CHAPTER 1
INTRODUCTION TO CANCER AND BIOLOGICAL ACTIVITY OF THYMOQUINONE

1.1 Cancer – An Introduction

Cancer is a general term for more than 100 diseases characterized by the uncontrolled, abnormal growth of cells that can spread to other parts of body \(^1\). Cancer is frequently a chronic disease. It has been around for centuries. Occurrence of cancer dates back to ancient times and there have been evidences of cancer in skeletons of prehistoric animals and in Peruvian, Etruscan and Egyptian mummies. From the time of its discovery, people have tried to understand this dreadful disease and they have reported classification of cancer for better understanding of nature of this disease.

1.1.1 Classification of Cancer

Carcinoma — a cancerous tumor or lump, originating in the surface tissue of body organs. It is the most common form of cancer, accounting for 80\% to 90\% of cases \(^1\)
Sarcoma — a cancerous tumor originating in the bone, cartilage, muscle, fibrous connective tissue or fatty tissue \(^1\)
Myeloma — a cancerous tumor originating in the plasma cells of the bone marrow \(^1\)
Lymphoma — a cancerous tumor originating in the lymph system \(^1\)
Leukemia — cancer originating in the blood forming tissue \(^1\)

1.1.2 How Cancer Affects Us?

Cancer cells harm the body in different ways. They rob normal cells of nourishment or space. They give rise to a mass/tumor, which may eventually invade and destroy normal tissues. They can also metastasize (spread) into other parts of the body by traveling through bloodstream or lymphatic system. Cancer that is detected and treated before it has invaded adjacent organs or metastasized has the greatest possibility of being cured. The risk of cancer increases with growing age.
1.1.3 Causes of Cancer

Cancer is primarily an environmental disease with 90–95% of cases attributed to environmental factors and 5–10% due to genetics. Environmental, as used by cancer researchers, means any cause that is not inherited genetically. Common environmental factors that contribute to cancer death include tobacco (25–30%), diet and obesity (30–35%), infections (15–20%), radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity and environmental pollutants ¹.

1.2 Inflammation and Cancer

Inflammation comes from the Latin word *inflammation*, which means, “to set on fire”. Redness, heat, swelling, and pain are four classic hallmarks of inflammation. Inflammation can be described as body’s natural response to damaging stimuli and this response is accomplished by the transfer of plasma and leukocytes from blood to site of injury. This immune response is exhibited by the body in case of acute inflammation. In case of chronic inflammation, there is a gradual change in the type of cells at the site of inflammation where the healing is being carried out but tissue damage is occurring at the same time. These chronic inflammations are associated with cancer and tumor progression and many cancers arise from sites of chronic inflammation ². The inflammatory microenvironment with inflammatory cells in addition to network of signaling molecules plays significant role in malignant progression because of accumulation of infection-fighting agents leading to mutagenic changes. Various factors like inflammatory cells and growth regulators lead to angiogenesis and promote multiplication, invasion and metastasis of tumor cells ³. Transcription factors, nuclear factor kappa B (NF-κB) is a very important link between inflammation and cancer, which is activated in response to stress like pro-inflammatory cytokines, viruses, γ-radiation, lipopolysaccharide (LPS) or chemotherapeutic agents ⁴. NF-κB binds with DNA and it directs the transcription of antiapoptotic, pro-angiogenic and pro-invasion genes already known for promoting cancer ⁵, ⁶. Other factors such as tumor necrosis factor (TNF), chemokines (IL-8 or CXCL8) and interleukins (IL-1β and IL-6) play an important role in inflammation and cancer. Cytokine TNF, is mainly secreted by macrophages and it regulates immune cells. However, its deregulation may lead to diseases, including cancer and it is known to be responsible for NF-κB activation. TNF-receptor on the cell’s surface binds to TNF and this binding activates
a pathway that turns on IκBα kinase (IKK) \(^7\). Interleukins IL-1β, IL-6 and IL-8 (CXCL8) are known to have major contributions in inflammatory pathway for increasing expression of adhesion factors on endothelial cells for movement of leukocytes, responding to tissue damage and contributing towards human cancer progression and triggering angiogenesis \(^8\).

Thus cancer takes long period spanning over a decade or more to develop (Figure 1)\(^8\). It has been explained that tumor formation starts with a normal cell which undergoes transformation due to activation of proto-oncogenes and repression of tumor suppressor TP53 genes. Once the cell undergoes transformation it stops behaving as normal cell and shows the characteristics of a cancer cell. Proliferation of such cell is uncontrollable due to self-sufficiency in growth signals and non-responsive behaviour towards anti-growth signals.

![Figure 1: Roles of NF-κB-Mediated Inflammatory Pathway in Cellular Transformation](Xie Cytokine Growth Factor Rev 2001;12:375-391)
This fast growth leads to tumor formation, which is further accelerated through evasion to apoptotic signals. The process of transformation of a normal cell to a cancer cell along with uncontrolled multiplication and fudging of apoptosis requires around ten to twenty years. Angiogenesis leads to development of new blood vessels in the growing tumor and it receives nutrients and oxygen through blood supply. Thus it is able to sustain itself and in many cases it starts invading other tissues in the body which is known as metastasis. Once the cancer metastasizes; it becomes more lethal and difficult to cure since it is widely spread in various tissues.

1.3 Cancer Database Management in India

In India the cancer cases are increasing at alarming speed and according to an estimate the number of cases shall shoot up from \(9,79,786\) in 2010 to \(11,48,757\) in 2020. In year 2008, \(1,26,62,600\) new cases of cancer were reported all over the world and cancer related deaths were \(75,64,800\) worldwide. In year 2008 India had \(9,48,900\) new cases of cancer and there were \(6,33,500\) cancer related deaths in the same year in India. National Cancer Registry Planning (NCRP - http://www.ncrpindia.org/) was started by Indian Council of Medical Research (ICMR) with three Population Based Cancer Registries (PBCR) at Bangalore, Chennai and Mumbai and three Hospital Based Cancer Registries (HBCR) at Chandigarh, Dibrugarh and Thiruvananthapuram from 1st January 1982 (Figure 2). The PBCR have gradually expanded over the years and as of now there are 24 PBCR and 7 HBCR under the NCRP network all over India.

The main objectives of this programme are as follows:

1. To generate reliable data on the magnitude and patterns of cancer
2. To Undertake epidemiological studies based on results of registry data
3. To help in designing, planning, monitoring and evaluation of cancer control activities under the National Cancer Control Planning (NCCP)
4. To develop training programmes in cancer registration and epidemiology.

Collection of data and statistical analysis with respect to epidemiology has become possible due to this programme. The epidemiological studies are becoming easier with
increasing data and it will help the government to undertake concrete programmes to fight against growing challenges of cancer.

Tata Memorial Hospital in Mumbai has reported 3,59,994 positive cases till 2007 and Dikshit and colleagues reported 5,56,400 cancer related deaths in 2010 in India. These statistics have become a major concern in India and it has attracted many researchers to work in the field of cancer treatment.

![Figure 2: Location of Population Based Cancer Registries in India](http://www.ncrpindia.org/)

1.4 History of Cancer Chemotherapy

Among many challenges of drug discovery, anti cancer drug development has had a more controversial start and most struggled progress. Before 1950, the cancer treatment was largely bases upon removal of tumors by surgery. In the later stages, radiation therapy emerged as important tools for treatment and control of cancer. However, both surgery and
radiotherapy could not solve the problem of metastasis and recurrence of cancers. It is understood that chemotherapeutic agents reach organs and can treat cancer more effectively. Thus, efforts towards development of chemotherapeutic agents and immunomodulatory compounds gained momentum.

Nitrogen mustards were discovered before 60 years for effective treatment of cancer and led to an era of development of anticancer chemotherapies. This was followed by development of anti-leukemic drugs such as 6-mercaptopurine (6-MP) in the early 1950 through the works of George Hitchings and Gertrude Elion. Elion and colleagues have shown that small changes in a compound can yield moieties that can inhibit growth of tumor cells through inhibition of RNA and DNA synthesis.

