***Nigella sativa* – a Plant with Personality in Biochemistry and Experimental Medicine Researches**

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Abstract

The last few decades have witnessed an increased interest for the use of plant-based natural compounds for the prevention and treatment of various human ailments. The plant *Nigella sativa* showed great promise in that regard. Known also as black cumin the seeds of *Nigella sativa* were processed for use either as powder or oil/water/alcoholic extracts. Traditionally, the seed powder served as food spice. For medical purposes the seeds were used as oil/water extracts and administered orally or as injections. The most studied compound from this plant is thymoquinone (a monoterpenoid), which appears to be the key component that has medicinal properties. It has been shown that the seeds of black cumin possess health benefit in conditions such as heart, gastrointestinal and respiratory diseases, reproductive and neurological disorders as well as exhibiting analgesic and appetite stimulation effects. Black cumin seeds were also used in cancer prevention and treatment as well as diabetes. The seed extracts were shown to possess antimicrobial, antiparasitic, anti-inflammatory and antioxidant properties.

Keywords: *biochemistry, experimental medicine, Nigella sativa.*

INTRODUCTION

Due to the increasing interest in the natural compounds from the plant kingdom the last few years witnessed the accumulation of a large body of data on the biochemical, pharmacological and medical properties of these plant compounds. Thereby, natural chemical products are the basis of the oldest science in medicine. History of science showed us that for a long time the herbs and plants (roots, leaves, flowers and other anatomical parts)

were the only natural remedies in traditional medicine (Odeh *et al.*, 2014).

Since the ancient there are data that *Nigella sativa*, known as black cumin, panacea, black-caraway, fennel-flower, nigella, nutmeg-flower or Roman-coriander, was used in experimental medicine (*in vivo* and *in vitro*), being a way of treatment and prevention (U.S. National Plant Germplasm System, 2000; Al-Rowais, 2002; Ahmad *et al.*, 2013; Khan *et al.*, 2016). Due to its

Fig. 1. *Nigella sativa*: flowers, seeds, oil

content in active principles, it is a valuable plant and can be used in pharmacology, medicine and food technology (Padhye *et al.*, 2008; Mehta *et al.*, 2009; Sharma *et al.*, 2009; Yarnell and Abascal, 2011; Afrin *et al.*, 2016). The anatomical parts of the plant – used as health enhancement and benefits – are seeds and fresh whole plant (Figure 1).

Nivella sativa is an annual plant, and from botanically point of view is part of Taxon: *Nigella sativa* L., Genus: *Nigella*, Family: *Ranunculaceae*, Subfamily: *Ranunculoideae*, Tribe: *Nigellaea*. It has white, pink, blue, or purple flowers, and is native from Asia. The plant grows up to 20-30cm tall; has finely divided leaves; five to ten petals for the flower; the fruit is a large and inflated capsule that contains numerous black seeds. The seeds are used as food spice and seed oil extracts in traditional medicine.

N. sativa seeds contain fibres, protein (and branched chain amino acids), lipids and essential fatty acids, and also carbohydrates. The oil extract of black cumin seeds contains quinones, monoterpenoid phenols, alkaloids, saponins, phytosterols; essential lipids; and thymoquinone (Pacioretty and Babish, 2008).

Due to its content of thymoquinone and other alkaloid components – characterised by excellent therapeutical, pharmaceutical, and nutritional properties, recent progress in the study of this plant led to the development of new products with clinical application (Crooks *et al.*, 2001; Pacioretty and Babish, 2008).

CHEMICAL COMPOSITION

The seeds of black cumin contain various biological active principles, most of them quinoidic phenolic compounds, from which the thymoquinone is the most important for biomedical reasons. Also, the seeds contain phenols; fatty

acids and sterols; other terpenoids; nigellamines, vitamins such as: ascorbic acid, thiamine, tocopherols, retinol, riboflavin, folic acid, niacin, pyridoxine, ergocalciferol, phylloquinone and menaquinone; and minerals: copper, manganese, zinc, iron, cobalt, chromium, phosphor (Matthaus and Ozcan, 2011; Ahmad *et al.*, 2013; Beheshti *et al.*, 2016; Khan and Afzal, 2016).

N. sativa seeds are characterised by 4-6% moisture, 4.8% total ash, and contain 8.4% crude fibre, 26.7% protein (3.7% branched chain amino acids), 28.5% lipids, and 24.9% carbohydrates (Ahmad *et al.*, 2013; Pacioretty and Babish, 2008). The oil extract of black cumin seeds has in its composition 34-39% quinones, monoterpenoid phenols, alkaloids, saponins, phytosterols; 0.5-1.5 % essential lipids; and up to 0.75 % thymoquinone (Pacioretty and Babish, 2008).

