Review

Therapeutic effects of black cumin (*Nigella sativa*). A systematic review

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Summary. Functional foods are predicted to become one of the prime dietary trends in the incoming decades and it is the responsibility of the nutritionists to pay attention towards their health claims and safety issues. Black cumin (*Nigella sativa* L.) locally known as "Kalonji" is a good source of nutritionally essential components. Their seeds have been used as herbal medicine by various cultures and civilizations to treat and prevent a number of diseases. Consumption of antioxidant rich foods may improve antioxidant defense mechanism and provide protection against oxidative damage caused by free radicals. In this context, black cumin is used against cancer, diabetic, antifungal, antibacterial and antioxidant. It has been realized that diet-based therapies are among the most effective and sustainable ways to overcome various maladies. It is deduced that black cumin fixed can be supplemented in food products for the management of these maladies. The health benefits including amelioration of oxidative stress, hypoglycemic and hypocholesterolemic potentials associated with the consumption of black cumin seed

Key words: Functional food, black cumin, biological, hypoglycemic, hypocholesterolemic

Back ground

Plant based functional foods gaining popularity across the world due to an array of evidences for their safer therapeutic applications. The health claims associated with the consumption of plants are due to their rich phytochemistry (1). Phytochemicals like Ώ-3-fatty acids, dietary fibers, antioxidant, vitamins, plant sterols and flavonoids are helpful in maintaining the health of an individual thus reducing the risk of various maladies (2).

Herb and spice products have been used widely as flavor to improve organoleptic and nutritional properties, also as preservatives in food industries. There have been various investigations on the chemical composition of essential oil, phenolic compounds and natural antioxidant of many herb species Moreover, herbal drugs and essential oil of cumin have great medicinal value to treat various diseases, especially digestive disorders, tooth ache, wounds, hoarseness, epilepsy, jaundice. In addition, it is known as antitumor, anti-inflammatory, diuretic, emmenagogic, cytotoxic, antidiabetic, antifungal, antibacterial, antioxidant, and antispas-modic (4-7). Herbal medicines have long been viewed as a source of curative remedy based on religious and cultural traditions. 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 (8).

In case of black pepper (*Piper nigrum*) major aroma producing components are volatile oils ranging from 2 to 5 % in berries. It constitutes β-pinene, α-pinene, 1-α- phellandrene, piperonal, limonene, dihydrocarveol, piperidine and β-caryophyllene as showed in Table 1. Different functional component of Black cumin as presented in Figure 2.