![Figure 3: Progress in Cancer Chemotherapy](Devita et al. Cancer Res 2008;68:8643-8653)
Eli Lilly showed that *Vinca* alkaloids possess the ability to blocked proliferation of cancer cells. It was also shown that a combination of methotrexate (an antifolate), vincristine (a *Vinca* alkaloid), 6-mercaptopurine (6-MP) and prednisone, which together was referred to as the POMP regimen, could induce long-term remissions of acute lymphoblastic leukemia (ALL) in children suggesting the importance of combination for successful anticancer therapy. This approach was extended to the lymphomas in the late 1960’s and (Figure 3) found that nitrogen mustard, vincristine, procarbazine and prednisone, known as the MOPP regimen, could be successfully used in treatment of Hodgkin’s lymphoma and non-Hodgkin’s Lymphoma. Gordon Zubrod has discovered taxanes (1964) and camptothecins (1966) as anticancer agents. In 2001 genome sequencing was started and it gave new insights into understanding of genetic origin of cancer which led to target specific drug development.

Taxol a novel antimitotic compound exhibited promising anticancer activity, but proved difficult to synthesize and could only be isolated from the bark of the Pacific Yew tree. It forced the NCI into harvesting substantial quantities of yew trees from public lands. Taxol was found to be consistently effective for the treatment of ovarian cancer and became Bristol-Myers-Squibb’s first billion dollar drug. Another compound, Camptothecin, derived from a Chinese ornamental tree, was found to inhibit topoisomerase I in cancer cells. Although compound exhibited promising activity, it induced kidney toxicity. Consequently, a stable camptothecin analogue viz. irinotecan, got the approval of Food and Drug Administration (FDA) for the treatment of colon cancers, lung and ovarian cancers.
1.4.1 Progress in Anticancer Drug Development

In the last two decades, several novel compounds have emerged because of boost provided by progress in bioinformatics, molecular biology, genomic science, combinatorial design for chemical synthesis and new drug delivery systems, while recent development of microarray techniques opened a completely new field for developing new anticancer therapies. Several pharmaceutical companies developed numerous lead compounds for development of anticancer agents and which are being tested in preclinical and clinical trials (Table 1).

Table 1: Summary of Compounds Used Clinically for Treatment of Cancer and Drugs in Clinical Trials

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structures</th>
<th>Type of Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A] DNA Alkylating Agents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td><img src="image" alt="Cyclophosphamide" /></td>
<td>Acute Myeloid Leukemia, Bladder Cancer(^{24})</td>
</tr>
<tr>
<td>Chlorambucil</td>
<td><img src="image" alt="Chlorambucil" /></td>
<td>Non-Hodgkin Lymphoma, Trophoblastic Neoplasms and Ovarian Carcinoma(^{25})</td>
</tr>
<tr>
<td>Penclomedine</td>
<td><img src="image" alt="Penclomedine" /></td>
<td>Glioma(^{26})</td>
</tr>
<tr>
<td>Tauromustine</td>
<td><img src="image" alt="Tauromustine" /></td>
<td>Colorectal Cancer(^{27})</td>
</tr>
<tr>
<td>Fotemustine</td>
<td><img src="image" alt="Fotemustine" /></td>
<td>Melanoma and Brain Tumor(^{28})</td>
</tr>
</tbody>
</table>
## B] Platinum Derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td><img src="image" alt="Cisplatin Structure" /></td>
<td>Sarcomas, Small Cell Lung Cancer and Ovarian Cancer(^{30})</td>
</tr>
<tr>
<td>Oxaliplatin</td>
<td><img src="image" alt="Oxaliplatin Structure" /></td>
<td>Colorectal Cancer, Cisplatin Resistant Cancers(^{31})</td>
</tr>
<tr>
<td>ZD0473</td>
<td><img src="image" alt="ZD0473 Structure" /></td>
<td>Breast Cancer, Cisplatin Resistant Cancers(^{32})</td>
</tr>
</tbody>
</table>

## C] Topo-Isomerase Inhibitors

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topotecan</td>
<td><img src="image" alt="Topotecan Structure" /></td>
<td>Ovarian and Lung Cancer(^{33})</td>
</tr>
<tr>
<td>Irinotecan</td>
<td><img src="image" alt="Irinotecan Structure" /></td>
<td>Colon Cancer(^{34})</td>
</tr>
<tr>
<td>Etoposide</td>
<td><img src="image" alt="Etoposide Structure" /></td>
<td>Kaposi’s Sarcoma, Ewing’s Sarcoma, Lung Cancer, Leukemia Glioblastoma(^{35})</td>
</tr>
<tr>
<td><strong>D) Antifolates and Purine Nucleoside Analogs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Doxorubicin</strong></td>
<td><img src="image" alt="Doxorubicin molecule" /></td>
<td>Blood Cancer like Leukemia and Lymphoma&lt;sup&gt;36&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Busulfan</strong></td>
<td><img src="image" alt="Busulfan molecule" /></td>
<td>Chronic Myeloid Leukemia (CML)&lt;sup&gt;37&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Fludarabine</strong></td>
<td><img src="image" alt="Fludarabine molecule" /></td>
<td>Leukemia and Lymphomas&lt;sup&gt;38&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Floxuridine</strong></td>
<td><img src="image" alt="Floxuridine molecule" /></td>
<td>Colorectal Cancer&lt;sup&gt;39&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Methotrexate</strong></td>
<td><img src="image" alt="Methotrexate molecule" /></td>
<td>Breast, Head and Neck, Leukemia&lt;sup&gt;40&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Pemetrexed</strong></td>
<td><img src="image" alt="Pemetrexed molecule" /></td>
<td>NSCLC&lt;sup&gt;41&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>E) Kinase Inhibitors</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Imatinib</strong>&lt;br&gt;(Tyrosine kinase inhibitor)</td>
</tr>
</tbody>
</table>
| **Axitinib**  
| (Tyrosine kinase inhibitor) | ![Axitinib molecule] | Renal Cell Carcinoma<sup>43</sup> |
| **Bevacizumab**  
| (VEGF inhibitor) | Monoclonal antibody | Colorectal, Lung, Breast (outside USA), Glioblastoma (USA only)<sup>44</sup> |
| **Dasatinib**  
| (Tyrosine kinase inhibitor) | ![Dasatinib molecule] | CML, Prostate Cancer<sup>45</sup> |
| **Gefitinib**  
| (EGFR inhibitor) | ![Gefitinib molecule] | Non-Small Cell Lung Cancers (NSCLC)<sup>46</sup> |
| **Erlotinib**  
| (Tyrosine kinase inhibitor) | ![Erlotinib molecule] | NSCLC, Pancreatic Cancer<sup>47</sup> |

**Farnesyl Transferases Inhibitors**

| **BMS 214662** | ![BMS 214662 molecule] | Leukemia<sup>48</sup> |
| **Tipifarnib** | ![Tipifarnib molecule] | Breast Cancer, Metastatic Pancreatic Cancer, NSCLC<sup>49</sup> |
| **Lonafarnib** | ![Lonafarnib molecule] | Head and Neck Squamous Cell Carcinoma<sup>50</sup> |
**G| Histone Deacetylases Inhibitors**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Molecular Structure</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vorinostat</td>
<td>![Vorinostat Structure]</td>
<td>Cutaneous T cell Lymphoma&lt;sup&gt;51&lt;/sup&gt;</td>
</tr>
<tr>
<td>Romidepsin</td>
<td>![Romidepsin Structure]</td>
<td>Cutaneous T-cell Lymphoma (CTCL), Peripheral T-cell Lymphoma&lt;sup&gt;52&lt;/sup&gt;</td>
</tr>
<tr>
<td>Panobinostat</td>
<td>![Panobinostat Structure]</td>
<td>Hodgkin's Lymphoma, Cutaneous T cell Lymphoma&lt;sup&gt;53&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**H| Steroid Hormone Receptor Inhibitors**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Molecular Structure</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toremifene</td>
<td>![Toremifene Structure]</td>
<td>Advanced (metastatic) Breast Cancer, Prostate Cancer&lt;sup&gt;54&lt;/sup&gt;</td>
</tr>
<tr>
<td>Raloxifene</td>
<td>![Raloxifene Structure]</td>
<td>Breast Cancer&lt;sup&gt;55&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>![Tamoxifen Structure]</td>
<td>Hormone Receptor-Positive Breast Cancer&lt;sup&gt;56&lt;/sup&gt;</td>
</tr>
<tr>
<td>Flutamide</td>
<td>![Flutamide Structure]</td>
<td>Prostate Cancer&lt;sup&gt;57&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
1.4.2 Chemotherapy and Phytochemicals