The seeds of black cumin are rich in essential and non-essential fatty acids. The unsaturated fatty acids from *N. sativa* seeds are represented mainly by 50-60 % linoleic acid, 20 % oleic acid, 10 % dihomolinoleic acid, and 3 % eicodadienoic acid. The content of saturated fatty acids found in black cumin seeds is less of 30 %, mostly represented by palmitic and stearic acids. Also, the seed contain sterols, from which 44-54 % is a-sitosterol and 6.57-20.92 % stigmasterol in Iranian and Tunisian varieties of *N. sativa* seed oils (Bourgou *et al.*, 2008; Cheikh-Rouhou *et al.*, 2008; Mehta *et al.*, 2008).

Protective and therapeutic properties of black cumin are partly due to the terpens represented by thymoquinone, dithymoquinone, trans-anethol, but also *p*-cymene, limonine and carvone (Khan and Afzal, 2016). Both thymoquinone and dithymoquinone have demonstrated antineoplastic and cytotoxic properties for different types of cancer, having

equally cytotoxicity /for parenteral/ and multi-drug resistance tumor cells (Crooks *et al.*, 2001).

Nigella sativa seeds contain active alkaloid compounds, represented mainly by nigellidine, nigellimine, and nigellicine (Beheshti *et al.*, 2016). Nigellidine and nigellidine-4-O-sulfite are indazole alkaloids naturally presented in black cumin seeds (Ali *et al.*, 2008; Atta-ur-Rahman *et al.*, 1995). Together with these alkaloids, the *Nigella sativa* seeds contain isoquinoline alkaloids represented by nigellicimine and nigellicimine-N-oxide, and also indazole alkaloids such as nigellidine and nigellicine (Khan and Afzal, 2016).

MEDICAL, PHARMACOLOGICAL AND NUTRITIONAL BENEFITS

Nigella sativa (*N. sativa*) was proved that has effective in different medical disorders, such as: hypertension; diarrhea; cardiac, digestive and respiratory diseases; liver and renal tonic; inflammations; reproductive and neural disorders, bacterial infection; dyspepsia; analgesic; appetite stimulant; cancer; bronchodilator; spasmolytic and diabetes. Also, the seeds powder, oil or extracts (hydro, alcoholic extracts) have antimicrobial, antiparasitic, antitumoral, antidiabetic, antioxidant, dermatological, anti-inflammatory therapeutic properties (Salem, 2005; Alenzi *et al.*, 2010; Liu *et al.*, 2013; Shabana *et al.*, 2013; Aljabre *et al.*, 2015; Mohtashami *et al.*, 2015; Abamor and Allahverdiyev, 2016; Periasamy *et al.*, 2016). Antioxidant activity is important due to thymoquinone content and it was tested on different parts of plant (shoots, roots and seeds used as extracts), results demonstrated strong antioxidant activity (Ahmad and Beg, 2016; Gholamnezhad *et al.*, 2016).

The antioxidant and antiperoxidative effect of methanol extract and volatile oil of *N. sativa* seeds was evaluated on atherogenic rats. The research aim was to evaluate the conjugated diene, malondialdehyde and lipid hydroperoxide as lipid peroxidation markers in experimental rats compared to hyperlipidemic control rats, after a treatment of hyperlipidemic rats with methanol extract and volatile oil of *N. sativa*. The results demonstrated the efficacy of proposed and tested extracts against lipid oxidative stress and proposed different form of food supplements as prevention and treatment against free radicals activity (Ahmad and Beg, 2016).

Thymoquinone from *N. sativa* exerts anti-inflammatory action; modulate humoral and cellular immune responses; provide natural cytotoxicity activity; regulates allergy symptoms, autoimmunity and cancer. Both *in vivo* and *in vitro* experiments reported the good ability of thymoquinone from black cumin seeds to modulate inflammation processes (Majdalawieh *et al.*, 2010; Majdalawieh and Fayyad, 2015).

Female urinary tract inflammation and microbial infection can be treated by a dietary supplement formulation based on thymoquinone from *N. sativa* seed extract and cranberry fruit extract and methionine (Pacioretty and Babish, 2008).

N. sativa boiled extract was also tested as bronchodilatory effect on asthmatic patients. The effect of 50 mg/Kg and 100 mg/Kg of boiled extract of *N. sativa* and 6mg/Kg theophylline was studied of asthmatic patients. The pulmonary function tests of the patients during the experiment presented relatively potent antiasthmatic action of *N. sativa* extract, but lower effect compared to theophylline action. The main advantage of this research consists in the natural extract treatment using black cumin extract compared to any chemical drug (Boskabady *et al.*, 2010).

