir. İnflamatıar süreçlerin mediatörü olan prostaglandinleri ve lökotrienleri baskılayarak anti–enflamatuar özellik göstermektedir. Bitkinin immunomodülatör özellikleri, T hücrelerini ve natural killer hücrelerini artırarak immun cevaba katkıda bulunma şeklindedir. Çeşitli kanser ve mikroorganizmalara karşı anti–kanser ve anti–mikrobiyal özellikler göstermektedir. Toksik ajanlara maruz kalmış kolon dokusunda DNA hasarını azaltmakta, karsinogfenezin başlmasını önlemektedir. N. Sativa DM'da da önemli tedavi edici etkilere sahiptir. Glukoz tarafından indüklenen insülin salgısını artırmanın yanında, intestinal mukozadan glukoz emilimini azaltma yönünde etkisi de bulunmaktadır. N. Sativa, hepatik dokunun zararlı etkilere karşı korunmasında etkili olmanın yanında hepatik lipit peroksidasyonunu da düzenlemektedir. N. Sativa'nın etkileri anti–inflammatuar, antioksidan ve antineoplastik etkilerin kombinasyonu şeklindedir.

Ragaa H.M. Salama

Department of Medical Biochemistry, Faculty of Medicine, Assiut University, Egypt.

Key Words: Herbs, Nigella Sativa, Pharmacological Effects, Body Systems.

Anahtar Kelimeler: Tıbbi Bitki, Nigella Sativa, Farmakolojik Etki, Vücut Sistemleri.

Sorumlu yazar/ Corresponding author:
Ragaa H.M. Salama
Assuit University, Faculty of Medicine, Medical Biochemistry Department, P.O: 71515 Assiut, Egypt.
ragaa_2002@yahoo.com

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 disrupt 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

intestines. 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, phosphatidylserine, and phosphatitdyl-inisitol, 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 oligomenorrhoea, 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 spasmolytic 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 immun 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 toxins 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 methylnitrosourea–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 cycloxygenase 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 carcinogenesis of rats in the post initiation stage. The inhibition associated, in part, with suppression of cell proliferation in the colonic

mucosa (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 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 lambia–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 lambia infection. The surprising result was 50% protection, i.e. 50% of the animals treated with N. sativa extract showed negative stool analysis for Giardia lambia 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 streptozotcin–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)

Anti–coagulant

A significant shortening of bleeding time in rats was observed after using N. sativa extract. However, there were no significant effects on the thrombin time or prothrombin time but the partial thromboplastin time was shortened while euglobulin time was prolonged (18). N. sativa also shortened the whole blood clotting time and plasma clot time of rabbits (19).

Anti–histaminic

The antihistaminic effect was first investigated by El–Dakhakhany (88). Thymoquinone had protective action against histamine–induced bronchospasm in guinea pigs (88). Furthermore, in an in vitro study, nigellone, isolated from N. sativa, effectively inhibited the release of histamine from mast cells, possibly through decrease the intracellular calcium and inhibition of protein kinase C. These effects together with analgesic and anti–inflammatory actions recommended the use of N. sativa in folk medicine in treatment of eczema and asthma, for scorpion and spider stings and for the bites of cat, dog and snake (16).