Cancer is a fatal disease and attempts are being made for designing effective drug for its treatment. Even after exhaustive research on establishing cancer cell pathways, therapeutic target identification and anticancer drug development, cancer still remain second largest cause of death in the world. It was found that multiple pathways are involved in cancer growth, development and metastasis. Hence, even if we block one target by using selective inhibitors, cancer can still sustain and grow in the host. Anticancer drug resistance and recurrence after surgery is a major problem in front of patients, medical practitioners as well as researchers. Recent reports have suggested existence of cancer stem cells (CSCs) in several types of cancers. These CSCs have capacity of self-renewal and differentiation into the heterogeneous lineages of cancer cells which comprise of the whole tumor. These tumor-initiating cells provide a reservoir of cells that cause tumor recurrence after therapy. The invasive nature of CSCs has invited detailed investigations in understanding pathways responsible for generations of CSCs and explorers also started searching for naturally occurring or synthetic compounds, which may act as multi targeted inhibitors of these pathways. Current literature suggests that nature is a rich source of such multi targeted compounds known as phytochemicals including curcumin, resveratrol, genistein etc. that can target multiple pathways in cancer and also have ability to eliminate CSCs. Several clinical studies showed that people consuming natural products or diets containing these natural products are less prone to be affected by cancer. Hence, it is clear that these natural compounds can act as chemo-preventive agents against cancer. Their ability to target CSCs also makes natural products act against drug resistance and cancer metastasis.

The current cancer therapy includes single or multiple drug combinations to treat cancer, but none of the drugs offer complete cure. The cost and toxicity to vital organs of body make treatment more difficult for patients as well as for physicians. Cardiotoxicity, nephrotoxicity, neurotoxicity, hepatotoxicity etc are the major side effects associated with anticancer drugs. The toxicities caused by anticancer drugs have been described by various groups and they are summarized in Table 2. Phytotoxicity, TQ, genistein, tea polyphenols and Gallic acid are useful, which offer protection against toxicity induced by various anticancer drugs like cisplatin in animal models. Phytochemicals produce antioxidant, anti-inflammatory effects to reduce the toxicity of these drugs. Several studies
also suggested that phytochemicals are useful in sensitizing chemotherapeutic agents to resistant cancers. For example, TQ was found to sensitize gemcitabine resistant pancreatic cancer cell lines. Similar reports are available for many natural products including curcumin, tea polyphenols etc.

Hence, phytochemicals play an important role in treatment of cancer by providing multi targeted activity, protecting vital organs, chemosensitizing cell lines towards existent anticancer drugs, eliminate CSC and hence offer a potential candidature towards anticancer drug development.

**Table 2: Toxicity of Anticancer Drugs** 58, 59

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Name of Anticancer Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drugs Causing Bone Marrow Depression</strong></td>
<td>Carboplatin, Chlorambucil, Oxaliplatin, Cytarabine, FU, Vinorelbine, Topotecan, Mitoxantrone, Vinblastine, Melphelan, Oxaliplatin, Idarubicin, Irinotecan, Rituximab, Transtuzumab, Doxorubicin, Gemcitabine, Methotrexate, Paclitaxel, Hydroxyurea, Cyclophosphamide,</td>
</tr>
<tr>
<td><strong>Anticancer Drugs Causing Anaemia</strong></td>
<td>Cisplatin, Altretamine, Cytarabine, Docetaxel, Topotecan, Paclitaxel</td>
</tr>
<tr>
<td><strong>Drugs Causing Thrombocytopenia</strong></td>
<td>Dacarbazine, Carboplatine, 5-flouracil, Lomustine, Mitomycin,</td>
</tr>
<tr>
<td><strong>Drugs causing Stomatitis</strong></td>
<td>Doxorubicin, Dactinomycin, Mitomycin, Bleomycin Methotrexate, 5-Flouracil, Irinotecan Vincristine, Vinblastine, Etoposide</td>
</tr>
<tr>
<td><strong>Anticancer Drugs Causing Diarrhea</strong></td>
<td>Paclitaxel, Methotrexate, Floxuridine, Nitrosourea, Cytarabine</td>
</tr>
<tr>
<td><strong>Drugs Causing Hair Follicle Toxicity</strong></td>
<td>Doxorubicine, Daunorubicine, Paclitaxel, Ifosamide, Etoposide, Methotrexate, Cyclophosphamide, Vincristine</td>
</tr>
<tr>
<td><strong>Drugs Causing Peripheral Neuropathy</strong></td>
<td>Vincristine, Carboplatin, Vinblastine, Procarbazine, Vindesin, Paclitaxel</td>
</tr>
<tr>
<td><strong>Drugs Causing Hypersensitivity</strong></td>
<td>Carboplatin, Cisplatin, Cyclophosphamide, Cytarabin, Paclitaxel, Doxorubicin, Daunorubicin, Ifosamide,</td>
</tr>
</tbody>
</table>
Drugs Causing Hepatotoxicity
- Cyclophosphamide, Streptozocin, 5-Flurouracil, Methotrexate, 6-Mercaptopurine, Doxorubicin

Drugs Causing Renal Toxicity
- Cisplatin, Ifosamide, Mitomycin, Plicamycin

Drugs Causing Pulmonary Toxicity
- Bleomycin, Procarbazine, Carmustine, Mitomycin

Drugs Causing Cardiotoxicity
- Doxorubicin, cytarabine, Paclitaxel, vinca alkaloids, Imatinib, Trastuzumab

1.5 Role of Herbs and Spices against Cancer

All over the world spices are used in order to add flavor, taste, and nutritional value to food. In recent times, the importance of these spices has gone beyond food additives. These spices are now considered as rich source of large number of nutrients with pharmaceutical properties. There is enough evidence for spices as a source of chemicals towards lowering the risk of most chronic diseases and it underlines the use of spices not only as agents for prevention but also for treatment of diseases.

It is a well established fact that cancer is a complicated disease involving complex interactions between multiple signaling pathways and various target molecules. Strategy of developing a drug for single target – normally a gene, gene product or single pathway – has not yielded promising results so far because of complicated nature of cancer.