Shahraki and his collaborators (2016) tested the antitumor action of a hydro-alcoholic total extract and *n*-hexane and ethyl acetate fractions of *N. sativa*, on human renal adenocarcinoma (ACHN) and normal renal epithelial (GP-293) cell lines, aiming the cell morphology, cell viability, and apoptosis. The results demonstrated that on cell survival and apoptosis, the hydro-alcoholic total extract had greater beneficial action compared to *n*-hexane and ethyl acetate fractions of *N. sativa* dry seeds. Also, the association of total extract, *n*-hexane and ethyl acetate fractions of *N. sativa* had better effect on the ACHN cell line compared to GP-293 cell line.

The nanoemulsion of black cumin essential oil (20-50 nm diameter of the droplet size) was obtained, was analytical characterized, and was tested on the viability of breast cancer cells (Michigan Cancer Foundation-7 breast cancer cells). The experiments presented very good activity of the nanoemulsion on the cancer cells, by clearly induced cell apoptosis. The research could detail the apoptosis processes by membrane blebbing of the breast cancer cell, then cytoplasmic

vacuolation, followed by marginalization of chromatin, and finally the fragmentation of the nucleus (Periasamy *et al.*, 2016).

Antioxidant and neuroprotective effect of *N. sativa* hydro-alcoholic extract was experimentally evaluated on learning and memory improvement of neonatal and juvenile rats (Beheshti *et al.*, 2016).

New experiments used different methods of microencapsulation of oleoresin from black cumin for nutraceutical applications. Oleoresin extraction was performed using hexane solvent, at room temperature; then the extract was microencapsulated by emulsifying the black cumin oleoresin in a solution that contained Arabic gum and maltodextrin. The resulting product indicates a very good efficiency of oleoresin and volatile oil fraction in powder with application in food technology for obtaining fortified-nutraceutical food products (Edris *et al.*, 2016).

N. sativa has significant protective role on jejunal mucosal injury after treatment with gamma radiation in rats (Orhon *et al.*, 2016).

Essential oil from *N. sativa* has been demonstrated that has vasorelaxant effect on rat aorta. The oils from black cumin seeds acted on the smooth muscle resulting relaxation and had vasorelaxing effect, by blocking both receptor-operated and voltage-sensitive calcium channels, being useful as antihypertensive agent (Cherkaoui-Tangi *et al.*, 2016).

Also, the *N. sativa* oil combination was tested in the parasitology domain with very good and promising results. *N. sativa* oil was used to obtain nanoparticles of silver doped titanium dioxide (TiAgNps) for medical treatment on Cutaneous Leishmaniasis (*Leishmania tropica*). The obtain formulation had non-toxic concentration of TiAgNps and black cumin oil and was used to test the antileishmanial effect on different life forms of the parasites. The experimental observations consist in evaluation of proliferation and metabolic activity, apoptosis infection index analysis and also the quantum of nitric acid production as antileishmanial efficiency. The results demonstrated high potential of effective antileishmanial effect by presented formulation, based on *N. sativa* oil (Abamor and Allahverdiyev, 2016).

Essential oil extract of *N. sativa* and other medicinal plants has demonstrated antibacterial

properties tested on aquatic crustaceans, i.e on a prawn species (shrimp – sea food). The black seed oil extract showed good protection and treatment results of *Artemia* spp. (*Fenneropenaeus indicus*) after infection with *Vibrio* spp. (*V. parahaemolyticus*). Thus, *Nigella sativa* oil extracts are very good treatment products, but in the same way are used in food industry to protect the sea raw food – with high impact for sea food consumers (Manju *et al.*, 2016).

Fungal infection can be treated with alternative/traditional medicine, with natural products. Thereby, a mycological treatment methodology was proposed by using hydro, alcoholic (methanolic) and chloroform extract of Mediterranean black cumin seeds for treatment of *Candida albicans* infection. The results proved that methanolic seeds extract had the best action, being the strongest anti-fungal activity (Bita *et al.*, 2012; Forouzanfar *et al.*, 2014).

Oil seeds of non-traditional food sources try to come into the food market and nutrition field to prove the nutritional prevention and therapeutically importance. Black cumin seeds oil is used for a long time in food industry due to its nutraceutical and functional properties, represented by the high content (about 85 %) of bioactive components of total content. Black cumin essential oil showed high antioxidant potential and complete inhibition zone for different Gram-negative and Gram-positive bacteria (*Penicillium citrinum*, *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) (Ramadan, 2016).

TOXICOLOGICAL RESEARCH

International literature data reported researches results on the toxicological studies in relation with ingestion on seeds, root and shoots water and/or alcoholic extracts, or oil of black cumin. *In vivo* experiments were also performed using injection administration of thymoquinone, and the results proved no toxicity for this biochemical compound.