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

1. Shaikh B, Hatcher J. Complementary and alternative medicine in Pakistan: prospects and limitations. Evid. Based Complement. Altern. Med. 2005; (2): 139–142.
2. Saxena A, Panborta B. Herbal remedies, renal tragedies. Swiss Med. Wkly. 2003; (133): 188–189.
3. Saad B, Azaizeh H, Said O. Tradition and perspectives of Arab herbal medicine: A Review. eCAM. 2005; 2(4): 475–479.
4. Tariq M. Nigella sativa seeds: Folklore treatment in modern day medicine. 2008; (14): 105–106.
5. El–Kadi A, Kandil O. Effect of Nigella sativa (the black seed) on immunity. Bull. Islamic Med. 1986; (4): 344–348.
6. Agarwal C, Narula A, Vyas D, Jacob D. Effect of seeds of “Kalaunji” (Nigella sativa) on fertility and sialic acid content of the reproductive organs of the male rat. Geobios. 1990; (17): 269–272.
7. Takruri H, Dameh M. Study of the nutritional value of black cumin seeds (Nigella sativa). J. Sci. Food and Agric. 1998; (76): 404–410.
8. Ghoneim M, El–Gindy A, El–Alami R, Shoukry E, Yaseen S. Possible effects of some extracts of Nigella sativa seeds on blood coagulation system and fibrinolytic activity. Proceeding of 2nd international Conference on Islamic Medicine.12th April. Kuwait. 1982, p. 528–535.
9. El–Aziz MA, Hassan HA, Mohamed MH, Meki AR, Abdel–Ghaffar SK, Hussein MR. The biochemical and morphological alterations following administration of melatonin, retinoic acid and Nigella sativa in mammary carcinoma: an

animal model. Int. J. Exp. Pathol. 2005; 86(6): 383–96.

10. Hawsawi Z, Ali B, Bamosa A. Effect of Nigella sativa (black seed) and thymoquinone on blood glucose in albino rats. Annals of Saudi Medicine. 2001; 21(3,4): 242–244.
11. Omar A, Ghosheh S, Abdulghani A, Houdi A, Crookscor PA. High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed (L. Nigella sativa). J Pharm Biomed Anal. 1999; (19): 757–62.
12. Haq A, Lobo PI, Al–Tufail M, Rama NR, Al–Sedairy ST. Immunomodulatory effect of Nigella sativa proteins fractionated by ion exchange chromatography. Int J Immunopharmacol. 1999; (21): 283–95.
13. Al–Jassir MS. Chemical composition and microflora of black cumin (Nigella sativa L.) seeds growing in Saudi Arabia. Food Chem. 1992; (45): 239–42.
14. Al–Gaby AM. Amino acid composition and biological effects of supplementing broad bean and corn proteins with (black cumin) cake protein. Nahrung. 1998; (42): 290–4.
15. Randhawa M, Al–Ghamdi M. A review of phamaco–therapeutic effects of Nigella sativa. Pakistan J. Med. Res. 2002; 41(2): 1–10.
16. Chakarvarti N. Inhibition of histamine release from mast cells by nigellone. Ann. Allergy. 1993; 70(3): 237–242.
17. El–Tahir K, Ashour M, Al–Harbi M. The respiratory effects of the volatile oil of black seed (Nigella sativa) in guinea pigs: elucidation of the mechanism(s) of action. Gen. Pharmacol. 1993; 24 (5): 1115–1122.
18. El–Kadi A, Kandil O. Effect of Nigella sativa (the black seed) on immunity. Bull Islamic Med. 1986; (4): 344–348.
