Chapter 18
Usefulness of *Ocimum sanctum* Linn. in Cancer Prevention: An Update

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18.1 Introduction

Cancer is a group of ailment and is as old as mankind (Baliga et al. 2013). It is characterized by an uninhibited cell division leading to malignancy with the capability of invading neighboring tissues through the bloodstream or lymphatic system (Croce 2008). Cancer destroys organ systems through a localized invasive growth and by metastasizing to other distant tissues and organs. At the molecular level, all these events are initiated due to failures in the regulatory mechanisms of oncogenes and tumor suppressor genes. The tumor cells differentiate rapidly and progress with metastatic growth (Croce 2008). Cancer is today's leading death causing diseases along with cardiovascular diseases. According to a survey report by the American Cancer Society, nearly 1,665,540 tumor cases were detected in 2014 alone (Torre et al. 2015; Sridevi et al. 2016). The incidence of cancer is expected to increase by 75% by the year 2030, and the faulty lifestyle behaviors and sedentary lifestyle are proposed as the major contributing factors (Torre et al. 2015).

From a therapeutic perspective, cancer is treated by using chemotherapy, radiotherapy, and surgery either alone or in conjunction with each other, and the modalities are decided by the patient's general health status and the stage of the disease (Baliga et al. 2013). However, the side effects associated with the use of chemotherapy and radiotherapy are immense and negate the therapeutic benefit (Baliga et al. 2013). In addition to the standard therapeutic modalities, the prevention of

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cancer termed as “chemoprevention” is also being emphasized and is being appreciated as one of the promising methods to delay or prevent cancer formation (Baliga et al. 2013). From a definition perspective, chemotherapy involves the use of chemical, natural, or biological agents to prevent or suppress the progression of carcinogenic to become invasive metastatic cancer (Tsao et al. 2004).

Medicinal plants have largely contributed to the modern-day therapeutics and have been used for thousands of years in the folk medicines by Asian and African populations, and many plants are consumed for their health benefits in several developed nations (Martins 2014). To substantiate this, epidemiological investigations constantly report that a regular intake of vegetables and fruits strongly lowers the risk of cancer. In addition, some of the phytochemicals were reported to prevent tumor initiation and progression by inducing antioxidative stress effects and anti-inflammatory activities and by modulating signaling pathways (nuclear factor (NF-κB), Keap1-NRF2-ARE, activator protein (AP-1), etc.) (Liu 2004; Guarino et al. 2007). Additionally, the greatest benefit of plant-based compounds is that they are orally administrable and have a high safety index (Baliga et al. 2013; Ozkan et al. 2016). In this chapter, detailed information on the anticancer and chemopreventive potential of tulsi plant and its phytoconstituents are discussed giving more emphasis on their mechanistic aspects against different cancer types. This summarized data will allow opportunities for pharmaceutical exploration of tulsi plant in bringing its phytoconstituents into the drug market against several cancers.

18.2 Botany and Morphology of Tulsi

India has a vast history of use of medicinal plants, and *Ocimum sanctum* L. (synonym *O. tenuiflorum* L.), commonly known as tulsi or holy basil, is one of the most revered and widely used plants in various folk systems of medicines (Baliga et al. 2013). Tulsi, belonging to the family Lamiaceae, is a tropical annual herb which is a branched and erect subshrub with scented simple opposite green or purple leaves and hairy stems (30–60 cm tall). The leaves measure about 5 cm in length with an ovate and to some extent toothed structure. They bear purplish flowers in an elongated raceme in the closed whorls (Prakash and Gupta 2005). Morphologically and on the basis of leaf color, tulsi can be classified into two types, the *Shyama* or *Krishna* Tulsi, having purple- or dark-colored leaves, and the *Rama* or *Shri* or *Lakshmi* Tulsi, with green or light leaf variety. These varieties have been proposed to have different potency in terms of their medicinal effects with dark variety being more potent than the green variety (Prakash and Gupta 2005).

Although, the geographical distribution of this plant is throughout the world tropics including tropical Asia, eastern and northern parts of Africa, parts of China, Hainan island province of China, and Taiwan, it has been believed to have originated in India dating back to thousands of years. It is widely used in Ayurveda system of medicine in India (Prakash and Gupta 2005). In ancient Indian texts, tulsi

meaning the "incomparable one" has been called as "queen of herbs" owing to its widespread healing properties (Pattanayak et al. 2010).

