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Review Article

Thymoquinone: Biosynthesis, Biological Activities and Therapeutic Potential from Natural and Synthetic Sources

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Abstract

Thymoquinone (TQ; 2-isopropyl-5-methyl-1,4-benzoquinone) is a secondary metabolite found in abundance in very few plant species including *Nigella sativa* L., *Monarda fistulosa* L. and *Satureja montana* L. It is found in crystalline triclinic form in a range of organs in these plants. TQ has been synthetically prepared from thymol (2-isopropyl-5-methylphenol); commercially it is synthesized by modification of thymol and carvacrol (5-isopropyl-2-methylphenol). TQ has substantial therapeutic potential because of its anti-cancer, hepato-protective, anti-inflammatory, antioxidant, antimicrobial and cardio-protective activities in cell culture systems and animal models. In this article, we have reviewed recent studies on the natural and synthetic sources of TQ, its biosynthetic pathway and its modes of action in human and experimental models, as well as its commercial preparation. We also compiled the medicinal effects of TQ. The biological activities of TQ support the potential of this plant secondary metabolite as a drug with a wide range of therapeutic applications. To substantiate the benefits and pharmaceutical properties of TQ, further well-designed clinical research is required.

Keywords: Anti-cancer; *Nigella sativa*; Phytochemical; Phytotherapy; Thymoquinone

Introduction

Thymoquinone (TQ; 2-isopropyl-5-methyl-1,4-benzoquinone; Fig. 1) is the most abundant and important bioactive constituent of a number of plant species, such as *Nigella sativa* L. (black-caraway, black cumin, also known as nigella or *kalonji*). In the Middle East, many diets include plants containing TQ and are considered to be health-promoting. *N. sativa* (an annual herb) is cultivated around the Mediterranean, Syria, Egypt and India at larger scale for TQ extraction. The safe uses of *N. sativa* oil and its most important constituent TQ have been confirmed by acute and chronic toxicity studies. TQ is also a bioactive element of the volatile oil of *Monarda fistulosa* L. (Gali-Muhtasib *et al*. 2006).

With the use of high-resolution X-ray powder diffraction, it was determined that TQ can be found only in a crystalline triclinic form (Pagola *et al*. 2004). Numerous analytical techniques, including high performance liquid chromatography (HPLC), gas chromatography (GC) and differential pulse polarography, have been used for TQ quantification in plant extracts (Michelitsch and Rittmannsberger 2003). Although TQ has poor solubility in water, an increase in the operating pressure from 100 to 120 bar at 38°C, for example, results in an increase in TQ solubility (Gurdenova and Wawrzyniak 2012). TQ is soluble in supercritical CO_2.

TQ is therapeutically important because of its anti-cancer, hepato-protective, anti-inflammatory, antioxidant, antimicrobial and cardio-protective activities in cell culture systems and animal models (Fig. 2). The understanding of these activities has been strengthened by elucidation of their molecular mechanisms (Pang *et al*. 2017). TQ inhibited cell proliferation and induced apoptosis in several human cancer cell lines such colon, breast, brain, pancreatic, and ovarian (Gurung *et al*. 2010). Several reports suggest an adjuvant role of TQ which may improve the quality of cancer patients (Woo *et al*. 2012).

Over the last 20 years about one quarter of drugs have been directly isolated from plants, while in another quarter, natural compounds have been chemically modified (Vuorelaa *et al*. 2004). TQ has shown considerable anti-neoplastic activity against human cancer by specifically inhibiting the growth of tumor cells without any harmful effects on normal cells. TQ operates through diverse modes of action: cell cycle arrest, reactive oxygen species (ROS) production, anti-proliferation activity, anti-metastasis activity and apoptosis induction (Gurung *et al*. 2010).

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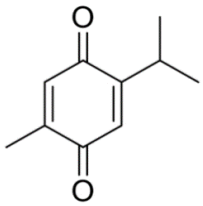

Fig. 1: Molecular structure of TQ

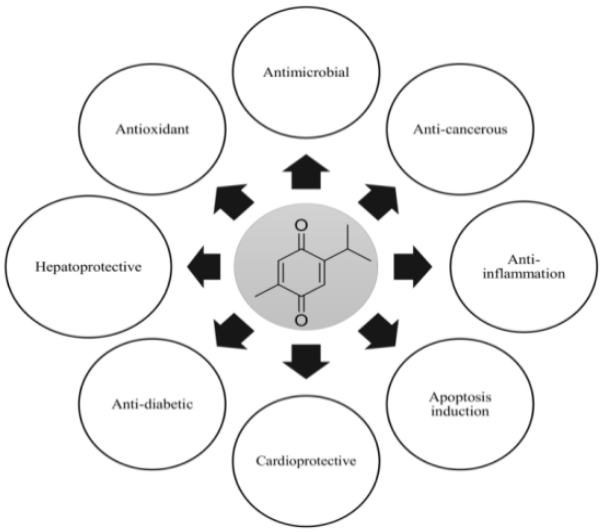

Fig. 2: General outline of TQ actions

TQ stimulates apoptosis in cancer cells through various pathways such as Akt activation, NF-κB suppression and extracellular signal-regulated kinase signaling. These studies have encouraged the use of TQ following the success of chemotherapeutics like gemcitabine and oxaliplatin. The anti-tumor property of TQ has also been examined in tumor xenograft mice models for colon, prostate, lung and pancreatic cancers showing inhibition of cell growth, induction of apoptosis and NF-κB modulation (Banerjee *et al*. 2010). The anti-inflammatory and anti-oxidative properties of TQ have been demonstrated in different disease models such as gastric ulcer, carcinogenesis, diabetes, asthma and encephalomyelitis. TQ also acts as superoxide radical and free radical scavenger (Banerjee *et al*. 2010).