19. Randhawa M, Al–Ghamdi M. A review of pharmaco–therapeutic effects of Nigella sativa. Pakistan J. Med. Res. 2002; 41(2): 1–10.
20. El–Zawahry B. Isolation of new hypotensive fraction from Nigella sativa seeds. Kongr. Pharmacol. Wiss. 1964; (23):193–203.
21. El–Tahir K, Ashour M, Al–Harbi M. The cardiovascular effects of the volatile oil of black seed (Nigella sativa) in rats: elucidation of the mechanism(s) of action. Gen. Pharmacol. 1993; 24 (5): 1123–1131.
22. Zaoui A, Cherrah Y, Laccaile–Dubois M, Schaff A, Amarouch H, Hassar M. Diuretic and hypotensive effects of Nigella sativa in spontaneous hypertensive rat. Ther. 2000; (55): 379–382.
23. Al–Naqeep G, Ismail M, Allaudin Z. Regulation of low–density lipoprotein receptor and 3–hydroxy–3–methylglutaryl coenzyme a reductase gene expression by thymoquinone–rich fraction and thymoquinone in hepg2 cells. J Nutrigenet Nutrigenomics. 2009; 16(2):163–172.
24. Gilani A, Jabeen Q, Khan M. A review of medicinal uses and pharmacological activities of Nigella sativa. Pakistan. J. of Biol. Sci. 2004; 7(4): 441–451.
25. Agarwal C, Narula A, Vyas D, Jacob D. Effect of seeds of “Kalaunji” (Nigella sativa) on fertility and sialic acid content of the reproductive organs of the male rat. Geobios. 1990; 17: 269–272.
26. El–Naggar A, El–Deib A. A study of some biological activities of (black seeds) “Habbat El–Barka” .J. Egypt. Soc. Pharmacol. Exp. Ther. 1992; 11(2): 781–800.
27. Aqel M, Shaheen R. Effect of the oil of Nigella sativa seed on the uterine smooth muscle of rat and guinea pig. J Ethnopharmacol. 1996; 52(1): 23–26.
28. Keshri G, Singh M, Lakshami V, Kamboj V. Post–coital contraceptive effect of the seeds of Nigella sativa in rats. Indian J. Physiol. Phamacol. 1995; 39(1): 59–62.
29. Mohammed A, Farrag A, Mohammed S, Mary K, Botus H, El Alfy A. The possible protective effect of Nigella sativa on gentamin–induced nephrotoxicity in unilateral nephrectomized rats. J.Pharma Biolog. 1996; (3): 421–429.
30. Badary O, Abdel–Naeem A, Abdel–Wahab M, Hamada F. The influence of thymoquinone on doxorubicin–induced hyperlipidemic nephropathy in rats. Toxicol. 2001; (143): 219–226.
31. Rajkapoor B, Anandan R, Jayakar B. Anti–ulcer effect of Nigella sativa against gastric ulcers in rats. Current Science. 2002; (82): 177–185.
32. El–Abhar H, Abd–Allah D, Saleh S. Gastroprotective activity of Nigella sativa oil and its constituent, thymoquinone, against gastric mucosal injury induced by ischaemia/reperfusion in rats. J. Ethnopharmacol. 2003; (84): 251–258.
33. Kanter M, Demir H, Karakaya C, Ozbek H. Gastroprotective activity of Nigella sativa L oil and its constituent, thymoquinone against acute alcohol–induced gastric mucosal injury in rats. World J Gastroenterol. 2005; (11): 6662–66.
34. Al Mofleh IA, Alhaider AA, Mossa JS, Al–Sohaibani MO, Al–Yahya MA, Rafatullah S, Shaik SA. Gastroprotective Effect of an Aqueous Suspension of Black Cumin on Necrotizing Agents–Induced Gastric Injury in Experimental