Table 1. Active ingredient of selected spices

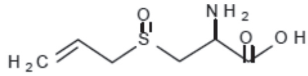
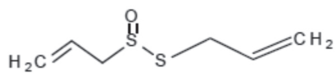
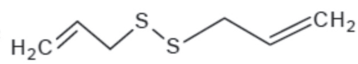
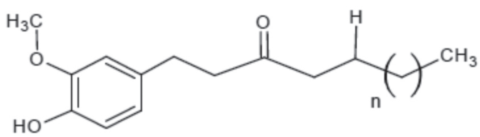
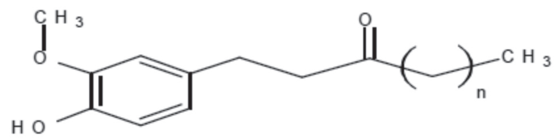
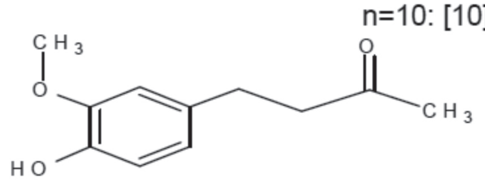
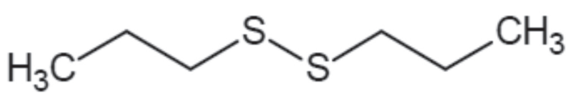
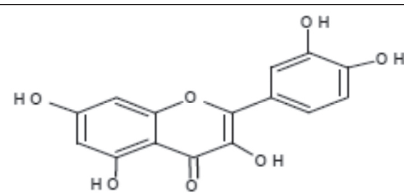
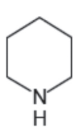
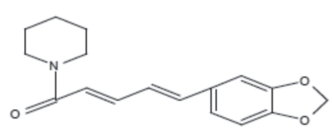
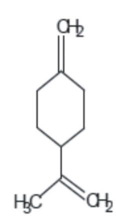
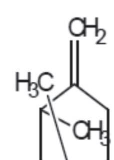
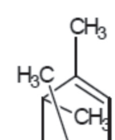
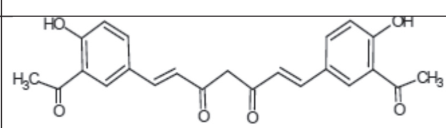
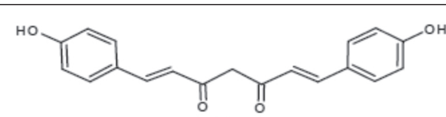
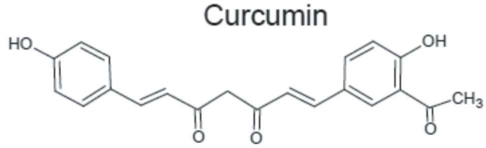
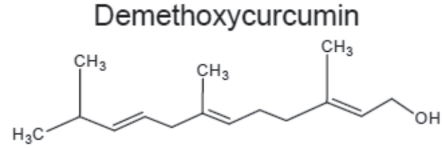
Garlic (Allium sativum)	 Allin  Allicin  Allyl isothiocyanate
Ginger (Zingiber officinale)	 Gingerols n=4: [6]-gingerol n=6: [8]-gingerol n=8: [10]-gingerol  Paradols n=6: [6]-paradols n=8: [8]-paradols n=10: [10]-paradols  Zingerone
Onion (Allium cepa)	 Dipropyl disulfides  Quercetin
Black pepper (*Piper nigrum)*	 Piperidine  Piperine  Limonene  A-pinene  β-Pinene
Turmeric *(Curcuma longa)*	 Curcumin  Demethoxycurcumin  Bisdemethoxycurcumin  Farnesol

Figure 1. Nigella sativa L.

Pivotal links have been established between dietary components and human health unveiling the imperative role of nutrients in normal functioning of the body. Probing these links led to coinage of terms like functional and nutraceutical foods that are becoming popular all over the globe. The consumer trend has widened, many have begun to look at food not only for its basic nutrition but also for the allied health benefits (9). In the domain of diet-based therapies, opportunities abound in functional foods to combat oxidative stress, hyperglycemia and high cholesterol levels.

Botanical classification and history

Nigella sativa is an annual herb of the Ranunculaceae family, which grows in countries bordering the Mediterranean Sea, Pakistan and India as showed in Table 2. This widely distributed plant is native to Arab countries and other parts of the Mediterranean region.

Black cumin (*Nigella sativa* L.) locally known as "Kalonji" is a good source of nutritionally essential components.

Table 2. Botanical classification of cumin

Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliophyta
Order	Apiales
Family	Ranunculaceae
Genus	Cuminum L.
Species	Cuminum cyminum L.

It is an annual flowering plant, native to Southwest Asia. It grows 19-30 cm tall, with finely divided, linear leaves. The flowers are usually pale blue and white, with 5-11 petals. The fruit is a large inflated capsule composed of 3-6-7 united follicles, each containing numerous seeds Black cumin seeds have been used as herbal medicine by various cultures and civilizations to treat and prevent a number of diseases. Recent research also witnessed the presence of *Nigella sativa* seeds some 3000 years ago at Uli Burun, off the southwest coast of Turkey (10). It is also famous for the saying of the Prophet Muhammad (SAW) "Hold on to use of the black cumin seed, for it has a remedy for every illness except death" (11). The historical tradition of black cumin seed in medicine is also substantial; identified as curative black cumin in the Bible, and mentioned as Melanthion by Hippocrates and Dioscorides and Pliny called it as the Gith (12).