The major aim of this review was to provide the updated evidence on the natural and synthetic sources of TQ, its biosynthetic pathway and its modes of action in different experimental models including humans, as well as its commercial preparation. We also compiled the medicinal effects of TQ.

Discovery of TQ

Plants synthesizing TQ

***Monarda fistulosa* (Wild bergamot):** It is a member of the family Lamiaceae (mint family) and is considered to be a prime source of TQ. TQ and thymohydroquinone (THQ), together with thymoquinhydrone, were isolated and identified for their oil characteristics in 1901 (Ernest 1908). Yellow crystals were separated in the condenser and identified as TQ upon purification of the non-phenol portion of oil distilled from *M. fistulosa* with water vapor. In the TQ biosynthesis, p-cymene and carvacrol act as precursors. The discovery of these compounds instantly provided an explanation for the purple color of stem and flowers of *M. fistulosa*, and for the spontaneous coloration of the Monarda oils upon standing. The separation of both of these compounds demonstrated that the red-brown color of the Monarda oils is due to the thymoquinhydrone resulting from the union of TQ and THQ. In 1904 Rabak isolated the enzyme from *M. fistulosa* that oxidases THQ to TQ (Wakeman 1911).

***Nigella sativa* (Kalonji):** It is from the family Ranunculaceae, and has a huge diversity of phytochemicals; many of which are volatile oils (Aftab *et al.* 2019). It was found that TQ is the main active constituent of the volatile oil of *N. sativa* seed (Edris 2011). El-Dakhakhny (1963) isolated the constituent components of *N. sativa* from its essential oil using silica gel column chromatography and later TQ was found to be the main constituent of the volatile oil (El-Dakhakhny 1963). TQ has also been detected in *N. arvensis* in trace amounts (Taborsky *et al*. 2012).

***Satureja montana* (Winter savory):** Gas chromatography–mass spectrometry (GC/MS) analysis has produced a very detailed picture of *S. montana* (family Lamiaceae) phytochemical components (Grosso *et al*. 2009). This species is a rich source of TQ when essential oils are extracted by hydrodistillation.

TQ distribution in other plant species: In addition to above described plant species, TQ has been identified in the genus Lamiaceae: *Agastache*, *Coridothymus*, *Monarda*, *Mosla*, *Origanum*, *Thymbra* and *Thymus* (Table 1). Its presence has also been confirmed in some other genera like *Tetraclinis*, *Cupressus* and *Juniperus* of the Cupressaceae family in glycosidic form. *M. didyma* was detected as the richest source of TQ; 3564 mg/kg dry weight (DW) in inflorescences and 3425 mg/kg DW in aerial parts, while *M. media* synthesized 2995 mg/kg TQ DW in aerial parts. The highest TQ content (1881 mg/kg DW) was found in *N. sativa* seeds. TQ contents found in the aerial parts of all the other plant taxa (*M. menthifolia*, *M. didyma*, *S. montana*, *Thymus vulgaris*, *T. serpyllum*, *Thymus pulegioides*, *Saureja hortensis* L., and *Eupatorium cannabinum* L.) presented lower amounts ranging from 8 to 1381 mg/kg DW (Taborsky *et al*. 2012).

The heart wood of *Tetraclinis articulate* Vahl is known to contain several compounds including TQ, carvacrol and β- and γ-pinenes (Zavarin and Anderson 1955). The amount of TQ in *T. kotschyanus* was 11.4% DW extracted by hydrodistillation of flowers and analyzed using GC-MS analysis (Rasooli and Mirmostafa 2003). The neutral fraction of heartwood of *Libocedrus decurrens* Torr

Table 1: TQ content in different parts of plant species

Family	Species	Plant part analyzed	TQ content (mg/kg DW)
Asteraceae	*Eupatorium cannabinum* L.	Aerial part	8
Cupressaceae	*Juniperus communis* L.	Twig	6
Lamiaceae	*Monarda didyma* L.,	Aerial part/inflorescence	3425
		Leaf,	3564
		Stem.	821
			23
	Monarda media Willd.	Aerial part	2995
	Monarda menthifolia Graham	Aerial part	1381
	Satureja hortensis L.	Aerial part	217
	Satureja montana L	Aerial part	1052
	Thymus pulegioides L.	Aerial part	223
	Thymus serpyllum L.	Aerial part	233
	Thymus vulgaris L.	Aerial part	300
Ranunculaceae	*Nigella sativa* L.	Seed	1881

(Incense-Cedar) was found to contain 21.7% of TQ on dry wood basis (Zavarin and Anderson 1955).

Synthesis of TQ

Pathways of TQ synthesis

Natural biosynthetic pathway of TQ: A key gene named geranyl diphosphate synthase (GPPS) is responsible for quinone and phenolics biosynthesis in plants. The proposed biosynthetic pathway of quinones and phenolics biosynthesis is given in Fig. 3. Geranyl diphosphate (GPP) is the precursor of this biosynthetic pathway, which leads to the production of ɾ-terpinene (phenol) followed by formation of p-cymene and carvacrol and ultimately the production of TQ (quinone) takes place (Khader and Eckl 2014).