Animals. Saudi J Gastroenterol. 2008; 14(3):128–34.

35. Mahgoub A. Thymoquinone protects against experimental colitis in rats. Toxicol. Lett. 2003; (143): 133–143.
36. Mahmoud MR, El–Abhar HS, Saleh S. The effect of Nigella sativa oil against the liver damage induced by Schistosoma mansoni infection in mice. J Ethnopharmacol. 2002; (79):1–11.
37. Janbaz K, Saeed S, Gilani A. Hepatoprotective effect of thymol on chemical induced hepatotoxicity in Rodents. Pakistan J. Biol. Sc. 2003; (6): 448–451.
38. El–Dakhakhany M, Mady N, Halim M. Nigella sativa L oil protects against CCL4 and D–galactosamine induced hepatotoxicity and improves serum lipid profile in rats. Arzeimittelforschung. 2000; 50(9): 832–836.
39. Farrag AR, Mahdy KA, Abdel Rahman GH, Osfor MM. Protective effect of Nigella sativa seeds against lead–induced hepatorenal damage in male rats. Pak. J. Biol. Sci. 2007; 10: 2809–2816.
40. Yildiz F, Coban S, Terzi A, Ates M, Aksoy N, Cakir H, at al. Nigella sativa relieves the deleterious effects of ischemia reperfusion injury on liver. World J Gastroenterol. 2008; 14: 5204–5209.
41. Al–Naggar T, Gomez–Serranillos M, Carretero M, Villar A. Neuropharmacological activity of Nigella sativa extracts. J. Ethnopharmacol. 2003; (88): 63–68.
42. Haq A, Abdullatif M, Lobo P, Khabar K, Sheth K, Al–Sedairy S. Nigella sativa: Effect on human lympocytes and polymorphonuclear leucocyte phagocytic activity. Immunopharmacology. 1995; 30(2): 147–150.
43. Swamy S. and Tan B. Cytotoxic and immunopotentiating effects of ethanolic extract of Nigella sativa seeds. J. Ethnopharmacol. 2000; 70: 1–7.
44. Houghton P, Zarka R, De las Heras B, Hoult J. Fixed oil of a derived thymoquinone inhibit generation in leukocytes and membrane lipid peroxidation. Planta Med. 1995; 61 (1): 33–36.
45. Kruk I, Michalska T, Lichszteld K, Kladna A, Aboul–Enein H. The effect of thymol and its derivatives on reactions generating reactive oxygen species. Chemosphere. 2000; (41): 1059–1064.
46. Meral T, Yener Z, Kahraman T, Meral N. Effect of Nigella sativa on glucose concentration, lipid peroxidation, antioxidant defense system and liver damage in experimentally induced diabetic rabbits. J. Vet. Med. A Physiol. Path. Clin. Med. 2001; (48): 593–599.
47. Mabrouk G, Moselhy S, Zohny S, Ali E., Helal T, Amin A, et al. Inhibition of Methylnitrosourea (MNU)–induced oxidative stress and carcinogenesis by orally administered bee honey and Nigella grains in Sprague Dawley rats. J. Exp. Clin. Cancer. Res. 2002; (21): 341–346.
48. Kanter M, Meral I, Dede S, Cemek M, Ozbek H, Uygan I, et al. .Effects of Nigella sativa L and Urtica dioica L on lipid peroxidation, antioxidant enzyme systems and some liver enzymes in Ccl4–treated rats. J. Vet. Med. 2003; (50): 264–268.
49. Hamdy NM, Taha RA. Effects of N. sativa oil and thymoquinone on oxidative stress and neuropathy in streptozotocin–induced diabetic rats. Pharmacology. 2009; 84(3): 127–34.
50. Uz E, Bayrak O, Uz E, Kaya A, Bayrak R, Uz B, Turgut FH, at al. A. Nigella sativa oil for prevention of chronic cyclosporine nephrotoxicity: an experimental model. Am J Nephrol. 2008; (28): 517–522.
51. Mutabagani A, El–Mahdy S. A study of the anti–inflammatory activity of Nigella sativa L and thymoquinone in rats. Saudi Pharm J. 1997; 5(2): 110–113.
52. Abd–El–Fattah A, Matsumoto K, Watanabe H. Antihypertensive effects of Nigella sativa oil and its major component, thymoquinone, in mice. Eur. J. Pharmacol. 2000; 14(1): 89–97.
53. Al–Ghamdi M. Anti–inflammatory, analgesic and anti–pyretic activity of Nigella sativa. J. Ethnopharmacol. 2001; (76): 45–48.
54. Mahmoud M, Gilani A, Khwaja A, Rashid A, Ashfaq M. The in vitro effect of aqueous extract of Nigella sativa seeds on nitric oxide production. Phytotherapy Res. 2003; (17): 921–924.
55. Salomi N, Nair S, Jayawardhanan K, Varghese C, Panikkar K. Antitumour principles from Nigella sativa seeds. Cancer Lett. 1992; 63(1): 41–46.
56. Medenica R, Janssens J, Tarasenko G, Lazovic W, Corbitt D, Powell D, et al. Anti–angiogenic activity of Nigella sativa plant extract in cancer therapy. Proc. Annu. Meet. Assoc. Cancer Res. 1997; 38: 1377–1380.
57. Badary O, Al–Shabanah O, Nagi M, Al–Rikabi A, El–mazar M. Inhibition of benzopyrene–induced forestomach carcinogenesis in mice by thymoquinone. Eur. J. Canccer Prev. 1999; 8(5): 435–440.
58. Badary O, Gamal El–Din A. Inhibitory effect of thymoquinone against 20–methylchlolanthrene–induced fibrosarcoma tumorigenesis. Cancer Detect Prev. 2001; 25(4): 362–368.