Chemical constituents and active principles in N. sativa seeds

Several therapeutic effects have been attributed to N. sativa seeds in their purified as well as crude components. Thymoquinone (TQ) (2-isopropyl-5-methyl-1,4-benzoquinone), which has the chemical formula $C_{10}H_{12}O_2$ and a molecular weight of 164.2 g/mol, is a major phytochemical bioactive ingredient in N. sativa oil and extracts. The chemical structure of TQ is shown in **Fig. 2**. TQ makes about 30–48% of N. sativa seeds, and since its isolation and characterization in 1963, it has been extensively studied by many researchers worldwide. Some of the medical benefits of N. sativa and TQ are due to their anti-histaminic, anti-inflammatory, anti-hypertensive, hypoglycemic, anti-cancer,

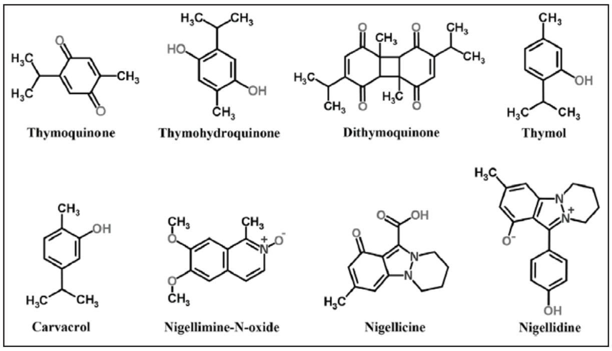


Figure 2. The chemical structures of TQ and other chemical constituents found in N. sativa seed extracts and oil.

and immunity-boosting effects. There is a growing research interest in evaluating TQ as a therapeutic agent against various in vitro and in vivo disease models (13).

The seeds are very rich and diverse in chemical composition. They contain amino acids, proteins, carbohydrates, fixed and volatile oils (14). 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.Moreover, Amin and Muneera(13) 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. El-Dakhakhny (15) 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 (16). In addition, El-Dakhakhny determined that the 'nigellone' isolated earlier was a dimer of TQ, which was later named dithymoquinone (TQ2) (Fig 3). 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. However, El-Fatatry (17) reported the isolation of thymohydroquinone (THQ) from N. sativa seed volatile oil. In another study (18**)**, 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 a-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 . The seeds also contain alkaloids of unknown pharmacological actions, such as nigellidine, nigellimine and nigellicine (19), saponins and crude fiber as well as minerals such as calcium, iron, sodium and potassium. Other constituents of the volatile oil include thymol (TOH) (Fig.3) (18). 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 (20).

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 (21). 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 (22). 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. (23). 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. (23) 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 (24).

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 (24).

Traditional uses

Traditionally N. sativa plant has been in use in many Middle Eastern countries as a natural remedy for diabetes (25). Significant reduction in blood glucose and cholesterol levels in humans following the use of the plant 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 (26). Several studies support the use of NSO extract for the treatment of thrombosis and dyslipidemia (26-28). The purified components (2-(2-methoxypropyl)-5-methyl-1,4-benzenediol, thymol and carvacrol) obtained from the methanol-soluble portion of Nigella sativa oil (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 (27). 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 (28). Analogous results, accompanied by decreases in serum lipid levels have also been observed in rats chronically treated with N. sativa fixed oil (29). 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 (26), suggesting that the oil influences blood homeostasis.