Synthetic preparation of TQ: TQ can be commercially synthesized by a variety of methods. As described by Kremers *et al.* (1941), one of the TQ synthesis methods is the sulfonation and oxidation of thymol and carvacrol (Fig. 4). TQ is synthesized by thymol and carvacrol precursors (Fig. 5). The yields of TQ from carvacrol and thymol were 71and 80% of the theoretical. This was as high as 75 and 90–93% of yield on a laboratory scale for these substances, respectively (Kremers *et al*. 1941).

Capsules containing TQ formulations for pharmaceutical, nutraceutical or food supplements purposes have been produced using an oregano extract without significant loss of TQ at room temperature during the shelf life of the capsules formulating thymohydroquinone and benzoquinones (Fig. 5). Capsules are constructed with hard or soft shells and contain a single dose of one or more active ingredients. The preferred capsules have hydroxypropylmethyl cellulose (HPMC) shell and may contain carvacrol as an additional active ingredient, either in synthetic form or as part of a plant extract. The source of TQ can be oregano extract from plants belonging to the genus *Origanum*, such as *O. vulgare* or *O. minutiflorum* O. Schwarz & P. H. Davis, and *Thymus*, such as *T. vulgaris*, or from *N. sativa* in the form of a concentrate of extractable compounds, especially volatile compounds. The amount of volatile TQ for this purpose should be at least 70% of total DW. Since TQ is light-sensitive, opaque/colored capsules (size 00, which corresponds to a capsule volume of 0.91 mL), silicon dioxide (AEROSIL 200) or phosphatidylcholine (EPIKURON 135 F IP: fractionated soybean lecithin and soybean oil with enriched phosphatidylcholine content are used as viscosity enhancers (Etheve *et al*. 2015).

TQ Phylogeny

TQ is extracted from different plant sources like *N. arvensis* seeds the presence of this compound has previously been confirmed in several genera of the Lamiaceae family such as *Agastache*, *Coridothymus*, *Monarda*, *Mosla*, *Origanum*, *Satureja*, *Thymbra* and *Thymus*. It has also been found in genus *Tetraclinis*, *Cupressus* and *Juniperus* of the Cupressaceae family (Foster and Duke 2000). All the sources of TQ are thought to have a monophyletic origin from order magnoliales class magnolioideae and then variable families (Fig. 6).

Genes Involved in Biosynthesis of TQ

Five different genes and their specific enzymes are involved in TQ biosynthesis. GPPS gene is precursor for TQ biosynthesis that synthesizes GP. It is converted into ɾ-terpinene and gene involved is ɾ-terpinene synthase. In the next step formation of *p*-cymene takes place by activity of ɾ-terpinene dehydrogenase enzyme. P-cymene is converted into carvacrol through p-cymene hydroxylase gene. Ultimately carvacrol under activity of carvacrol oxidase is converted into TQ (Botnick *et al*. 2012).

Biological Effects of TQ

TQ induces apoptosis

TQ can have an effect on T lymphoblastic leukemia using CEMs cells (also known as CEM-SS cells) as an *in vitro* model (Salim *et al.* 2013). Apoptosis is a major type of programmed cell death, and a key pathway for controlling homeostasis and morphogenesis of mammalian cells