18.3 Nutritional and Phytochemical Composition of Tulsi

Diverse factors such as different strains, geographical location, the procedures of growing and harvesting, and storage conditions determine the chemical composition of tulsi (Anandjiwala et al. 2006; Zheljazkov et al. 2008). This high degree of variability makes it a highly complex plant containing a diverse milieu of nutrients and other biologically active compounds. The major phytonutrients in tulsi are vitamin A, vitamin C, minerals such as zinc, calcium, iron, and several other nutritional phytoconstituents. The nutritional composition of tulsi per 100 g is as follows: protein, 4.2 g; carbohydrate, 2.3 g; fat, 0.5 g; phosphorus, 287 mg; calcium, 25 mg; iron, 15.1 mg; and vitamin C, 25 g (Anbarasu and Vijayalakshmi 2007; Pattanayak et al. 2010).

The various parts of tulsi are rich in biologically active constituents including flavonoids, saponins, tannins, triterpenoids, and other phenolic compounds. Tulsi leaves are the richest in eugenol and methyl eugenol in addition to the presence of vicenin, orientin, ursolic acid, molludistin, luteolin, luteolin-7-O-glucuronide, apigenin, and apigenin-7-O-glucuronide (Kelm et al. 2000; Baliga et al. 2013). The essential oils of tulsi also constitute carvacrol, linalool, limatrol, and a sesquiterpene hydrocarbon, caryophyllene. Additionally, some of the phenolic compounds present in fresh leaves and stems include cirsimaritin, cirsiliol, apigenin, isothymusin, and rosmarinic acid (Baliga et al. 2013). Other beneficial compounds of tulsi include a number of monoterpenes and sesquiterpenes such as α-elemene, bornyl acetate, myrtenal, neral, α-pinene, β-pinene, campesterol, camphene, β-sitosterol, and stigmasterol (Singh et al. 1996, 2007, 2012; Kelm et al. 2000; Gupta et al. 2002; Shishodia et al. 2003; Prakash and Gupta 2005; Anandjiwala et al. 2006; Zheljazkov et al. 2008; Baliga et al. 2013). Some of the important phytochemicals of tulsi are depicted in Fig. 18.1.

18.4 Pharmacological Significance of Tulsi

Tulsi is one of the well examined plants with many pharmacological effects. The broad range of biological activities include immunomodulation, anti-ulcer, anti-inflammation, antimicrobial, antifertility, antihypertensive, cardioprotective, hepatoprotective, antidiabetic, radioprotective and as a chemopreventive agent. Additionally as an adaptogen, tulsi is also helpful and supports in adapting to stress (Godhwani et al. 1987; Pandey and Madhuri 2010). Different solvent extracts from tulsi leaves show antibacterial activity when analyzed against *Staphylococcus aureus* and *Salmonella typhimurium* pathogenic bacteria which cause diarrhea

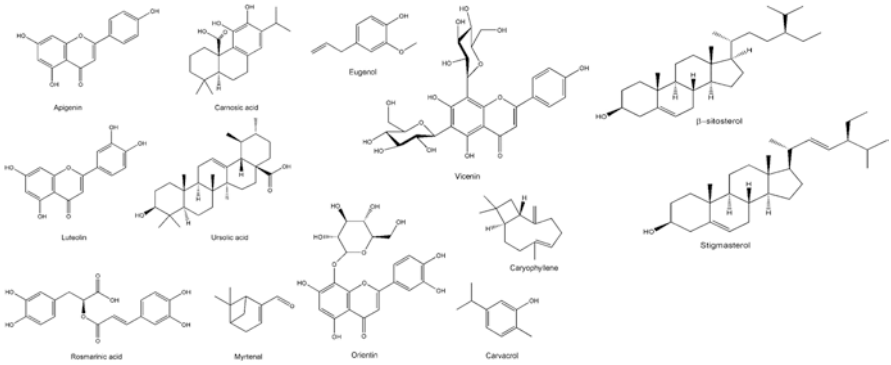

Fig. 18.1 Some of the important phytochemicals of *Ocimum sanctum*

(Eswaret al. 2016). Tulsi has been reported to possess ameliorative properties and to possess antidiabetic properties (Mahaprabhu et al. 2011). Wound healing effects of tulsi were studied using incisional wound model in rats, and it was found that it has a wound healing potential (Eyo et al. 2014). Aqueous extract of tulsi reduced the level of LDL cholesterol, total cholesterol, and triglycerides levels in acute hyperlipidemia induced rats by Triton WR-1339 in rats. Administration of the ethanolic extract of tulsi in alloxan diabetic rats was shown to reduce hyperglycemia (Vats et al. 2002). It was found that 60% and 80% of the benzene and petroleum ether extracts of tulsi leaves produces antifertility activity in female rats. In an experimental study, tulsi extract was found to be highly effective as a hepatoprotective agent. It reduced the liver damage induced by paracetamol in albino rats (Lahon and Das 2011). Studies have also shown that tulsi is beneficial in cancer, and this review summarizes the anticancer effects of tulsi against different cancer types (Baliga et al. 2013), and the subsequent paragraphs address these aspects in detail.