The World Cancer Research Foundation reported that 35% of the cancer cases worldwide are attributable to various lifestyle factors such as food, nutrition and physical activity. A comparison of United States and India shows that US has more cases of colorectal cancer. US had 356 colon cancer cases in 2000 and 139 reported deaths per million people. On the other hand India reported 40 cases of colon cancer with 26 deaths per million people. Lower incidence of colon cancer in India in comparison to Western countries cannot be fully explained, but as of today we know that lifestyle is considered as major contributor in 90-95% of cancers, ubiquitous use of various Indian spices (Figure 4) with large number of biologically active molecules as their constituents (Table 3) could be one of the reasons behind lower incidences of colon cancer in Indian population.
Figure 4: Spices Used as Food Additives in India and Other Countries \(^{65}\)
### Table 3: List of Common Spices and their Nutraceuticals

<table>
<thead>
<tr>
<th>Name of Spice</th>
<th>Important Constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allspice</td>
<td>Eugenol, methyl eugenol, myrcene, 1,8-cineol, α-phellandrene, quercetin, myricetin&lt;sup&gt;66&lt;/sup&gt;</td>
</tr>
<tr>
<td>Anise</td>
<td>Anethole, bergapten, estragole, anisaldehyde, α-himalchalene&lt;sup&gt;67&lt;/sup&gt;</td>
</tr>
<tr>
<td>Asafoetida</td>
<td>α-Pinene, phellandrenes, Farnesiferoles, hendecylsulphonyl acetic acid&lt;sup&gt;68&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basil</td>
<td>Ursolic acid, eugenol, caffeic acid, β-sitosterol, limonene, estragole, methyl eugenol, geraniol, 1,8-cineol, linalool, citral, methyl cinnamate&lt;sup&gt;69&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bay leaves</td>
<td>Linalool, α-terpinol, α-terpinyl acetate, thymol, caryophyllene, aromadendrene, β-selinene, farnesene, cadinene, methyl, eugenol, myrcene, eugenol&lt;sup&gt;70&lt;/sup&gt;</td>
</tr>
<tr>
<td>Black cumin</td>
<td>p-cymene, carvacrol, thymohydroquinone, thymoquinone, γ-terpinene and α-thujene&lt;sup&gt;71&lt;/sup&gt;</td>
</tr>
<tr>
<td>Black pepper</td>
<td>Piperine, β-caryophyllene, limonene, δ-3-carene, α-pinene, β-pinene, α-phellandrene, myrcene, terpinolene&lt;sup&gt;72&lt;/sup&gt;</td>
</tr>
<tr>
<td>Caraway</td>
<td>S-Carvone, germacrene D, limonene, dihydrocarveol, α-pinene, β-pinene, sabinene, perillyl alcohol, carveol&lt;sup&gt;73, 74&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cardamom</td>
<td>α-Terpinyl acetate, 1,8-cineol, limonene, linalool, linalyl acetate, terpinolene, myrcene&lt;sup&gt;75&lt;/sup&gt;</td>
</tr>
<tr>
<td>Celery seed</td>
<td>Apigenin, limonene, β-selinene, humulene, 3-butylphthalide, senkyunolide, α-pinene, β-pinene, myrcene, (Z)-β-ocimene, γ-terpinene, cis-allo-ocimene, (E)-β-farnesene, apiole, senkyunolide, neocnidilide&lt;sup&gt;76&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chervil</td>
<td>Estragole, apiin, hendecane (undecane)&lt;sup&gt;77, 78&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chives</td>
<td>Dipropyl disulfide, methyl pentyl disulfide, pentyl hydrodisulfidea, cis/trans 3,5-diethyl-1,2,4-trithiolanea, pentanethiol, diallyl sulfide&lt;sup&gt;79&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>Cinnamaldehyde, cinnamyl acetate, cineol, eugenol, coumarin, ethyl cinnamate, linalool, humulene, β-caryophyllene, τ-cadinol&lt;sup&gt;80&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cloves</td>
<td>Carvacrol, thymol, eugenol, cinnamaldehyde, eugenyl acetate&lt;sup&gt;81&lt;/sup&gt;</td>
</tr>
<tr>
<td>Coriander</td>
<td>Linalool, geraniol, geranyl acetate, camphor&lt;sup&gt;82&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plant</td>
<td>Chemical Constituents</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Cumin</td>
<td>Cuminaldehyde, γ-terpinene, β-pinene, p-cymene, p-mentha-1,3-diene-7-al, p-mentha-1,4-dien-7-al</td>
</tr>
<tr>
<td>Dill</td>
<td>Carvones, limonene, dillapiole, trans-dihydrocarvone, cis-dihydrocarvone, myristicin</td>
</tr>
<tr>
<td>Fennel</td>
<td>(E)-Anethole, limonene, fenchone, estragole, anisaldehyde, bergapten, β-sitosterol</td>
</tr>
<tr>
<td>Garlic</td>
<td>Ajoene, allicin, alliin, diallyl sulfide, diallyl disulfide, diallyl trisulfide, S-allylcysteine, methiin, isoalliin, cycloalliin, Sallyl mercaptocysteine</td>
</tr>
<tr>
<td>Ginger</td>
<td>[6]-Gingerol, [6]-paradol, shogoal, 6-gingerdiol, gingerdione, zingiberene, citral (neral and geranial), bisabolene, α-farnesene, β-phellandrene, cineol, zingerone</td>
</tr>
<tr>
<td>Horseradish</td>
<td>Sinigrin, allyl isothiocyanate, gluconasturtiin, phenylethyl isothiocyanate, quercetin, kaempferol</td>
</tr>
<tr>
<td>Marjoram</td>
<td>4-Terpinenol, (E)-sabinene hydrate, γ-terpinene, sabinene, β-pinene, limonene, β-phellandrene, (Z)-sabinene hydrate, terpinolene</td>
</tr>
<tr>
<td>Mint (spearmint)</td>
<td>Menthol, R-carvone, limonene, β-pinene, β-myrcene, trans-thujan-4-ol, dihydrocarvone, β-bourbonene, β-caryophyllene, epibicyclosesquiphellandrene</td>
</tr>
<tr>
<td>Mustard</td>
<td>Allyl isothiocyanate, sulforaphane</td>
</tr>
<tr>
<td>Nutmeg</td>
<td>Eugenol, myristicin, elemicin, sabinene, safrole, methyl eugenol, α-pinene, β-pinene, myristic acid, 4-terpineol</td>
</tr>
<tr>
<td>Onion</td>
<td>Quercetin, allicepinα, allyl propyl disulphide, protocatechuic acid, quercetin dimera, quercetin trimera</td>
</tr>
<tr>
<td>Oregano</td>
<td>Carvacrol, cis-sabinene hydrate, thymol, linalyl acetate, β-caryophyllene, 4-terpineol, α-terpineol, caffeic acid</td>
</tr>
<tr>
<td>Paprika</td>
<td>β-Carotene, α-, β-, and γ-tocopherols, canthaxanthin, capsaicin, dihydrocapsaicin</td>
</tr>
<tr>
<td>Parsley</td>
<td>p-1,3,8-Menthatriene, β-phellandrene, apiole, myrcene, myristicin, rutin, apigenin</td>
</tr>
<tr>
<td>Poppy seed</td>
<td>1-Pentanol, 1-hexanal, pentylfuran, caproic acid, linoleic acid, oleic acid,</td>
</tr>
<tr>
<td>Spice</td>
<td>Nutraceuticals</td>
</tr>
<tr>
<td>-------------</td>
<td>-------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Red pepper</td>
<td>Capsaicin, β-carotene, zeaxanthin, lutein, caffeic acid, capsanthin</td>
</tr>
<tr>
<td>Rosemary</td>
<td>Carnosol, rosmarinic acid, carnosic acid, α-pinene, camphor, limonene,</td>
</tr>
<tr>
<td></td>
<td>camphene, borneol, cineole, (Z)-linalool oxide, bornyl acetate</td>
</tr>
<tr>
<td>Saffron</td>
<td>Crocin, safranal, picrocrocin, crocetin, α- and β-carotene, lycopene,</td>
</tr>
<tr>
<td></td>
<td>zeaxanthin</td>
</tr>
<tr>
<td>Sage</td>
<td>1,8-Cineol, camphor, α-thujone, β-thujone, borneol, viridiflorol, manool,</td>
</tr>
<tr>
<td></td>
<td>humulene, β-caryophyllene</td>
</tr>
<tr>
<td>Savory</td>
<td>Carvacrol, α-pinene, γ-terpinene, 4-terpineol, α-terpineol, cadinene, τ-</td>
</tr>
<tr>
<td></td>
<td>cadinol, caryophyllene</td>
</tr>
<tr>
<td>Sesame seed</td>
<td>Sesamin, sesamolin, phytic acid, linoleic acid, oleic acid, β-sitosterol,</td>
</tr>
<tr>
<td></td>
<td>campesterol, stigmasterol, γ-tocopherol, Δ5-avenasterol, palmitic acid</td>
</tr>
<tr>
<td>Tamarind</td>
<td>Tartaric acid, limonene, geraniol, saffrole, cinnamic acid, ethyl cinnamate,</td>
</tr>
<tr>
<td></td>
<td>methyl salicylate, pyrazine, phenylacetaldehyde, 2-furfural, palmitic acid</td>
</tr>
<tr>
<td>Tarragon</td>
<td>(Z)-Anethole, (Z)-β-ocimene, (E)-β-ocimene, limonene, methyl eugenol,</td>
</tr>
<tr>
<td></td>
<td>camphor, cineol</td>
</tr>
<tr>
<td>Thyme</td>
<td>Thymol, carvacrol, p-cymene, γ-terpinene, linalool, borneol, β-caryophyllene,</td>
</tr>
<tr>
<td></td>
<td>caffeic acid, β-pinene, thymodihydroquinone</td>
</tr>
<tr>
<td>Turmeric</td>
<td>Curcumin, zingiberene, turmerone, γ-atlantone, β-sesquiphellandrene,</td>
</tr>
<tr>
<td></td>
<td>turmerol, bisabolone</td>
</tr>
<tr>
<td>Vanilla</td>
<td>Vanillin, ethyl vanillin, 4-hydroxybenzyl alcohol, vanillyl alcohol,</td>
</tr>
<tr>
<td></td>
<td>piperonal, ferulic acid, vanillic acid, 3,4-dihydroxybenzaldehyde, 4-</td>
</tr>
<tr>
<td></td>
<td>hydroxybenzoic acid, 4-hydroxybenzaldehyde, p-coumaric acid</td>
</tr>
<tr>
<td>White pepper</td>
<td>Piperine, β-caryophyllene, limonene, δ-3-carene, α-pinene, β-pinene, α-</td>
</tr>
<tr>
<td></td>
<td>phellandrene, myrcene, terpinolene</td>
</tr>
</tbody>
</table>