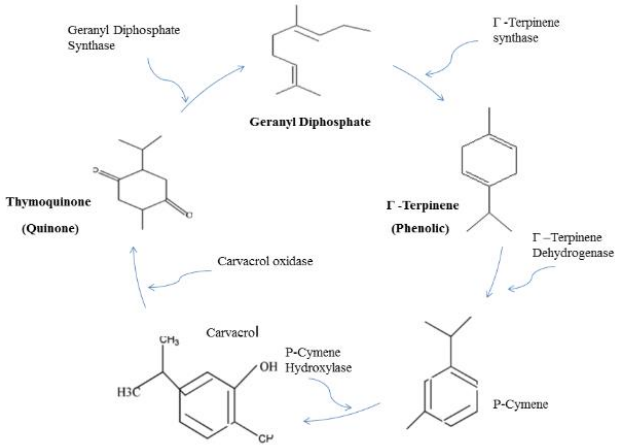

Fig. 3: Natural biosynthetic pathway of TQ

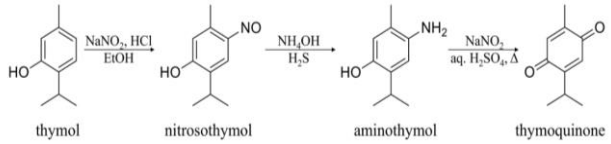

Fig. 4: Chemical Synthesis of TQ

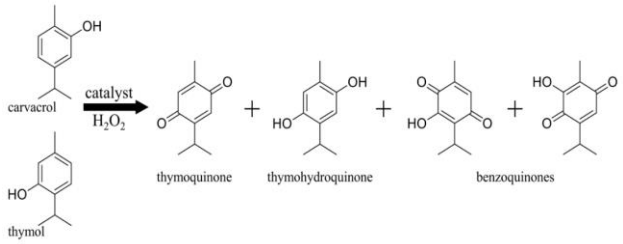

Fig. 5: Synthetic preparation of TQ based pharmaceuticals

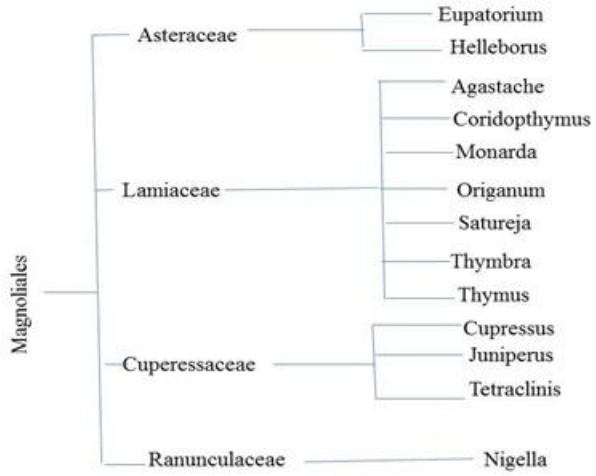

Fig. 6: TQ phylogeny among different families

(Goldsworthy *et al*. 1996). TQ treatment induces apoptosis in CEMss cells with an IC50 of 1.5 µg/mL and affects the different stages of apoptosis pathway, starting from chromatin development (Salim *et al*. 2013). TQ induces apoptosis in CEMss cells in coordination with the activity of caspase (a specific group of cysteine proteases) in nucleated animal cells avoiding the effect of various helper and repair proteins (Williams and Stoeber 2012). Caspases are required for the normal program of apoptosis, and are important for apoptotic chromatin development and DNA irregularity in all types of cells.

Some phytochemicals can act as cell-cycle modulators, regulating apoptosis and cell cycle restriction (Salim *et al*. 2013). Flow cytometry experiments to separate the distinctive cell cycle check centers following TQ treatment show that the cell cycle is modulated in CEMs cells by TQ.

Anti-cancer development of TQ in lymphoblastic leukemia is controlled and monitored by various systems including cell viability test, acridine orange/propidium iodide, DNA laddering, flow cytometry, caspase-3 activity and western blotting. An extensive number of tests have recommended that TQ could limit development and actuate apoptosis in the leukemic cell line CEMss (Salim *et al*. 2013).

Defensive impact of TQ against cyclophosphamide-actuated hemorrhagic cystitis

Previous studies have shown that TQ could decrease the toxicities of various compounds, including cyclophosphamide (CYP)-initiated pneumonic damage (Suddek *et al*. 2013), cisplatin-prompted hepatotoxicity and kidney damage (Al-Malki and Sayed 2014), as well as acetaminophen-incited hepatotoxicity (Ulu *et al*. 2012). TQ has been suggested to be a powerful cancer prevention agent and diminishes lipid peroxidation in various tissues by targeting nuclear factor (erythroid-derived 2)-like 2 (Nrf2) to regulate catalysis in TQ treated mice (Giudice *et al*. 2010; Aycan *et al*. 2014). All the above-mentioned impacts have also been seen *in vitro* in HaCaT cells treated with TQ [30]. The defensive impact of TQ to accept Nrf2 against doxorubicin-instigated nephrotoxicity in test rats and cytotoxicity against human leukocytes has also been demonstrated (Kundu *et al*. 2014).

TQ also has a defensive impact against CYP-instigated hemorrhagic cystitis in mice. The cancer prevention agent and DNA defensive impacts of TQ in bladder tissue were measured. Cystitis was actuated in mice by intraperitoneal organization of CYP at 200 mg/kg. At the same time each mouse was treated with a variable suspension of TQ (5, 10 and 20 mg/kg). TQ dosage diminished CYP-induced cystitis and it was observed that the bladder histology returned to a state like that seen in the control mice (Gore *et al*. 2016). TQ has also been implicated in the up-regulation of Nrf2 expression and DNA repair (Ma 2013), which may improve cell survival (Zhou *et al*. 2007).

TQ has been an effective agent in the treatment of CYP-initiated DNA damage in mice bladder cells. The DNA damage assessment utilizing the DNA ladder test uncovered increased DNA fracture and development of low-molecular-weight DNA-derived structures (Gore *et al*. 2016). Treatment with TQ conditionally gave protection against CYP-initiated DNA fracture. This characteristic is strongly related to the previously revealed antioxidant regulation and anti-apoptotic action of TQ in experimental organisms (Ma 2013).

Anti-inflammatory activities and IRAK1 inhibitor action of TQ

TQ is the key anti-inflammatory component both *in vitro* (TLR2/3/4-invigorated macrophages) and *in vivo* (mouse gastritis and hepatitis models) trial conditions. As at first estimated, TQ diminishes the emission of NO and prostaglandin E2 (PGE2) by down-regulating inflammatory gene expression in activated macrophages (Hossen *et al.* 2017). TQ additionally represses interleukin-6, tumor necrosis, inducible nitric oxide synthase, and cyclooxygenase expression in lipopolysaccharide and macrophage cells. Fundamentally, TQ has all these impacts without affecting cell sustainability. Furthermore, TQ attenuates allergen-induced lung inflammation Th2 cytokines inhibition (El Gazzar *et al*. 2006) and eosinophil infiltration into the airways in an allergic asthma mouse model (El-Mezayen *et al.* 2006). TQ eased colitis indications in mice initiated by a 7-day regimen of dextran sodium sulfate (3% w/v) added to drinking water (Lei *et al*. 2012). Thus, considering the literature, TQ has a general mitigating impact that might give a clinically helpful treatment for a range of conditions involving inflammation.

