Effect of *Nigella sativa* seeds on Cervical Cancer

Suhas Ballal
Assistant professor, Department of Chemistry,
School of Sciences, B-II, Jain (Deemed to be University), JC Road, Bangalore-560027
Email Id: b.suhas@jainuniversity.ac.in

ABSTRACT: ***Nigella sativa is a widely used medicinal plant throughout the world. It is an annual flowering plant in the family Ranunculaceae. Nigella sativa is a small black seed that has been used for centuries in herbal medicines. It is sometimes used to treat certain health conditions including asthma, bronchitis, and inflammation, and has long been used as a spice and food preservative. Nigella sativa has the promising properties including anticancer and chemo-sensitizing peculiarities and most significantly for the treatment of cervical cancer. The cervical cancer is North America's second most common form of gynecological tumours. The majority of chemotherapeutic agents used by oncologists have been shown to kill both cancer cells and healthy ones. Nevertheless, its beneficial impact can outweigh the side effects. For decades, traditional herbal practitioners used Nigella sativa seeds, also referred to as black seeds or black cumin, to treat inflammation, illnesses, and cancers. Thymoquinone (TQ) is the principle active constituent of black seed (Nigella sativa) essential oil which demonstrates in vitro and in vivo anti-neoplastic activities in different tumour cell lines. TQ's anticancer capacity is achieved across many aspects; including fostering apoptosis, cell cycle arrest and producing ROS. Additionally, it strengthens the immune system and reduces the side effects of conventional anticancer therapy. In this paper, the potential mechanism of Nigella sativa seeds having the active constituent Thymoquinone (TQ) acts in a way to combat the cervical cancer.***

KEYWORDS: ***Cervical cancer, Chemo-sensitizing peculiarities, Medicinal plant, Nigella sativa, Thymoquinone (TQ).***

INTRODUCTION

Cancer is a global disease that results from the conversion of normal cells into tumour cells by addressing multiple stages from pre-cancer to malignant status. Cancer is considered the world's second-largest cause of death, accounting for 8.8 million deaths in 2015. Near to one in six deaths from cancer. Close to 70 percent of cancer mortality occurs in low-and middle-income countries. Therefore, the treatment of cancer is a worldwide health priority. Among all the cancer types, cervical cancer is one of the most common gynaecologic malignant tumours, which accounts for about 15% of all cancer-related deaths [1]. Nearly 500,000 women have been estimated to die each year from cervical cancer worldwide, of which > 80 percent of death occurs in developing countries, where mortality from this disease is the highest among deaths caused due to the neoplasm. There are an estimated 13,000 new cases of cervical cancer per year in the United States and 50,000 with advanced precancerous conditions. After the breast cancer, cervical cancer is the second most common cause of cancer in women [2].

The first edition of the National Comprehensive Cancer Network (NCCN) in 2009 prescribes in detail the specific therapeutic methods of cervical cancer in various clinical stages which have been widely recognized in China [3]. Among cervical cancer patients, the radical hysterectomy-based surgery approach is the first-line treatment during the early stage, and concomitant platinum-based chemo-radiotherapy remains a curative treatment among advanced cervical cancer at the local level, particularly for remote control of the disease. However, its non-specific cytotoxicity and drug resistance limit the chemotherapeutic strategies. Analysis to discover new and successful anti-cancer agents has therefore gained special attention [4].

Bioactive components of natural plants have recently inspired researchers to study their anticancer behaviour as they showed virtually no toxicity, inexpensive alongside their ingestive availability. About 25% of medicinal products sold over the past two decades are derived directly from plants, but another 25% are chemically modified natural products [5].

Therapeutic interventions for the diagnosis of invasive cervical cancer usually include surgery, radiotherapy and chemotherapy [6]. Both methods, however, have some negative side effects due to the drug's non-specific cytotoxicity and treatment resistance, which pose a major problem in the management of cervical cancer. Additionally, all of these therapies demonstrate very minimal anti-cancer efficacy. Current studies are searching for potent, safe and reliable anticancer agents to overcome resistance and minimize side effects; at present, many herbal therapies are approved for cancer treatment as they have fewer side effects than traditional therapies and are comparatively fewer costly. Most medicinal plants are used in folk and herbal medicines, and their biologically active derivatives are increasingly being used for anticancer

activities in clinical trials and provide alternatives in cancer therapy. According to a researcher who has demonstrated that the extracts of a particular herbal composition extract which he had used consist of some cytotoxic effects on human cervical cancer cell lines and another herbal composition extract consist of the anti-proliferative activities on cervical cancer cell lines [7].