Medicinal perspectives

Antioxidant

Consumption of antioxidant rich foods may improve antioxidant defence mechanism and provide protection against oxidative damage caused by free radicals. Management of plasma cholesterol continues to be a cardinal issue in cardiovascular disease (CVD) prevention. Hypercholesterolemia and LDL oxidation play key role in the onset of atherosclerosis and related disorders. Owing to rich phytochemistry with special reference to polyphenols, natural products might be suitable preventive measure in coronary care and regulating blood cholesterol. Consumption of black cumin fixed oil in daily diets can address the problem concern. Functional ingredients of black cumin seed especially antioxidants, fat-soluble vitamins, phytosterols and some pyrazanol containing moieties are important in cholesterol lowering properties (30-32). Oxidative stress results when production of reactive oxygen species (ROS) exceeds the scavenging potential of cells, tissues or organs. Consequences of oxidative stress include cardiovascular, neurodegenerative and carcinogenic processes (33). The active ingredients like thymoquinone and its derivatives act as safeguards against free radical damage not only during oxidative stress but also in associated discrepancies (34).

The role of dietary components in immune response is indispensable for regulation and proper functionality of the system (35). Black cumin oil and its essential ingredient like thymoquinone act as immune boosters and research investigations have proved their anti-inflammatory and immune modulatory effects (36,37).

It has been realized that diet-based therapies are among the most effective and sustainable ways to overcome various maladies. However, development of successful food-based strategy requires knowledge of nutrients dense sources, target communities and indeed selection of suitable vehicle (38,39). Functional foods are important components in such interventions aiming to provide health benefits beyond their basic nutrition (40,41). Wheat based baked products are considered suitable vehicles for incorporation of functional ingredients that can easily be accessible to masses especially in countries like Pakistan where wheat is staple diet (42).

Mechanism of action

Antioxidants provide protection against oxidation. The different factors which affect lipid oxidation include the presence of oxygen and transition metal ions, moisture, heat and light. To prevent, minimize or slow down the rate of lipid oxidation, oxygen and metal catalysts must be removed, or sequestered to render them unreactive. The food prone to oxidation must be stored at low temperatures and/ or shielded from light. Most of the antioxidants from spices and herbs act by reacting with free radicals created during the initiation stage of autoxidation. Others form complexes with metal ions (43).

Anticancer

Peng *et al.,* 2013 studied that the antitumor and anti-angiogenic effects of thymoquinone (TQ) on osteosarcoma in vitro and in vivo. Their results showed that TQ induced a higher percentage of growth inhibition and apoptosis in the human osteosarcoma cell line SaOS-2 compared to that of control, and TQ significantly blocked human umbilical vein endothelial cell tube formation in a dose-dependent manner. Moreover, hydroxyl radical, superoxide anion radical, peroxynitrite (ONOO-), nitric oxide (NO) and hydrogen peroxide (H_2O_2) are causative agents of structural cell damage as in lipid and protein membranes system and DNA thus shifting healthy cells towards cancerous state as shown in Figure 3

It was found that TQ significantly down regulated NF-κB DNA-binding activity, XIAP, survivin and VEGF in SaOS-2 cells. Moreover, the expression of cleaved caspase-3 and Smac were up regulated in SaOS-2 cells after treatment with TQ. It was also found that TQ inhibits tumor angiogenesis and tumor growth through suppressing NF-κB and its regulated molecules. It was concluded that TQ effectively inhibits tumor growth and angiogenesis both in vitro and in vivo. Therefore, inhibition of NF-κB and downstream effect or molecules is a possible underlying mechanism of the antitumor and anti-angiogenic activity of TQ in osteosarcoma (44,45).

The anticancer effects of TQ on breast cancer cells, and its potential effect on the PPAR-γ activation pathway was investigated and it was found that TQ exerted strong anti-proliferative effect in breast cancer cells and when TQ combined with doxorubicin and 5-fluorouracil, cytotoxicity was found to be increased. TQ was found to increase sub-G1 accumulation and annexin-V positive staining, indicating apoptotic induction. In addition, TQ activated caspases 8, 9 and 7 in a dose-dependent manner. Migration and invasive properties of MDA-MB-231 cells were also reduced in the presence of TQ. Interestingly, TQ was found to increase PPAR-γ activity and down-regulate the expression of the genes for Bcl-2, Bcl-xL and surviving in breast cancer cells. More importantly, the increase in PPAR-γ activity was prevented in the presence of PPAR-γ specific inhibitor and PPAR-γ dominant negative plasmid, suggesting that TQ may act as a ligand of PPAR-γ. It was observed by using molecular docking analysis that TQ indeed formed interactions with 7 polar residues and 6 non-polar residues within the ligand-binding pocket of PPAR-γ that are reported to be critical for its activity. Thus, it was concluded that TQ may have potential implication in breast cancer prevention and treatment and anti-tumor effect of TQ may also be mediated through modulation of the PPAR-γ activation pathway (46-47).