Intestinal, airway and cardiovascular relaxant activities of TQ

TQ may exert a relaxing effect on gut, trachea and cardiac muscles through Ca^{2+} influx blockage via voltage-operated Ca^{2+} channels (VOCC) (Ghayur *et al*. 2012). It has been observed that TQ mediates relaxation of histamine-and serotonin-contracted guinea-pig ileum through the inhibition of the products of lipoxygenase and arachidonic acid metabolism, via an unknown non-specific mechanism (Al-Majed *et al*. 2001). TQ appears to have an anti-inflammatory activity and to be helpful in mice models of asthma (El Gazzar *et al.* 2006). TQ has pharmacological potential to control hyperactive disorders of gastroenterology, respiration and cardiovascular systems through its anti-inflammatory activity (Ghayur *et al.* 2012).

Renal oxidative damage protector activity of TQ

TQ is a defense against $HgCl_2$-induced nephrotoxicity (Fouda *et al*. 2008). In Fouda's analysis in rats, TQ at 10 mg/kg had a protective effect against doxorubicin-initiated cardio toxicity (Nagi and Mansour 2000) and nephropathy (Badary *et al*. 2000). The LD50 of TQ is 90.3 mg/kg 9 (Mansour *et al*. 2001). Infusion of nephrotoxic measurements of $HgCl_2$ into rats brings on quick augmentation of the biomarkers of oxidative anxiety related with checked renal cell damage.

Uses and Applications of TQ

Classical applications

Traditional uses of TQ-rich plant parts: Development of dark seed (also known as black seed, from Nigella) has been followed back over 3,000 years to the kingdom of the Assyrians and old Egyptians. A container of TQ-rich, dark cumin oil was found in the tomb of King Tutankhamun, maybe to ensure the ruler in eternity. Dark cumin was an imperative fixing in numerous Egyptian dishes. Doctors of the pharaohs made seed decoctions for stomach ailments after extravagant dining experiences and as a treatment for colds, cerebral pains, toothaches, and contaminations. Ruler Nefertiti, applauded for her impeccable appearance, was an enthusiastic user of dark seed oil. Dark cumin and its oil have been qutilized to cleanse parasites and worms, detoxify, enhanced amoebic diarrhea, shigellosis, abscesses, tumors, ulcers of the mouth and rhinitis. Recent research affirms these uses for treatment of humans, dogs, horses and cats (Dwivedi 1999; Zaid *et al*. 2012; Aftab *et al*. 2018).

N. sativa seeds are considered as bitter, sharp, aromatic, diuretic, emmenagogue, galactagogue, anthelmintic, acrid, thermogenic, carminative, anodyne, deodorant, sudorific, expectorant, purgative and abortifacient. In addition, seed oil is used as local anesthetic. Traditionally the seeds and oil of *N. sativa* have been used to treat several ailments such as ascites, cough, jaundice, hydrophobia, fever, paralysis, conjunctivitis, piles, skin diseases, anorexia, dyspepsia, flatulence, abdominal disorders, diarrhea, dysentery, intrinsic hemorrhage and amenorrhea (Aftab *et al.* 2018).

Folk medicines of TQ-rich plant parts: In spite of the fact that its part in Egyptian culture is obscure, it is understood that things buried with a pharaoh were deliberately chosen to help him in the hereafter (Padhye *et al*. 2008). The Islamic prophet Muhammad (S.A.W.) once stated that death is the only disease that black seed can't heal. In The Canon of Medicine, Avicenna tells that Nigella as stimulates the body's energy and helps recovery from fatigue and dispiritedness (Aftab *et al*. 2018). Moreover, due its healing properties it is also included in the list of natural drugs of 'Tibb-e-Nabavi', or "Medicine of the Prophet (Muhammad)". In the Unani Tibb system of medicine, *N. sativa* and its components were very valuable in treating different diseases such as asthma, bronchitis, rheumatism,

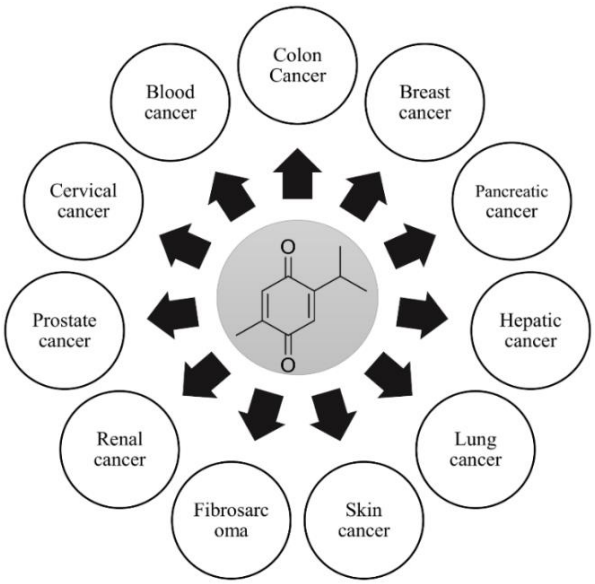

Fig. 7: Anti-cancer property of TQ

jaundice, gastrointestinal problems, anorexia, conjunctivitis, dyspepsia, rheumatism, diabetes, hypertension, intrinsic hemorrhage, paralysis, amenorrhea, anorexia and related inflammatory diseases, to increase milk production in nursing mothers, to promote digestion and to fight parasitic infections. Traditionally it was used in East and Asian countries and used by Indian and Arabian civilization as food as well in their medicines (Warrier and Nambiar 1993).