Black seed is an annual flowering plant belonging to the Ranunculaceae family, also known as black cumin or *Nigella sativa* [8]. This plant was widely cultivated and grown on boarding countries situated in the Mediterranean Sea. It has a rich religious and historical history as a miracle cure for health promotion and the treatment of many diseases in various cultures [9]. In addition, *Nigella sativa* seeds have been commonly used as a natural and miraculous feed additive in Arab and Islamic communities and have become a lifestyle treating almost all diseases. Black seeds are rich in a range of nutrients including amino acids, carbohydrates, proteins, essential fatty acids, blunt fibers and minerals (sodium, calcium, zinc, iron, magnesium and potassium) alongside saponins and alkaloids. Several studies have shown that the biological influences of *Nigella sativa* seeds are mainly referred to as thymoquinone (TQ), the key bioactive component of which has been studied intensively in vitro and in vivo for its beneficial effects [10].

The biological activity of *Nigella sativa* seed is linked to the main active components, in short, thymoquinone or TQ, a crystalline substance isolated from essential oil and known to be the main component of essential oil. TQ was known to be an active, anti-carcinogenic, antioxidant and anti-mutagenic drug [11]. TQ has been demonstrated to exert anti-neoplastic effects in vitro and in vivo. TQ's growth-inhibitory effects are unique to cancer cells that contribute to changes in the therapeutic index while being less toxic to and preventing normal non-tumour cells from sustaining damage caused by chemotherapy. With respect to cervical cancer cells, one of the researchers has found that selenomethione induced cell damage to Siha cells in combination with estrogen, lycopene, and TQ as evidenced by decreased proliferation rate. An ethanol extract from *Nigella sativa* has been found to inhibit proliferation and induce apoptosis in the HeLa cell lines of human cervical cancer.

Research have shown thymoquinone (2-methyl-5-isopropyl-1,4-benzoquinone, TQ) to be the main phytochemical compound behind the medicinal properties of black cumin [12]. TQ has been reported to target a large number of carcinogenic signalling pathways in different cancers, and is therefore considered a promising molecule for anticancer. EMT-inducing transcription factors (EMT-TFs) such as Twist1, Snail1, Slug, and Zeb1 plays an important role in cancer metastasis, being directly or indirectly involved in cancer cell metastasis via various signalling cascades, hence the modulation of EMT-TFs may be an fascinating possible strategy in cancer therapy. Recent evidence supports that TQ aims to control metastasis in breast cancers through EMT-TFs. However little is known about this in cervical cancer cells, so to further explain this, we assessed the cytotoxicity and anti-metastatic activity of TQ treatment and its potential mechanisms of action through various EMT-TFs in cervical cancer cell lines such as CaSki and SiHa in the present study.

1. *Chemical Structure*:

Chemically, thymoquinone is 2-isopropyl-5-methyl-1, molecular formulated 4-benzoquinone: C10H12O2. Its body weight is 164.204 g / mol. as in Fig. 1.

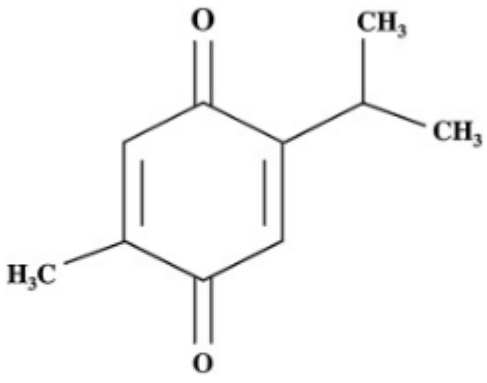

Fig.1: Chemical Structure of Thymoquinone (TQ)

2. *Anticancer Effect*:

Many studies have subjected TQ to the test for its cancer efficacy. TQ shows major antineoplastic effects on different forms of cancer including breast cancer, prostate cancer, stomach cancer, colon cancer, bladder cancer, lung cancer and bone cancer [13]. Research work on in vivo and in vitro has shown that TQ's anticancer activity is mediated by different modes of action. It activates various biological processes that are incriminated in proliferation, control of the cell cycle, apoptosis, angiogenesis, carcinogenesis and metastasis of cancer.

a) Antioxidant effect:

During normal cell respiration, reactive oxygen species (ROS) are released in cells along with xenobiotics in response. They are vigorously reactive and can destroy the cellular components including lipids, carbohydrates, proteins and nucleic acids and alter their functions in an oxidative way. Redox homeostasis is important for cell viability, proliferation and the function of the organ. On the hand of the latter, oxidative stress is imponderable between antioxidants and oxidants. This is considered a crucial factor in the pathogenesis of several pathological conditions, like diabetes, inflammation, cardiovascular diseases (CVDs), atherosclerosis, neurodegeneration, cancer and even aging [14]. Additionally, in addition to mutagenesis in DNA, oxidative stress is a causal factor for many alterations in cell function and structure, thereby creating cancer. Antioxidants may be critical in this regard to inhibiting the development of diseases like cancer.

There is a collection of phase-2 detoxification enzymes and antioxidants, which are known as cyto-protective proteins that provide the first-line protection against cellular damage caused by oxidative stress. One of the cytoprotective proteins is Heme oxygenase-1(HO-1), which catalyzes heme oxidative degradation rate limiting and first stage and provides protection against inflammation and carcinogenesis. This protein has been documented particularly in skin types for providing relief against cancer. HO-1 expression is induced primarily by the activation of the redox-sensitive transcription factor (NF)-erythorid2 (E2)-related factor-2 (Nrf2). The later factor interacts with the antioxidant response element (ARE) present at the HO-1 gene promoter region.

b) Anti-inflammatory and Immunomodulatory Effects:

Inflammation is a crucial physiological reaction of the tissue to deleterious stimuli which aim to maintain homeostasis of the tissue by removing stimuli followed by tissue repair. Persistent inflammation substantially contributes to disruption of cellular homeostasis through ROS production, impaired DNA repair and subsequent mutation, over-regulation of pro-inflammatory cytokines and reduced levels. Chronic inflammatory response has a crucial capacity in many diseases including cancer pathogenesis. Inflammation promotes cancer stages which include initiation, promotion, malignant conversion, invasion, and metastasis. In fact, it affects immune control and treatment responses. The key factors that increase the pathogenesis of inflammation-mediated cancer are nitric oxide (NO), protein-1 activator (AP-1), kappa nuclear factor (NF-πB), cyclooxygenase-2 (COX-2) and oxidative stress, as well as lipid peroxidation that impairs DNA repair [15].

Black seeds TQ are considered to possess both anti-inflammatory and immunomodulatory capacity that can regulate the progression of cancer, as many literatures have shown (Fig. 2). For these results through signalling channels are incriminated. TQ should down regulate the AP-1 and NF-ÿB. The down regulation of NF-κB interferes with pro-inflammatory and inflammatory mediators such as interleukin-1β, tumour necrosis factor alpha, COX-2, MMP-13 and PGE2. Also, black seeds TQ could inhibit interferon regulatory factor (IRF) signalling for pro-inflammatory cytokines production and cellular proliferation, thus regressing cancer development. Another immunomodulatory role of TQ, is that it could induce marked dose-dependent inhibition of the leukotrienes LTC4 and LTB4 in human granulocyte suspensions. This inhibition was done with the help of suppression 5-lipoxygenase activity. Furthermore, a researcher has added the immunomodulatory potentials of TQ in mice to further study the dermatitis model, which shows that the treatment of TQ in mice has reduced the number of total and differential leucocytes along with the down regulation of the expressional values of cytokines. Moreover, TQ has the ability to supress the condition of cellular inflammation and cancerous cell proliferation by modulating the peroxisome proliferator activated receptor gamma.