A comprehensive review by Sung and co-workers has discussed nutraceuticals obtained from many spices which are able to target multiple cell signaling pathways connected with tumorigenesis. Some of the major pathways shown to be affected by
nutraceuticals with their molecular targets are transcription factors (NF-κB, STAT3, AP-1, Nrf2, PPARγ) growth factors (VEGFR, EGFR, HER2, EGFR2, IGF-1R), protein kinases (PI3K, AMPK, HIF-1α, Bcr-abl, and Raf/Ras), inflammatory mediators (TNF-α, COX-2, 5-LOX, IL-1β, IL-6, IL-8, CRP, iNOS) and other targets (Proteasome, TWIST, CXCR4, adhesion molecules) involved in tumor progression (Figure 5).

![Figure 5: Spice Nutraceuticals Targeting Cell Signaling Pathways](image)

1.5.1 Nigella sativa against Cancer

*Nigella sativa* is an annual herb and its seeds are widely used as food additive. It is grown in various countries of Mediterranean region like Spain, Italy, Turkey, Syria, Israel, Egypt, Libya, Tunisia, Morocco and in countries from Southwest Asia like Saudi Arabia, Kuwait, Iraq, India, Pakistan and Afghanistan. It has been described in sacred texts with different names like Bible describes it as the ‘curative black cumin’ (Isaiah 28:25, 27, New King James Version) and it is described as Black seed with curative potential against all the
diseases (5686, Sahih Al-Bukhari). It is interesting to note that the seeds of Nigella sativa were found along with several articles from tomb of Egyptian Pharaoh Tutankhamen which signifies the use of these seeds in those times. The black cumin herb goes by many different names. In Arabic it is called ‘Habbatus Sawda’ (Black seeds) or ‘Habbatul Baraka’ translated as ‘Seeds of blessing’. Kalonji is the well known name for the seeds in India and Pakistan and in China it is known as Hak Jung Chou and it is described as Melanthion by Hippocrates and Dioscorides and as Gith by Pliny.

The plant belongs to Ranunculaceae family of flowering plants and 14 species have been reported which include Nigella nigellastrum, Nigella ciliaris, Nigella hispanica, Nigella integrifolia, Nigella damascene, Nigella oriental, Nigella arvensis and Nigella sativa.

The plant grows upto 20-30 cm and leaves are finely divided with thread like leaf segments. The flowers are white, pink, pale blue or pale purple, yellow with 5-10 petals. The fruit is a capsule with many follicles joined to each other, containing numerous seeds. These seeds are the most widely used part of the plant in alternative and folk medicines. They are primarily used as spice and added as food preservative. In many areas they are ingested with food or mixed with honey for variety of health problems and act as lactogogues, carminitative and antihelmnthetic agent.

1.5.2 Thymoquinone-Principle Constituent of Nigella sativa

2-methyl-5-isopropyl-1,4-benzoquinone 1 is an important constituent of oil obtained from seeds of Nigella sativa. It is commonly known as thymoquinone (TQ). Previous work by Ghosh and colleagues reports High Performance Liquid Chromatography (HPLC) analysis of the oil of Nigella sativa using the isocratic mobile phase of water-methanol-2-propanol (50:45:5%v/v) and the group reported concentration of different constituents in the oil including TQ and Thymohydroquinone.
Ashraf and co-workers showed that second fraction of *Nigella sativa* oil, obtained by Supercritical Fluid Extraction (SFE) contained 85.6% TQ and Soxhlet extraction gave 65.8% TQ when 15% diethylether in hexane was used as solvent for extraction\textsuperscript{120}. TQ is a redox-active benzoquinone and in Sörensen buffer: methanol (3:7, v/v; pH 8.5) shows a single, reversible peak at dropping mercury electrode at -0.095 V vs. Ag/AgCl electrode\textsuperscript{121}. This redox active nature of TQ probably defines its antioxidant property in biological system. The chemical reaction of TQ with GSH, NADH and NADPH has been reported by Khalife and Lupidi and the reactions under physiological conditions produced glutathione dihydrothymoquinone after reaction with GSH at a faster rate and dihydrothymoquinone (DHTQ) at a slower rate with NADH and NADPH, respectively\textsuperscript{122}.

1.5.3 Safety Profile (LD\textsubscript{50}) of TQ

Al-Ali and colleagues studied the effect of oral and intra peritoneal doses of TQ in mice and rats. They found LD\textsubscript{50} of TQ for intraperitoneal administration as 104.7 mg/kg and for oral ingestion LD\textsubscript{50} was 870.9 mg/kg in mice and for rats it was found to be 57.5 mg/kg and 794.3 mg/kg for intraperitoneal administration and oral ingestion respectively\textsuperscript{123}. It is important to note that TQ shows various biological activities like anti-oxidant, anti-inflammatory, anticancer, chemopreventive and chemosensitizing effects at 10 to 100 times lesser concentrations than that of its LD\textsubscript{50} values in almost all the studies. There are many reports on various biological activities of TQ or the extracts of black seed oil. The majority of reports is on anticancer activity and some groups have also explored anti-inflammatory, antioxidant, chemoprotective and chemosensitizing potential of TQ.

1.5.4 Anti-inflammatory and Chemopreventive Activity of TQ

The role of pro-inflammatory cytokines and reactive oxygen species (ROS) along with reactive nitrogen species (RNS) to create various patho-physiological disorders such as Crohn’s disease or ulcerative colitis during inflammation\textsuperscript{124-128} and the role of these species in colorectal adenocarcinoma\textsuperscript{129, 130} and gastric *Helicobacter pylori* infection\textsuperscript{131} has been well documented by various groups. Role of 5-lipoxygenase (5-LOX) in inflammatory pathway has been explored and established by many groups and the conversion of arachidonic acid to hydroxyeicosatetraenoic acids (HETE) or leukotrienes (LT) by 5-LOX leads to cell
proliferation and survival along with suppression of apoptosis. Thus inhibition of 5-LOX cascade can trigger apoptosis and this strategy can be adopted against proliferation of cancer cells. In human blood cells, TQ has been reported to inhibit the formation of leukotrienes in dose and time-dependent manner through down-regulation of 5-LOX at \( \text{IC}_{50}=3 \ \mu\text{M} \) and Leucotiriene-C4-synthase (LTC4-synthase) and LTB4 formation with \( \text{IC}_{50} \) values of 1.8 \( \mu\text{M} \) and 2.3 \( \mu\text{M} \) respectively. In the same study the authors showed that Staurosporine, which is an unselective protein kinase inhibitor, failed to prevent inhibition of LTC4 synthase activity induced by TQ which shows that TQ inhibits the formation of leukotrienes.