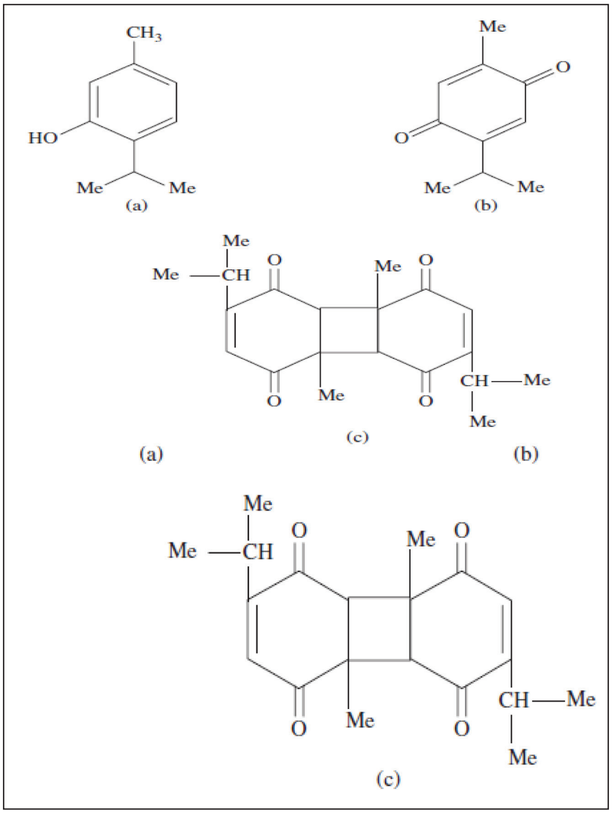


Figure 3. Chemical structures of thymol (a), thymoquinone (b) and dithymoquinone (c) (Me: methyl group).

Anti diabetic

Diabetes mellitus and its complications are one of the leading causes of death counts in developing countries. According to an estimate, at the end of year 2030, approximately 376 million people were affected worldwide by diabetes. Recently, Pakistan is holding the 6th position, and it was replaced Japan from 5th place at the end of 2030 (48). No doubt, drug therapies are obligatory yet accompanied by side effects and even their effectiveness decreases with the passage of time (49,50). Diet selection is crucial for the management of diabetes and its allied difficulty, including immune dysfunction, degenerative and cardiovascular disorders. Thus, nigella sativa, fixed and essential oils, hold insulinotropic properties and helpful in maintaining β-cells integrity and both these properties are important in mediating diabetes mellitus. Likewise, antioxidants present in black cumin mitigate diabetic complications arising due to free radical production and elevated cholesterol level (51,52).

Ahmad et al. (53) investigated that the therapeutic potentials of α-lipoic acid (α-LA), L-carnitine, and N. sativa or combination of them in carbohydrate and lipid metabolism was evaluated in a Rat model of diabetes which was induced by single i.p. injection of streptozocin (STZ) 65 mg/kg. For evaluation of glucose metabolism, fasting blood glucose, insulin, insulin sensitivity, HOMA, C-peptide, and pyruvate dehydrogenase activity were determined. Either α-LA or N. sativa significantly reduced the elevated blood glucose level. The combination of 3 compounds significantly increased the level of insulin and C-peptide. Combination of α-LA, L-carnitine and N. sativa will contribute significantly in improvement of the carbohydrate metabolism in diabetic rats, thus increasing the rate of success in management of DM.