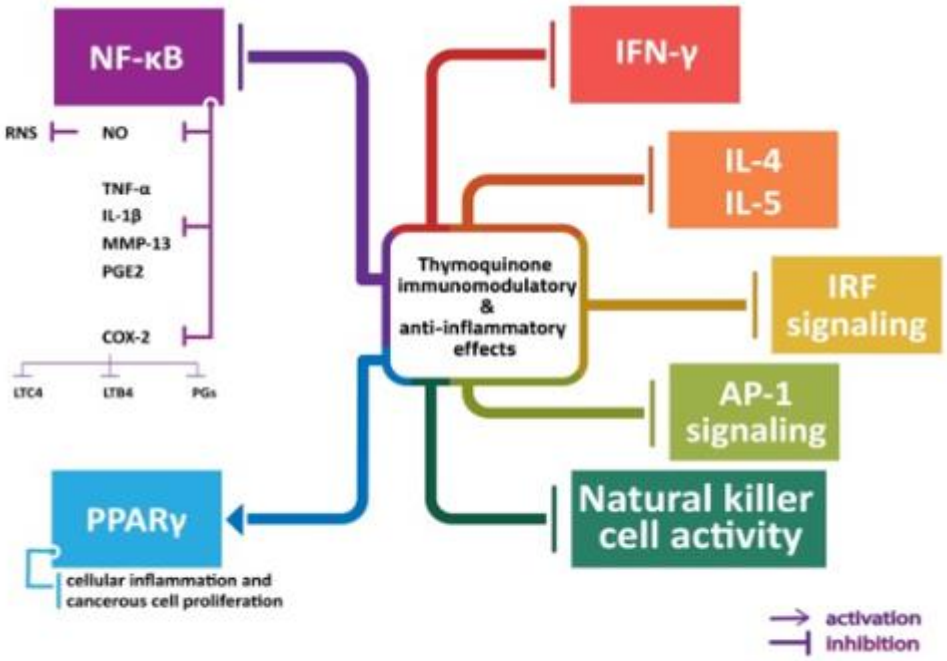

Fig.2: Thymoquinone immunomodulatory and anti-inflammatory effects

DISCUSSION

Cervical cancer is a neoplastic malignant disease which arises in the transitional zone between squamous and columnar cell epithelia. The studies have demonstrated that the infection caused due to the genital HPV is the major cause of the cervical cancer. However, the malignant tumours are caused by the HPV infections, along with the 70% of all cases of cervical carcinomas. The recent treatments for cervical cancer include surgery, chemotherapy and radiation therapy, but they usually cause the side effects to the patients such as recurrence of the cancer after the treatment, rectal dysfunction and vaginal cysts, etc. Epithelial-mesenchymal transition (EMT) plays a significant role in cancer metastasis. For EMT to be activated and perform its function in the cancer includes the transcription factors such as Twist1, Snail1, Slug and Zeb1. Cervical cancer is one of the major causes of the cancers in women, which has a high morbidity in China, however, its resistance to chemotherapy is a major obstacle for effective treatment worldwide. The features of EMT shows that it contributes to the chemo-resistance in cancer cells. The present paper elucidated the induction of black seeds which has the capacity of growth inhibition in cancer patients, by the effect of the Thymoquinone (TQ) on apoptosis induction, progression of cell cycle and DNA synthesis by cell lines for the effective treatment of the cervical cancer in patients.

Although, besides the ability of thymoquinone (TQ) interfering in the process to inhibit the growth of cancer cells in cancer cell lines, and various other methods of tumours and carcinogenesis, the experimental results demonstrates the possible support of TQ in cancer patients. Due to the *Nigella sativa* seeds immunotherapy in the patients, the cancer cell growth stops and thus, do not grow the cancer cells in the body, which shows the improvement in the activity of the NK cells and hence, proving the anticancer potential of TQ in combating the cervical cancer. One of the experimental studies have shown that the TQ improves the cancer cell markers in the affected patients in early one to two weeks by the dose administration of up to 2600 mg/day. Also, the researchers have suggested that the more increment in the dose of TQ gives the possible results in treating the patients with advanced malignancies.

Recently, many research studies have also demonstrated that the TQ is associated with the apoptosis with high levels of p53 expression levels in cancer cell lines with wild-type p53 gene. TQ induces the apoptosis by the activation of caspases-3, 8 and 9 into the cancer cell lines due to which the growth of the cancer cells stops. These caspases triggers the activation of the apoptosis in order to stop the growth of the cancer cells.