The chemoprotective action of TQ against acetic acid-induced colitis compared to sulfasalazine (500 mg/kg) control group is underlined by an investigation where rats pre-treated with oral TQ (10 mg/kg for 3 days) dose, were completely protected against colitis. TQ inhibited the production of 5-LOX products (\( \text{IC}_{50}=0.26 \text{ mg/ml} \)) and 5-HETE (\( \text{IC}_{50}=0.36 \text{ mg/ml} \)) in polymorphonuclear leukocytes from rats, which may be ascribed to its antioxidant potential. These observations explain the traditional use of *Nigella sativa* oil for improving inflammatory conditions in various folk medicinal systems. El-Gazzar et al. reported that TQ inhibits lipopolysaccharide (LPS)-induced pro-inflammatory cytokine production in LPS-activated rat mast cells RBL-2H3 by inhibiting IL-5 and IL-13 mRNA transcription, which demonstrates its anti-inflammatory effect.

The inhibitory effects of TQ on activation of the redox-sensitive transcription factor nuclear factor kappa B (NF-κB) and interleukin-6 (IL-6) were studied *in vitro*. Human proximal tubular epithelial cells (PTEC) were cultivated *in vitro* and stimulated with Advanced Glycation End Products (AGEs) and the effects of TQ were studied. A significant reduction of AGE-induced NF-κB-activation and IL-6 expression was observed in PTEC. Sayed studied in 2008 the effect of angiotensin II (AT II) on proximal tubular epithelial cells (PTECs) *in vitro*. AT II has been found to activate NF-κB and its controlled gene IL-6 in a time-dependent manner wherein the first point of maximum NF-κB activation occurs after 12 Hrs and the second after 3.5 days and TQ reversed these changes at 500 nM concentration. Kanter reported beneficial effects of black seed oil and TQ on neurodegeneration in hippocampus after chronic toluene exposure in rats. Treatment with TQ (50 mg/kg body weight) caused morphological improvement on neurodegeneration indicating necessity of further preclinical research in this area. Same work also studied the effects of same compound.
on histopathological recuperation of sciatic nerves in streptozotocin (STZ)-induced diabetic rats on TQ treatment (50 mg/kg body weight) once a day orally for 12 weeks starting two days after STZ injection \(^{139,140}\).

McDermott and co-workers assessed chemoprotective potential of epigallocatechin-3-gallate (EGCG) and TQ, against n-hexane toxicity. Exposure to n-hexane increases ROS formation with a corresponding decrease in Jurkat T-cell proliferation. Treatment of cells with EGCG and TQ reduced concentration of ROS, formed by exposure to n-hexane \(^{141}\).

1.5.5 Antioxidant Activity of TQ

Antioxidant property of TQ has been shown through different mechanisms in several reports. It inhibits the production of 5-hydroxyeicosa-tetraenoic as well as 5-lipoxygenase products both of which are required for the viability of colon cancer cells \(^{135}\). It has been shown that it scavenges many reactive oxygen species, which include superoxide radical anion and hydroxyl radicals \(^{142,143}\). TQ inhibited iron-dependent microsomal lipid peroxidation, very effectively in case of doxorubicin-induced hyperlipidemic nephropathy in rats \(^{144}\). It decreased cellular oxidative stress by inducing production of glutathione (GSH) in experimental allergic encephalomyelitis in female Lewis rats \(^{145}\).

\textit{In vitro} studies showed that pretreatment with 10 \(\mu\text{M}\) TQ provided protection through restoration of levels of glutathione and inhibition of protein oxidation caused by Cd\(^{+2}\) in supernatant prepared from liver of Swiss albino mice \(^{146}\). Oral dose of TQ (50 mg/kg body weight) 3 days prior to bile duct ligation (BDL) in Sprague-Dawley rats showed restored levels of antioxidant enzyme, superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity as compared to control group. In addition, elevated levels of hydroxyproline (HP) and malondialdehyde (MDA) were also reduced in TQ treated rats with BDL \(^{147}\). Renal ischaemia-reperfusion (I/R) model of renal failure in Male Sprague-Dawley rats was studied to see the antioxidant action of TQ. 10 mg/kg dose of TQ for 10 days before I/R maintained the activity of glutathione-S-transferase (GST) and superoxide dismutase (SOD) in kidney and liver tissues and decreased the level of spermidine/spermine N-1-acetyl-transferase (SSAT) - a catabolic enzyme in polyamine metabolism \(^{148}\). In heart tissues of adult male Wistar albino rats, single dose of 200 mg/kg, intra peritoneal (IP) of cyclophosphamide (CP), produced thiobarbituric acid reactive species and decreased levels of reduced glutathione,
glutathione peroxidase, catalase, and adenosine triphosphate after 12 days and these effects were completely reversed in rats treated with 50 mg/L of TQ in drinking water 5 days prior to CP dose and afterwards for 12 days which shows TQ has a protective effect against oxidative stress. TQ delayed cellular damage in diploid human cell culture line composed of fibroblasts derived from lung (WI-38) incubated with low-density lipoprotein (LDL) providing protection against oxidative stress. TQ in a dose of 80 mg/kg body weight for 45 days significantly reversed the damage in streptozotocin (STZ)-induced hyperglycemia in rats. STZ caused increase in blood glucose and decrease in the activity of antioxidant enzymes glutathione peroxidase (GPx), glutathione-S-transferase (GST) and decrease in levels of plasma insulin. TQ treated group did not show these changes after STZ treatment. Another *in vivo* investigation in hyperlipidemic rabbit showed that 20 mg/kg/day dose of TQ assuaged oxidative stress caused by high cholesterol diet and decreased the serum levels of alanine aminotransferase and reactive oxygen species. During an *in vivo* investigation on New Zealand rabbits, TQ inhibited increase in serum total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), triglycerides concentration along with restraining a decrease in high density lipoprotein-cholesterol and decrease in glutathione levels compared to high cholesterol control group. Histopathological examination of aorta, kidney and pulmonary arteries in high density cholesterol control group showed high cholesterol induced tissue damage and TQ treated group did not show any signs of tissue damage induced by high cholesterol diet.

### 1.6 Anti-proliferative and Cell Cycle Regulatory Activity

Shoieb and colleagues investigated anti-proliferative effects of TQ in cancer and normal cell lines, viz. canine osteocarcinoma (COS31) and its cisplatin-resistant variant (COS31/rCDDP), human breast adenocarcinoma (MCF-7), Human ovarian adenocarcinoma (BG-1) and Mandin-Darby Canine Cells (MDCK). TQ was found to inhibit proliferation in a concentration-dependent manner (IC$_{50}$ = 101 μM) as assessed by MTT assay. Normal kidney cells were the least affected cells.

Ait and colleagues evaluated the anti-tumor properties of the black seed oil and its ethyl acetate extract against Mouse lymphoblast-like mastocytoma (P815) cell line and both were found to be cytotoxic. In DBA2/P815 (H2d) mouse model it was observed that the
injection of the essential oil into the tumor site significantly inhibited solid tumor development as well as the incidence of liver metastasis, thus improving mouse survival. These results indicate that the anti-tumor activity or cell growth inhibition could in part be due to the effect of TQ on cell cycle.

The cell cycle checkpoints allow the cells to correct possible defects and avoid progression to cancer. On molecular level, Kaseb et al. have indicated effects of TQ on cell cycle regulatory proteins and pro-apoptotic proteins in prostate cancer cells. Cancer cell specific targeting action of TQ involves suppression of NF-κB activation, activation of caspase-8, -9 and -7, increase of activity of PPAR-γ, down-regulation of the expression of the genes for Bcl-2, Bcl-xL, survivin and down regulation of androgen receptor and E2F-1.