59. Jeong H, Choi C. Expression of inducible nitric oxide synthase by α–hedrin in macrophages. Planta Medica. 2002; (68): 392–396.
60. Farah IO, Begum RA. Effect of N. sativa L. and oxidative stress on the survival pattern of MCF–7 breast cancer cells. Biomed Sci Instrum. 2003; 39: 359–64.
61. Soheib A, El–Gayyar M, Dudrick P, Bell J, Tithof P. In vitro inhibition of growth and induction of apoptosis in cancer cell lines by thymoquinone. Int J. Oncol. 2003; 22: 107–113.
62. Salem E, Fukushima S. Chemopreventive potential of volatile oil from black kumin seeds against rat colon carcinogenesis. Nut. Cancer. 2003; 45(2): 195–202.
63. Al–Johar D, Shinwari N, Arif J, Al–Sanea N, Jabbar AA, El–Sayed R, et al. Role of Nigella sativa and a number of its antioxidant constituents towards azoxymethane–induced genotoxic effects and colon cancer in rats. Phytother Res. 2008; (22): 1311–1323.
64. Norwood AA, Tucci M, Benghuzzi H. A comparison of 5–fluorouracil and natural chemotherapeutic agents, EGCG and thymoquinone, delivered by sustained drug delivery on colon cancer cells. Biomed Sci Instrum. 2007; (43): 272–277.
65. Ait Mbarek L, Ait Mouse H, Elabbadi N, Bensalah M, Gamouh A, Aboufatima R, Benharref A, at al. Anti–tumor properties of black seed (Nigella sativa L.) extracts. Braz J Med Biol Res. 2007; (40): 839–847.
66. Iddamaldeniya SS, Thabrew MI, Wickramasinghe SM, Ratnatunge N, Thammitiyagodage MG. A long–term investigation of the anti–hepatocarcinogenic potential of an indigenous medicine comprised of Nigella sativa, Hemidesmus indicus and Smilax glabra. J Carcinog. 2006; 5: 11.
67. Sethi G, Ahn KS, Aggarwal BB. Targeting nuclear factor kappa B activation pathway by thymoquinone: role in suppression of antiapoptotic gene products and enhancement of apoptosis. Mol Cancer Res. 2008; (6): 1059–1070.
68. Gali–Muhtasib H, Diab–Assaf M, Boltze C, Al–Hmaira J, Hartig R, Roessner A, Schneider–Stock R. Thymoquinone extracted from black seed triggers apoptotic cell death in human colorectal cancer cells via a p53–dependent mechanism. Int J Oncol. 2004; (25): 857–866.
69. Khader M, Bresgen N, Eckl PM. Antimutagenic effects of ethanolic extracts from selected Palestinian medicinal plants. J Ethnopharmacol. 2010; 127(2): 319–24.
70. Kaseb AO, Chinnakannu K, Chen D, Sivanandam A, Tejwani S, Menon M,et al. Androgen receptor and E2F–1 targeted thymoquinone therapy for hormone–refractory prostate cancer. Cancer Res. 2007; 67(15): 7782–8.
71. Topozada H, Masloum H, El–Dakhakhany M. The anti–bacterial properties of Nigella sativa seeds: Active principle with some clinical application. J. Egypt Med Assoc. 1965; (48): 187–202.
72. El–Fatatry. Isolation and structure assignment of an anti–microbial principle from the volatile oil of Nigella sativa L seeds. Pharmazie. 1975; 30(2): 109–111.
73. Hanafi M, Hatem M. Studies on the anti–microbial activity of the Nigella sativa seed (Black Cumin). J. Ethnopharmacol. 1991; 34(2–3): 275–278.
74. Morsi N. Antimicrobial effect of crude extracts of Nigella sativa on multiplle antibiotic resistant bacteria. Acta Microbiol. Pol. 2000; 49(1): 63–74.
75. Khan M, Ashfaq M, Zuberi H, Mahmoud M, Gilani A. The in vivo antifungal activity of the aqueous extract from Nigella sativa seeds. Phytotherapy Res. 2003; (17): 183–186.
76. Akhtar M, Rifaat S. Field trials of Saussurea Lappa roots against nematodes and Nigella sativa seeds against cestodes in children. J. Pakistan Med. Assoc. 1991; 41(8): 185–187.
77. Bishara S, Masoud S. Effect of Nigella sativa extract on experimental giardiasis. New Egypt J. of Med. 1992; (7): 1–3.
78. El Shenawy NS, Soliman MF, Reyad SI. The effect of antioxidant properties of aqueous garlic extract and Nigella sativa as anti–schistosomiasis agents in mice. Rev. Inst. Med. Trop. Sao. Paulo. 2008; (50): 29–36.
79. Khan M, Ashfaq M, Zuberi H, Mahmoud M, Gilani A. The in vivo antifungal activity of the aqueous extract from Nigella sativa seeds. Phytotherapy Res. 2003; (17): 183–186.
80. Al–Hader A, Aqel M, Hasan Z. Hypoglycemic effects of the volatile oil of Nigella sativa. Intern. J. Pharmacognosy. 1993; (31): 96–100.
81. Bamosa A, Ali B, Al–Hawsawi Z. The effect of thymoquinone on blood lipids in rats. Indian J. Physiol. Pharmaco. 2002; (46): 195–201.
82. Fararh K, Atoji Y, Shimizu Y, Takewaki T. Insulinotropic properties of Nigella sativa oil in Streptozotocin plus Nicotinamide diabetic hamsters. Res. Vet. Sci. 2002; (73): 279–282.
83. Rchid H, Chevassus H, Nmila R, Guiral C, Petit P, Chokaïri M, et al. Nigella sativa seed extracts enhance glucoseinduced insulin release from rat–isolated Langerhans islets. Fundam Clin Pharmacol. 2004; (18): 525–529.