Traditional disease prevention and control

Cancer treatment: TQ has been found to be effective against various types of cancer (Fig. 7). TQ showed an anti-proliferative effect in myoblastic leukemia in human HL-60 cells (El-Mahdy *et al*. 2005). Various derivatives of TQ induce apoptosis (Effenberger *et al*. 2010). Alcoholic and aqueous extracts of TQ help to deactivate breast cancer cells (Farah and Begum 2003) TQ was found to inactivate MCF-7 breast cancer cells *in vitro* and reduce the effect of DMBA (7,12-di-methylbenz(a) anthracene) in mammary carcinoma of rats in combination with melatonin and retinoic acid (El-Aziz *et al.* 2005). It was also reported that TQ was tested in MCF-7/Topo breast carcinoma and resulted in the death of cells by apoptosis (Dastergi *et al*. 2016).

TQ is quite effective against cancer cells as an antineoplastic and pro-apoptotic agent against colon cell line HCT116 (Gali-Muhtasib *et al*. 2004). The oil extracted from *N. sativa* is effective against colon carcinogenesis. Volatile oil from *N. sativa* seeds has been effective in inhibiting colon cancer in post initiation stage without any adverse effects in rats (Salim *et al*. 2013). TQ can also be used as a chemotherapeutic agent on SW-625 colon cancer cells. TQ causes apoptosis and represses proliferation in pancreatic cancer cells (Norwood *et al*. 2006; Chehl *et al*. 2009). TQ also acts as novel inhibitor of pro-inflammatory pathways (Norwood *et al*. 2006). TQ (5 mg/mL) has been found to inhibit DNA synthesis almost 88% on human HepG2 cell line after 2 h of incubation with different concentrations (Thabrew *et al*. 2005). Oral treatment of TQ (1, 2, 4 mg/kg) was effective in increasing different activities and makes it a promising agent against chemical carcinogenesis and toxicity in hepatic cancer (Nagi and Almakki 2009). Supplementation with bee honey and *N. sativa* has a defensive impact against MNU (methylnitrosourea)-induced oxidative stress and cancer development in rats (Mabrouk *et al*. 2002). It was revealed that α-hederin and TQ improve neither cytotoxicity nor apoptosis in A549 (lung) or HEp-2 (larynx epidermoid) cancer cell lines (Rooney and Ryan 2005). TQ methanolic extract was found to inhibit skin cancer development in mice. Intraperitoneal administration of the extract (100 mg/kg) for 30 d, following subcutaneous administration of 20-methylcholanthrene (MCA), delimited soft tissue sarcomas to 33.3% compared with 100% in MCA-treated controls (Salomi *et al*. 1991). Before and after the treatment of methycholanthrene at 0.01% in drinking water, TQ inhibited skin carcinogenesis in mice which was also called as fibro sarcoma and tumor burden by 43 and 34%. TQ postponed the onset of methycholanthrene-induced fibro sarcoma tumors. TQ also inhibited the survival of fibro sarcoma cells with an IC50 of 15 m*M* and decreased the fibrinolytic potential of the human fibro sarcoma *in vitro* cell line *i.e*., HT1080 (Awad 2005).

Literature has shown the anti-proliferative, apoptotic and anti-invasive properties of TQ in a cervical cancer (HeLa) cell line (Shafi *et al*. 2009). TQ are capable of reducing human epithelial cervical cancer by inducing apoptosis, and this was confirmed in different solvents such as methanol, n-hexane and chloroform (Sakalar *et al.* 2013) TQ has also been found effective against renal cancer. It reduces renal oxidative stress, renal carcinogenesis and hyper-proliferative response in ferric nitrilotriacetate-treated rats (Khan and Sultana 2005). Rats were orally treated with extracts of *N. sativa* and a decrease of hydrogen peroxide production, DNA synthesis and incidence of cancer were observed. TQ inhibited DNA synthesis, expansion of harmful (LNCaP, C4-B, DU145, and PC-3) yet not non-harmful (BPH-1) prostate epithelial cells by down-directing AR (androgen receptor) and transcription factor E2F-1 (Kaseb *et al.* 2007). TQ blocks angiogenesis and represses human prostate tumor development at low doses with no toxic symptoms (Khan and Sultana 2005).

Mechanism of action against cancer: Thymoquinone works in cancer prevention through different pathways like AKT pathway PI3K-Akt signaling pathway is a signal transduction pathway that promotes survival and growth in response to extracellular signals. Activated Akt mediates downstream responses, including cell survival, growth, proliferation, cell migration and angiogenesis, by phosphorylating a range of intracellular proteins. The

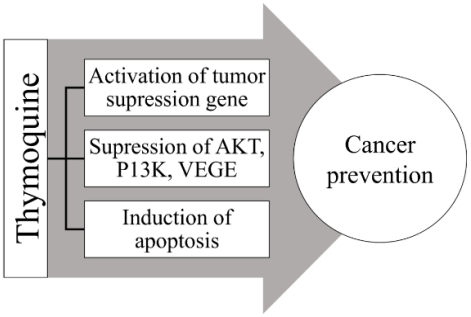

Fig. 8: Role of thymoquinone in prevention of cancer

pathway is present in all cells of higher eukaryotes and is highly conserved. The pathway is highly regulated by multiple mechanisms, often involving cross-talk with other signaling pathways. Problems with PI3K-Akt pathway regulation can lead to an increase in signaling activity. This has been linked to a range of diseases such as cancer. The Akt-PI3K pathway is essential for cell survival as activated Akt influences many factors involved in apoptosis, either by transcription regulation or direct phosphorylation. Akt inhibits transcription factors that promote the expression of cell death genes and enhances transcription of anti-apoptotic genes (Fig. 8).