The principle activity of TQ was found to be due to its effects on the expression of cell cycle regulatory proteins. The treatment of cells with 30 μM concentration of TQ induced G1 cell-cycle arrest in papilloma cells after 48 Hrs, which correlated with a sharp increase in the expression of the cyclin-dependent kinase inhibitor p16 and down regulation of cyclin D1 protein expression. In Flow cytometric studies of DNA content by propidium iodide staining it has been revealed that TQ induces G1 cell-cycle arrest of osteosarcoma cancer cells (COS31) as well as human colon cancer cells (HCT-116), at 100 μM concentration. Growth inhibitory effect of TQ was noticed after 24 Hrs at a concentration of 50 μM for COS31 cells while for HCT-116 cells it started at 60 μM. The G1 arrest was associated with up-regulation of p21WAF1 in HCT-116 cells which was suggested as the principal transcriptional target of p53 in the context of the G1 checkpoint. The resulting high levels of p21WAF1 block cdk2 activity and possibly cdk4 and cdk6 activities leading to G1 arrest.

Roepke and colleagues evaluated anti-proliferative and pro-apoptotic effects of TQ in two human osteosarcoma cell lines with different p53 mutation status. Cell viability was reduced more selectively in MG63 fibroblast tumor cells than in normal human osteoblasts. Flow cytometric analysis by them showed that TQ induced a much greater increase in the Pre-G1 (apoptotic) cell population, but no cell cycle arrest in MG63 cells. G2/M arrest in methylnitronitrosoguanidine/Human Osterosarcoma (MNNG/HOS) cells was associated with p21WAF1 up-regulation. Using three DNA damage assays, the compound was confirmed to induce greater extent of apoptosis in p53 null MG63 cells. Although the Bax/Bcl-2 ratios were not differentially modulated in both cell lines, the mitochondrial pathway appeared to be
involved in apoptosis induced by TQ in MG63 by showing the cleavage of caspase-9 and caspase -3. Since TQ was found to induce p53-independent apoptosis in human osteosarcoma cells, it suggests the potential clinical usefulness of TQ for the treatment of these malignancies.

The serine/threonine kinase Polo-like kinase 1 (Plk1) is over-expressed in many types of human cancers and it has been implicated as an adverse prognostic marker. Plk1 localizes to its intracellular anchoring sites via its polobox domain (PBD) \(^{165, 166}\). TQ and its synthetic C-1 imino analog, Poloxin has been shown to inhibit PLK1 PBD \textit{in vitro} and cause chromosomal defects, mitotic arrest and apoptosis in HeLa cells \(^{167, 168}\). Anti-tumor activity of TQ and THQ in L929 normal mouse fibroblasts and two other tumor cell lines, viz. squamous cell carcinoma (SCC VII) and fibrosarcoma (FsaR), respectively was explored by Ivankovic\(^{169}\) and both compounds were found to be cytotoxic in dose dependent manner and cytotoxicity was more in tumor cells compared to L929 normal fibroblasts. 20-methylcholanthrene (MC)-induced fibrosarcoma tumor formation in male Swiss albino mice was significantly inhibited on administration of TQ (0.01% in drinking water) one week before and after MC treatment. Moreover, TQ also delayed the onset of MC-induced fibrosarcoma tumors indicating that it could be a powerful chemopreventive agent against MC-induced fibrosarcomas \(^{170}\).

Gali and colleagues\(^{171}\) studied inhibitory effects of TQ in two different murine colon cancer models, viz.1, 2-dimethyl hydrazine (DMH) and xenografts. They examined the growth of C26 mouse colorectal carcinoma spheroids and assessed tumor invasion \textit{in vitro} and found that the tumor multiplicity was reduced from 17.8 in the DMH group to 4.2 in mice injected with TQ. This suppression was observed on 30\(^{th}\) week and was long lasting since tumors did not re-grow even when TQ injection was discontinued for 10 weeks. In a xenograft model of HCT116 colon cancer cells, TQ significantly delayed the growth of the tumor cells.
1.6.1 TQ against Pancreatic Cancer

Wu and co-workers \(^{172}\) showed that TQ controlled the metastasis of pancreatic cancer Cells Panc-1 during *in vitro* investigations and Western blot showed the downregulation of NF-κB and MMP-9 in the treated cell line. *In vivo* studies by the same group were carried out by plantation of pancreatic tumor tissue into the wall of pancreas of nude mice and TQ treatment significantly suppressed the metastasis of tumor. Streptozotocin-induced diabetes and resulting oxidative stress were suppressed by 3 mg/mL intra-peritoneal dose of TQ when it was given for 6 days/week for 30 days. This also led to increase in insulin level due to overall improvement of beta cells’ health and integrity. These findings underline the chemoprotective and therapeutic potential of TQ \(^{173}\). Another molecular target in pancreatic cancer is MUC4, a glycoprotein of high molecular weight, normally absent in normal pancreatic ductal cells and it is found in increased concentration in pancreatic cancer cells FG/COLO357 and CD18/HPAF. The treatment of these cell lines with TQ caused substantial down-regulation of MUC4 expression and triggered apoptosis by setting off c-Jun NH(2)-terminal kinase and p38 mitogen-activated protein kinase pathways \(^{174}\). Pro-apoptotic and anti-inflammatory action of TQ is also reported by Chehl and group \(^{175}\) in pancreatic ductal adenocarcinoma (PDA) cells. In a dose and time-dependent manner TQ eradicated MCP-1, TNF-alpha, interleukin (IL)-1beta and COX-2 after 24 Hrs. The reduction of transport of NF-κB from cytosol to nucleus was also noted during the study. TQ has been shown to chemosensitize the pancreatic cancer cells at 25 µM concentration against gemcitabine or oxaliplatin. Pre-exposure of cells for 48 Hrs caused loss of 80% cell growth on gemcitabine or oxaliplatin treatment. The down-regulation of NF-κB, Bcl-2 and COX-2 by TQ is also noticed in this investigation, which shows the chemosensitization potential of TQ against pancreatic cancer cells. The *in vivo* tumor growth was also inhibited when study was replicated in animal model \(^{176}\).

1.6.2 TQ against Breast Cancer

Another study by Effenberger *et al.*\(^{177}\) showed that doxorubicin-resistant human breast cancer MCF-7/DOX cell lines exhibited increased level of PTEN, PTEN mRNA along with cell growth arrest at G2/M phase and apoptosis on treatment of TQ. Flow cytometry showed large number of cells at sub G1 level. The increased level of PTEN was complemented by
decrease in phosphorylated Akt leading to lesser cell survival. This investigation gave a mechanistic insight into the action of TQ against breast cancer cell line \(^{178}\). In MCF-7, which is doxorubicin resistant breast cancer cell line, treatment of TQ and doxorubicin caused growth inhibition of cells. On the same cell line, Motaghed and co-workers\(^{179}\) reported antiproliferative activity of TQ at 25 \(\mu M\) concentration, it also arrested cell cycle at S phase in lower concentration and this activity is at par with tamoxifen. In more recent reports on curative action of TQ against breast cancer, it was shown that combination of TQ with tamoxifen (TAM) increased apoptosis and also effected down regulation of Akt and X-linked inhibitor of apoptosis protein (XIAP) degradation resulting in the activation of caspase-9. Other notable changes during this study were down regulation of pro-survival Bcl-xL, Bcl-2 and up regulation of Bax expression \(^{180}\). *In vitro* investigations by Sutton *et al.*\(^{181}\) on breast cancer cell lines like T-47D, MDA-MB-231, MDA-MB-468 and MCF-7 showed apoptotic effects of TQ except MCF-7. It is noted by the group that NADPH quinone oxidoreductase 1 (NQO1) causes resistance against TQ and inhibition of NQO1 made MCF-7 cells susceptible to apoptosis caused by TQ.