CONCLUSION

TQ's potent anticancer effect is mediated across many factors that have been controlled by different pathways. Those include: antioxidant, anti-inflammatory and immunomodulatory activity as well as cell cycle regulation, proliferation, and apoptosis. Numerous pathways and mechanisms regulate anti-proliferative and apoptotic effects of TQ such as; ROS generation, P53 pathway, STAT3 pathway, MAPK

pathway and Wnt pathway alongside angiogenesis inhibition and metastasis pathways. The results indicate that TQ inhibited markedly the proliferation of cervical cancer cells in a time-dependent and dose-dependent manner, and prevented cancer cell migration and invasion. Targeting EMT-TFs such as Twist1 and Zeb1 may be TQ's potential mechanism of action for regulating metastasis in cervical cancer. This paper indicates that TQ is a potential chemotherapeutic agent against cervical cancer but clinical studies are required to further develop and identify TQ as a medicinal drug.

REFERENCES

[1] B. W. Stewart and C. P. Wild, "World cancer report 2014," *World Heal. Organ.*, 2014.

[2] American Cancer Society, "Cancer Facts & Figures 2016," *Cancer Facts Fig. 2016*, 2016.

[3] J. Gu, C. Hao, X. Yan, and S. Xuan, "Applied analysis of ultrasound-guided ilioinguinal and iliohypogastric nerve blocks in the radical surgery of aged cervical cancer," *Oncol. Lett.*, 2017.

[4] M. Zhang, H. Zhang, Y. Yu, H. Huang, G. Li, and C. Xu, "Synergistic effects of a novel lipid-soluble extract from Pinellia pedatisecta schott and cisplatin on human cervical carcinoma cell lines through the regulation of DNA damage response signaling pathway," *Oncol. Lett.*, 2017.

[5] P. Juárez, "Plant-derived anticancer agents: a promising treatment for bone metastasis," *Bonekey Rep.*, 2014.

[6] M. Schwartz and L. Chuang, "Cervical cancer," in *Encyclopedia of Reproduction*, 2018.

[7] C. Klijn *et al.*, "A comprehensive transcriptional portrait of human cancer cell lines," *Nat. Biotechnol.*, 2015.

[8] W. Kooti, Z. Hasanzadeh-Noohi, N. Sharafi-Ahvazi, M. Asadi-Samani, and D. Ashtary-Larky, "Phytochemistry, pharmacology, and therapeutic uses of black seed (Nigella sativa)," *Chinese Journal of Natural Medicines*. 2016.

[9] H. M. A. Abdelrazek, O. E. Kilany, M. A. A. Muhammad, H. M. Tag, and A. M. Abdelazim, "Black seed thymoquinone improved insulin secretion, hepatic glycogen storage, and oxidative stress in streptozotocin-induced diabetic male Wistar rats," *Oxid. Med. Cell. Longev.*, 2018.

[10] S. S. Ebrahimi, S. Oryan, E. Izadpanah, and K. Hassanzadeh, "Thymoquinone exerts neuroprotective effect in animal model of Parkinson's disease," *Toxicol. Lett.*, 2017.

[11] H. Ijaz *et al.*, "Nigella sativa (Prophetic Medicine): A Review," *Pakistan Journal of Pharmaceutical Sciences*. 2017.

[12] M. A. Khan *et al.*, "Thymoquinone inhibits cancer metastasis by downregulating TWIST1 expression to reduce epithelial to mesenchymal transition," *Oncotarget*, 2015.

[13] O. H. Alobaedi, W. H. Talib, and I. A. Basheti, "Antitumor effect of thymoquinone combined with resveratrol on mice transplanted with breast cancer," *Asian Pac. J. Trop. Med.*, 2017.

[14] A. J. Kattoor, N. V. K. Pothineni, D. Palagiri, and J. L. Mehta, "Oxidative Stress in Atherosclerosis," *Current Atherosclerosis Reports*. 2017.

[15] S. I. Grivennikov, F. R. Greten, and M. Karin, "Immunity, Inflammation, and Cancer," *Cell*. 2010.