Antiviral treatment: TQ have been investigated to improve helper T cell and suppressor T cell ratio and enhance natural killer cell activity (El-Kadi and Kandil 1986). They also have an inhibitory effect on human immune deficiency virus (HIV) protease (Chandra *et al*. 2009). A study reported that TQ extract is an effective remedy against murine cytomegalovirus (Salem 2005).

Epilepsy: Constituents of *N. sativa*, especially TQ, are well known for their anticonvulsive properties. TQ was given as a treatment for epileptic children in 2007 who did not respond well to drug treatment; it was found that water extract of *N. sativa* (40 mg/kg/8 h) reduced seizure activity (Akhondian *et al*. 2007).

Hypertension: TQ has been shown to function effectively in lowering blood pressure with dietary use of 100–200 mg of *N. sativa* water extract for almost two months, twice a day (Dehkordi and Kamkhah 2008). It had a blood pressure lowering effect in mild hypertension facing patients.

Depression and anxiety: TQ proved very helpful in treating variety of nervous disorders which decrease anxiety and improve cognition in adolescent human males. It also decreases the activity of the central nervous system and shows anti-depressant and anti-fatigue effect when it was tested in mice (Yimer *et al*. 2019).

Asthma: TQ was observed to be anti-asthmatic when injected intravenously in a guinea pig model of asthma against fluticasone standard drug (Keyhanmanesh *et al*. 2010). Certain clinical studies of *N. sativa* in patients have reported a potential efficacy on asthma outcomes and biomarkers and *its* treatment might be beneficial in lung injury and have potential clinical use (Kanter 2009). The prophylactic effect of boiled extract of *N. sativa* on asthmatic disease was examined (Boskabady *et al*. 2008).

Acute tonsillopharyngitis: Acute tonsillopharyngitis includes tonsil or pharyngeal inflammation. A combination of *N. sativa* and *Phyllanthus niruri* L. extract, in which the presence of TQ was confirmed, significantly alleviate throat pain and reduce the need for pain-killers (Dirjomuljono *et al*. 2008). The protective effect of thymoquinone on tracheal responsiveness and lung inflammation has been observed (Boskabady *et al*. 2008). The patients with tonsillopharyngitis, indicated that the capsules containing 360 mg of *N. sativa* and 50 mg of *P. niruri* extracts, as compared with a placebo, if given three times a day for 7 days to patients with acute tonsillopharyngitis, could significantly alleviate the symptoms of the disease due to their anti-inflammatory and immuno-modulatory effects (Dirjomuljono *et al.* 2008). It has been noticed that nasal drops of *N. sativa* oil, in comparison with nasal drops of ordinary food oil, could significantly improve the symptoms of acute tonsillopharryngitis patients, as well as their ability to tolerate exposure to allergens (Alsamarai *et al.* 2008).

Chemical weapons injury and radiation damage: It has been found that in patients injured by chemical weapons, boiled water extract of *N. sativa* decreases respiratory symptoms, chest wheezing and also reduces the need for drug treatment (Omran 2014). TQ defends the brain tissue from radiation-induced nitrosative stress (Ahlatci *et al*. 2014).

Post-surgical adhesions prevention: It is generally considered that some people are more prone to develop postoperative adhesions than are others. Unfortunately, there is no available marker to predict the occurrence or the extent and severity of adhesions preoperatively (Alpay *et al*. 2008). TQ prevents post-surgical adhesions. It was shown that covering the peritoneal surfaces with *N. sativa* extracted oil after injury is effective in lowering peritoneal adhesion formation (Sahbaz *et al*. 2014). The inflammatory system, the fibrinolytic system and extracellular matrix deposition of *N. sativa* with remodeling are three intertwined host processes that cause adhesion development. Covering peritoneal surfaces with *N. Sativa* oil after peritoneal trauma is effective in decreasing peritoneal adhesion formation (Sahbaz *et al*. 2014). Thymoquinone extract seems to have a possible effect in the prevention of post-surgical adhesion. This may occur by its effect in decreasing collagen formation and by decreasing apoptosis in the injured tissues. It has been reported that combined use of *N. sativa* oil with seprafilm may increase the adhesion preventive effect of seprafilm (Ebrahim *et al*. 2019).

Psoriasis: Psoriasis is a chronic life-long inflammatory disease that primarily affects the skin, musculoskeletal system, the gastrointestinal system and the eye (Dwarampudi *et al*. 2012). The ethanolic extract of *N. sativa* seeds produced significant differentiation in epidermis as seen from its degree of orthokeratosis. This extracts of *N. sativa* exhibit 95% anti-psoriatic activity, especially due to TQ, consistent with its use in traditional medicine (Dwarampudi

et al. 2012). Topical use of black seed oil strongly inhibited IMQ-induced psoriasis-like inflammation and alleviated all epidermal and dermal changes, thus black seed oil can be used as an adjuvant topical therapy for treating psoriasis (Okasha *et al.* 2018). Extract from *N. sativa* has been found to have antiproliferative, antiosteoporotic, effects in many studies. The major active ingredient, thymoquinone, has been demonstrated to have comparable results with topical hydrocortisone 2.5% when applied topically on carrageenan-induced paw edema, manifested by a notable decrease in leukocyte count and tumor necrosis factor-α concentration in inflamed area (Rida and Gladman 2020).