### 1.6.3 TQ against Colon Cancer

In Wistar rats, colon cancer was induced by 1,2-dimethylhydrazine- (DMH) treatment and group pretreated with TQ showed normal values of levels of malondialdehyde, conjugated dienes along with various enzymes like superoxide dismutase, catalase and glutathione peroxidase. In the control group all the species cited above were found to be at increased levels. Thus, TQ acts as chemoprotective agent against DMH-induced damages \(^{182}\). El-Najjar and colleagues\(^{183}\) showed that TQ inhibited the growth of colon cancer cell lines like Caco-2, HCT-116, LoVo, DLD-1 and HT-29 without affecting normal human intestinal cells FHs74Int. Flow cytometry showed that the cell death was caused by induction of apoptosis and activation of caspase 3/7. The mitogen activated protein kinases (MAPK), JNK and ERK underwent more phosphorylation after TQ treatment, which also shows the prooxidant effect of TQ.
1.6.4 TQ against Lung Cancer

Rats exposed to 3000 ppm of toluene for 8 Hrs/day did not show inflammatory responses in their lungs when subsequent oral treatment of TQ in a daily dose of 50 mg/kg body weight right after toluene exposure was given to them for 12 weeks and in addition the histopathological examination of lungs did not show any tissue damage to the TQ treated group. Synergistic action of TQ (100 µM) with cisplatin (5 µM) against non-small cell lung cancer (NCI-H460) and small cell lung cancer (NCI-H146) cell lines, caused a reduction of 90% in cell proliferation as shown by MTT assay. In xenograft model (NCI-H460) the tumor size showed dose-dependent variation and TQ (5 mg/kg) with Cisplatin (2.5 mg/kg) reduced the tumor volume by 59% and when TQ amount was increased to 20 mg/kg with same amount of cisplatin, the reduction in tumor volume was by 79%. In another study, TQ has shown synergistic action with cisplatin against lung (LNM35), liver (HepG2), colon (HT29), melanoma (MDA-MB-435) and breast (MDA-MB-231 and MCF-7) cancer cell lines and in vivo investigations showed that intra peritoneal dose of 10 mg/kg for 18 days in athymic mice inhibited growth of LNM35 tumor by 39%.

1.6.5 TQ against Prostate Cancer

Koka and co-workers showed that TQ acts against androgen receptor (AR)-independent (C4-2B) and AR naïve (PC-3) prostate cancer cells in a time and dose dependent manner with IC₅₀ values of 50 µM and 80 µM respectively along with a threefold increase in level of reactive oxygen species and a decrease in glutathione (GSH) levels. They also showed that TQ treatment caused down-regulation of Bcl2 related proteins. TQ suppressed angiogenesis in vitro and in human prostate cancer (PC-3) xenograft model in mouse during in vivo investigations, resulting in inhibition of tumor growth along with regulatory effects on vascular endothelial growth factor-induced extracellular signal-regulated kinase activation.

1.6.6 TQ against Cervical Cancer

Thymoquinone induced apoptosis in human cervical squamous carcinoma cells (SiHa) at 9.33 µg/L shown by MTT assay while it showed less toxicity towards normal cells (3T3-L1 and Vero). Furthermore an increase in p53 levels and suppression of anti-apoptotic
Bcl2 was found in TQ treated cells, which complemented the accumulation of cells at sub-G1 phase in flow cytometry studies \(^{188}\).

**1.6.7 TQ against Liver Cancer**

A daily dose of 4 mg/kg of TQ in drinking water for 7 consecutive days countered the increase in total bilirubin, total nitrate/nitrite (NOx) and decreased levels of glutathione (GSH), glutathione peroxidase (GSHPx), glutathione-s-transferase (GST) and catalase (CAT) activity and histopathological lesions in hepatic tissues caused by a single intraperitoneal dose of 200 mg/kg diethylnitrosamine (DENA) in male Wistar albino rats \(^{189}\).

**1.6.8 TQ against Leukemia**

Alhosin and co-workers showed that TQ degraded \(\alpha/\beta\) tubulin in brain cancer cells and (cell line U87, solid tumor model) in Jurkat cells (T lymphoblastic leukaemia cells) in a concentration and time dependent manner and interestingly did not have any adverse effect on \(\alpha/\beta\) tubulin in normal human fibroblast cells which were used as control \(^{190}\). In acute lymphoblastic leukemia Jurkat cell line, TQ caused an inhibition in PDE1A (phosphodiesterase1A) expression and consequent triggering of apoptosis activation and cell cycle arrest \(^{191}\). Alhosin and his group previously reported that TQ-induced apoptosis of p53-deficient acute lymphoblastic leukemia (ALL) Jurkat cell line depends upon p73 pathway and it also shows down-regulation of UHRF1 (Ubiquitin-like, containing PHD and RING finger domains, 1) which otherwise activates cell cycle progression \(^{192}\).

**1.6.9 TQ against Ovarian and Renal Cancer**

In case of drug resistant ovarian cancer, Nessa and colleagues showed that pretreatment by TQ followed by cis-platin and oxaliplatin on human epithelial ovarian cancer cell lines A2780 and A2780(cisR) greatly decreased the cell viability even in cisplatin resistant cell lines \(^{193}\). Increase in the cellular glutathione levels in TQ treated cultures of Rhesus Monkey Kidney Epithelial Cells (RMKEC) at 72 Hrs defines protective effect of thymoquinone against oxidative damage at concentration of 10-50 \(\mu\)M \(^{194}\).
1.7 Summary and Conclusion

Thymoquinone has chemotherapeutic potential against various diseases in general and more focus has been given to anti cancer activity of thymoquinone. Investigations on *Nigella sativa* plant are not limited to *in vitro* and *in vivo* models. *Nigella sativa* seeds and extracts have been undertaken for clinical trials in US as shown below\textsuperscript{195}. Four of these clinical trials have been completed with positive results.

1. Effect of *Nigella sativa* on Lipid Profiles in Elderly
2. Effect of *Nigella sativa* in the Treatment of Palmer Arsenical Keratosis
3. Effect of *Nigella sativa* Seed Extract on the Blood Pressure of Elderly With Hypertension
4. Effectiveness of *Nigella sativa* (Kalonji) Seed in Dyslipidemia
5. Effect of Consumption of Black Cumin (*Nigella sativa* L.) Water Extract on Weight Loss in Overweight Women
6. Effect of Consumption of Caraway on Treatment of Obesity

There is no clinical trial reported on thymoquinone probably due to its low water solubility and limited bioavailability. Despite all the biological activities of thymoquinone, its hydrophobicity hinders its development as a potential chemotherapeutic agent for clinical trials. There is a need for development of conjugates of TQ in such a way that they just don’t increase the bioavailability but also add pharmacophores in the existing structure and lead to conjugates of TQ with enhanced biological activities against cancer.
Reference List


55. Palacios S, de Villiers TJ, Nardone FC, Levine AB, Williams R, Hines T, Mirkin S, Chines AA. Assessment of the safety of long-term bazedoxifene treatment on the reproductive tract in postmenopausal women with osteoporosis: results of a


63. Fojo T, Parkinson DR. Biologically targeted cancer therapy and marginal benefits: are we making too much of too little or are we achieving too little by giving too much? Clin Cancer Res 2010;16:5972-5980.


122. Khalife KH, Lupidi G. Reduction of hypervalent states of myoglobin and hemoglobin to their ferrous forms by thymoquinone: The role of GSH, NADH and NADPH. Biochimica et Biophysica Acta (BBA) - General Subjects 2008;1780:627-637.


142. Badary OA, Taha RA, Gamal el-Din AM, Abdel-Wahab MH. Thymoquinone is a potent superoxide anion scavenger. Drug Chem Toxicol 2003;26:87-98.


188. Ng WK, Yazan LS, Ismail M. Thymoquinone from Nigella sativa was more potent than cisplatin in eliminating of SiHa cells via apoptosis with down-regulation of Bcl-2 protein. Toxicol In Vitro 2011;25:1392-1398.