84. Meddah B, Ducroc R, El Abbes Faouzi M, Eto B, Mahraoui L,et al. Nigella sativa inhibits intestinal glucose absorption and improves glucose tolerance in rats. J Ethnopharmacol. 2009; (121): 419–424.

85. Demir H, Kanter M, Coskun O, Uz YH, Koc A, Yildiz A. Effect of black cumin (Nigella sativa) on heart rate, some hematological values, and pancreatic beta–cell damage in cadmium–treated rats. Biol Trace Elem Res. 2006; (110): 151–162.

86. Benhaddou–Andaloussi A, Martineau LC, Vallerand D, Haddad Y, Afshar A, Settaf A, et al. Multiple molecular targets underlie the antidiabetic effect of N. sativa seed extract in skeletal muscle, adipocyte and liver cells. Diabetes Obes. Metab. 2010; 12(2): 148–57.

87. Al–Awadi F, Gomma K. Studies on the activity of individual plants of an anti–diabetic plant mixture. Acta Diabetol Lat. 1987; 24(1): 37–41.

88. El–Dakhakhany M. Some pharmacological properties of some constituents of Nigella sativa L seeds: The carbonyl fraction of essential oil. Drug Res. 1982; (15): 1227–1229.

89. Zaoui A, Cherrah Y, Mahassine N, Alaoui K, Amarouch H, Hassar M. Acute and chronic toxicity of Nigella sativa fixed oil. Phytomedicine. 2002; (9): 69–74.