The effects of N. sativa aqueous extract and oil, as well as TQ, on serum insulin and glucose concentrations in streptozotocin diabetic rats were studied. Serum insulin and glucose concentrations, SOD levels, and pancreatic tissue malondialdehyde (MDA) were determined. Electron microscopy was used to identify any subcellular changes. Diabetes increased tissue MDA and serum glucose levels and decreased insulin and SOD levels. Treatment of rats with N. sativa extract and oil, as well as TQ, significantly decreased the diabetes-induced increases in tissue MDA and serum glucose and significantly increased serum insulin and tissue SOD. Ultra structurally, TQ ameliorated most of the toxic effects of streptozotocine (STZ), including segregated nucleoli, heterochromatin aggregates (indicating DNA damage), and mitochondrial vacuolization and fragmentation. The aqueous extract of N. sativa also reversed these effects of STZ, but to a lesser extent. The N. sativa oil restored normal insulin levels, but failed to decrease serum glucose concentrations to normal. The biochemical and ultrastructural findings suggest that N. sativa extract and TQ have therapeutic and protect against STZ-diabetes by decreasing oxidative stress, thus preserving pancreatic β-cell integrity. The hypoglycemic effect observed could be due to amelioration of β-cell ultrastructure, thus leading to increased insulin levels. N. sativa and TQ may prove clinically useful in the treatment of diabetics and in the protection of β-cells against oxidative stress (54,55).

In vivo antidiabetic activity of N. sativa seed ethanol extract (NSE) was evaluated in diabetic Meriones shawi. Plasma lipid profile, insulin, leptin, and adiponectin levels were assessed. ACC phosphorylation and Glut4 protein content were determined in liver and skeletal muscle. NSE animals showed a progressive normalization of glycaemia. It was also demonstrate that in vivo treatment with NSE exerts an insulin-sensitizing action by enhancing ACC phosphorylation, a major component of the insulin-independent AMPK signaling pathway, and by enhancing muscle Glut4 content (56,57).

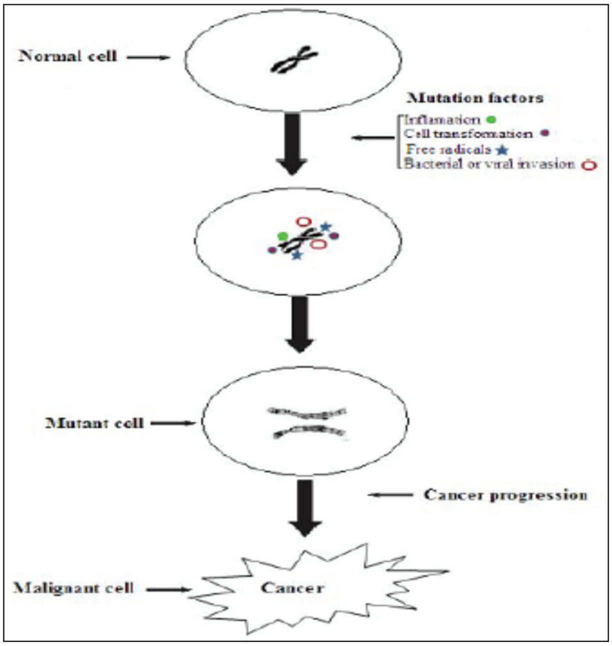


Figure 4. Cancer progression in healthy cell

Conclusion

In the nutshell, black cumin holds nutraceutical potential against various physiological threats owing to its rich phytochemistry especially due to the presence of thymoquinone, tocopherols, etc. The role of dietary components in immune response is indispensable for regulation and proper functionality of the system. In diet-based therapies, black cumin fixed should be recommended for the vulnerable segments. However, development of successful food-based strategy requires knowledge of nutrients dense sources, target communities and indeed selection of suitable vehicle. Black cumin is promising candidates in dietary modifications

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