The *Nigella sativa* fixed oil was tested for LD50 on mice and rats. The acute toxicity test was investigated for orally (28.8 ml/kg body wt.) and intraperitoneal (2.06 ml/kg body wt.) administration, using single doses in mice. The chronic toxicity was tested in rats after a daily, oral dose (2 ml/kg body wt.), for 12 weeks. The

experimental results evidenced high values for LD50, for stability of the most important hepatic enzymes (aspartate-aminotransferase – AST, alanine-aminotransferase – ALT, gamma-glutamyltransferase – GGT), and also for organs (liver, kidney, heart, and pancreas) integrity (Zaoui *et al.*, 2002).

The toxicity effect of thymoquinone was tested on male and female rats, by intraperitoneal and oral administration. The thymoquinone concentration administrated in this research was 20 mg/kg, 30 mg/kg, and 40 mg/kg body weight – for intraperitoneal injections, and 200 mg/kg, 300 mg/kg and 500 mg/kg body weight – for oral administration. The results of the experiment proved that maximum tolerant dose was 22.5 mg/kg for male rats and 15mg/kg for female rats as intraperitoneal administration and 250mg/kg for oral administration – both for male and female rats. For 500 mg/kg body weight oral administration two rats died, as a result of complications caused by bowel obstruction (AbuKhader, 2012).

The administration of *N. sativa* seeds powder mixed in water with rat chow pellet was tested for toxicity on liver function. The tested dose was supplementation diet with 0.01 g/kg/day powder; 0.1 g/kg/day powder; and 1g/kg/day powder, for 28 consecutive days on male Sprague Dawley rats (300-350 g body weight). The experimental study demonstrated no inflammation or necrosis, and also no changes in hepatic enzymes level or toxicity effects on the liver (Dollah *et al.*, 2013).

The black cumin seeds were characterised by very low toxicity potential, and extremely low allergenic effect. Thus, administration of seed extract and seed oil of black cumin did not induce any negative effects on hepatic or renal function (Ali and Blunden, 2003).

High dose of *Nigella sativa* seed oil was toxicity tested on male adult albino rats. The research consist in administration of 15 to 25 ml/kg *N. sativa* seed oil, for 1 month, during which the toxic effects were tested for liver and kidney function. Large dose of black cumin seed oil administration has toxic effects with histological modification of hepatic and renal structure (Abd Allah *et al.*, 2012).

The literature data present very low or no toxicity of black cumin seeds or oil. Many experimental researches proved that administration of curative dosage of seeds, oil seeds, different extracts, or dietary (culinary)

utilisation of *Nigella sativa* seeds as spice, have no toxicity effects on mice, rats, or human. Some varieties of plants can contain different chemical composition; and also the quantity of the active principles can be directly influenced by the environment conditions: like the soil and water composition, the climate and geographical region, the potential pollution in the cultivated area (Dronca, 2004). There are literature data about some programs for genetic improvement of *Nigella sativa* yield (quantity and quality) that demonstrated that ecotype (geographical area) are important for the content and quality of oil components from black seeds, and also the rate between the biologic active principles (Ali and Blunden, 2003; Jabeen *et al.*, 2012; Salamati and Zeinali, 2013; Salehi *et al.*, 2016).

CONCLUSION

Nigella sativa, commonly known also as black cumin, is a plant used from ancient times in traditional medicine and culinary filed. The seeds are used as such, but also as powder, or extracts. Most common extract are aqueous, ethanolic, aqueous-ethanolic, methanolic or glycolic extracts are used in traditional and modern medicine and pharmacy.

The most bioactive principle of *Nigella sativa* is the monoterpenoid compound: thymoquinone. The black cumin seeds also contain nigellamine, nigellidine, nigellimine, and nigellicine; dithymoquinone, trans-anethol, and less *p*-cymene, limonine and carvone. Other biocomponents with medical benefits are represented by lipids: unsaturated and saturated fatty acids, sterols; phenols; vitamins: ascorbic acid, ergocalciferol, folic acid, niacin, pyridoxine, retinol, riboflavin, thiamine, tocopherols; and important minerals: chromium, cobalt, copper, iron, manganese, phosphor and zinc.

Administration of *N. sativa* seeds, seed powder, seed oil, seed extracts in prescript doses does not lead to toxicity for animal or human organism. Experimental researches presented very low toxicity for black cumin seed administration or consumption.

Nigella sativa is – for all the positive presented research data – a plant with high potential in traditional medicine, being easy to cultivate, growth, preservation or storing, and offering various potential of use (seed, seed oil, different seed extracts).

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