Brain pathology associated with Parkinson disease: TQ extracted from *N. sativa* protects against alpha synuclein (αSN)-induced synapse damage, impairment observed in the brains of patients with Parkinson's disease and dementia with Lewy bodies (Alhebshi *et al*. 2014).

Osteoporosis: The mechanism involved in the treatment of osteoporosis is unclear; however it was proposed that the antioxidant tendency of *N. sativa* and TQ has a potential pharmacological effect to treat osteoporosis (Shuid *et al*. 2012). In patients with diabetes mellitus, osteoporosis is the most important metabolic bone disease that is anciently treated by using *N. sativa.* Diabetes could affect the bone of patients through multiple mechanisms such as insulin deficiency, insulin resistance, hyperglycemia, or atherosclerosis (Altan 2007). In a study, the *N. sativa* was more effective in reversing the osteoporotic changes and improving the bone strength. *N. sativa* and thymoquinone have highlighted two properties that may be responsible for their effects against osteoporosis, that is, antoxidative and anti-inflammatory properties (Okazaki 2011). TQ is a potent antioxidant, it is expected that it may be able to protect bone against osteoporosis due to oxidative stress. It is most effective in scavenging superoxides, the reactive oxygen species which plays an important role in the activation of osteoclasts (Basu *et al.* 2001).

Wound healing: *N. sativa* seeds and its extracts possess healing properties in farm animals (Ahmed *et al*. 1995). When extracts of *N. sativa* were applied on the skin of mice infected with S*taphylococcus aureus*, it resulted in increased healing by decreasing white blood cell count, infection, inflammation and repairing of tissues (Abu-Al-Basal 2011).

Prevention of kidney disorders: Traditionally, *N. sativa* seeds were used for kidney stones prevention and treatment. Its oil was very effective in curing gentamycin kidney toxicity and has protective action against kidney injury; *i.e*., ischemia. TQ extracted from *N. sativa* when injected in rats confirmed its anti-kidney stone properties (Hayatdavoudi *et al*. 2016).

Non-traditional uses

Cosmetic applications: Extracted seed cakes of *N. sativa* were manufactured and tested to check their effect on various skin problems. The extracts were found to lower skin irritation, improve skin hydration and to act as an epidermal function barrier. They have potential applications as mitigating, moisturizing, anti-aging and protective cosmetics due to their antioxidant and anti-inflammatory activities (Amin *et al*. 2010).

Digestion promoter: TQ has been helpful to stop vomiting in humans while a tincture was prepared from it to cure indigestion, loss of appetite and diarrhea (Ahmed *et al*. 1995). Both *N. sativa* and thymoquinoneQ can partly protect gastric mucosa from acute alcohol-induced mucosal injury and these gastroprotective effects might be induced (Zaoui *et al*. 2000). The protective effect of thymoquinone against ethanol induced ulcer may be explained by different mechanisms. An increase in glutathione level caused a decrease in the ethanol induced gastric damage (Shoaib and Shafiq 2004). It has been observed that when *N. sativa* is given as single oral dose, cumin exerts a lowering effect on pancreatic lipase, amylase, trypsin, and chymotrypsin. Among the terminal digestive enzymes, a small intestinal maltase activity was significantly higher in animals fed with cumin, whereas lactase and sucrose were unaffected (Krishnapura 2018).

Antimicrobial roles of TQ

Fights infections antibacterial activity: *N. sativa* extract represses the bacterial activity; e.g. *Staphylococcus aureus. N. sativa* seeds possess antibacterial potential against *Helicobacter pylori* and are effective against isolates of methicillin-resistant *S. aureus* (Emeka *et al*. 2015). Gram negative isolates were more effective than Gram positive isolates. It was found that TQ prevents the formation of biofilm. Also possess anti-eicosanoid and antioxidant activity while the antioxidant action of the TQ and its 5-lipoxygenase inhibition may explain its anti-inflammatory effect (El-Dakhakhny *et al*. 2002).

Anti-fungal activity: The oil and extracts *N. sativa* and TQ, THQ and thymol, showed inhibitory effect against pathogenic yeasts, dermatophytes, non-dermatophytic filamentous fungi and aflatoxin-producing fungi (Shokri 2016). The strongest antifungal effect against *Candida albicans* was shown by methanolic extract of TQ while no antifungal activity was found for the water extract (Bita *et al.* 2012). Literature showed that *N. sativa* seeds were effective against aflatoxicosis and mycosis.

Conclusion

TQ is the principal constituent of *N. sativa*, *M. fistulos* and *S. montana* and has a wide spectrum of medicinal effects. Several therapeutic properties have been attributed to TQ including anti-cancer, anti-inflammatory, antioxidant, antimicrobial and cardio protective, being the anti-cancer property the most studied. Further clinical research is required to support the TQ, and medicinal plants rich in TQ, usage as a treatment of wide range diseases.

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Author Contributions

AA worked on natural biosynthesis and genes/ enzymes involved in pathway, ZY worked on introduction, HBS worked on discovery of TQ, NR worked on mode of action, AY worked on biological applications, MR also worked on biological applications and AJ helped in writing and managing the article.

Conflict of Interest

There is no conflict of interest among the authors and institutions where the work has been done.

Data Availability Declaration

All data reported in this article are available with the corresponding authors and can be produced on demand.

Ethics Approval

Not applicable.

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