18.4.1 Role of Tulsi in Breast Cancer

Breast cancer ranks the second most common cancer types worldwide and is one of the major reasons of mortality due to cancer (Wiseman 2008; Torre et al. 2015). Using the Matrigel plug assay method, Nangia et al. (2004) have reported that the breast cancer cells, MDA-MB-435, treated with tulsi leaf extract effectively inhibit the migration and capillary tube formation and reduce the number of blood vessels. In another study, the breast cancer cells, MDA-MB-231, induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) also showed the inhibitory effect of tulsi leaf extract and increased the levels of cyclooxygenase-2 (COX-2) enzyme (Nangia et al. 2004). These results successfully establish the anti-tumorigenic and anti-angiogenic properties of tulsi leaf extracts.

The phytochemicals such as eugenol have been shown to possess anticarcinogenic activity against the breast cancer cells (MCF-7) owing to their redox modulatory activities (Vidhya and Devaraj 2011). Along the similar lines, flavonoids (luteolin and apigenin) found in tulsi were demonstrated to induce apoptosis in various human breast cancer cells such as MDA-MB-453, SK-BR-3, and MCF-7 by overexpressing HER2 oncogene. Further, these phytochemicals are known to regulate key signaling molecules involved in the cell cycle pathways (CCNA2, PCNA, CDKN1A, CCND1, PLK1), estrogen signaling pathways (GTF2H2, NCOR1, TAF9, NRAS, NRIP1, POLR2A, DDX5, NCOA3), histone deacetylase activity, and cell survival, invasion, and cancer growth (Markaverich et al. 2011; Attoub et al. 2011; Kim et al. 2012). Additionally, carnosic acid and rosmarinic acid are shown to possess growth inhibitory effects in in vitro studies (Yesil-Celiktas 2010).

This anti-tumorigenic potential of these compounds has been further validated experimentally using tumor-bearing nude mice and mammary cancer induced by treating dimethylbenz[a]anthracene (DMBA) and medroxyprogesterone acetate (MPA) in experimental Sprague-Dawley rats. The study results revealed that MPA increases vascular endothelial growth factors (VEGF) and suppresses vascular endothelial growth factor receptor-2 (VEGFR-2) in the hyperplastic regions. The suggested possible mechanism of action could be useful for such therapeutic applications (Chen et al. 2007; Mafuvadze et al. 2011; Mafuvadze et al. 2012).

18.4.2 Role of Tulsi in Skin Cancer

A long-term exposure or chronic contact of the body to sunlight's ultraviolet (UV) radiation is the major etiologic agent causing the skin cancers such as squamous-cell skin cancer (SCC), basal-cell skin cancer (BCC), and melanoma. Majority of these tumors are BCC involving non-melanocytic skin cells, followed by cutaneous malignancy, i.e., SCC. A plethora of studies has authenticated that UV-B radiation (290–320 nm) initiates tumor formation and also acts as a tumor promoter. Acute exposure of UV-B radiation to human keratinocytes increases the secretion of diacylglycerols, prostaglandins, and free arachidonic acid and upregulates COX-2 protein expression (Buckman et al. 1998). Another cause of skin cancer is the exposure to toxic xenobiotic compounds. Their constant exposure alters the structure of the skin and promotes skin cancer. Medicinal plants possessing phytocompounds with free radical scavenging and antioxidant activities are well documented to have radioprotective properties.

Leaves of tulsi have been demonstrated to possess selective radioprotective effects at a nontoxic concentration (Baliga et al. 2016). Earliest studies have observed that the topical applications of ethanolic extract of tulsi leaves decrease DMBA-induced skin papillomagenesis. It has been reported that pretreatment of the skin with tulsi extract before the application of carcinogens decreases the tumor formation incidences (Baliga et al. 2013). The essential oil extracted from tulsi seeds has reduced MCA-induced skin carcinogenesis and increased the survival of

tumor-bearing mice (Baliga et al. 2013). Additional studies have shown that tulsi increases the levels of GSH (glutathione), GST (glutathione S-transferase), and other enzymes with antioxidant activities, by decreasing the activity of ornithine decarboxylase (ODC) and cytochrome P450 enzymes and reducing the expression of GST-P (glutathione S-transferase placental form) and GGT (gamma-glutamyl transpeptidase). Also, the levels of lipid peroxidation were found to decrease, suggesting its protective role. Tulsi leaves contain various biologically active phytocomponents mainly eugenol, apigenin, and luteolin which are beneficial in curing the dermal toxic effects of xenobiotics and UV-B radiation (Baliga et al. 2013).

Eugenols hinder the formation of superoxides and lipid peroxidation; decrease the oxidative stress, inflammation, and cell multiplication; and induce apoptosis (Manikandan et al. 2010; Baliga et al. 2013; Sarkar et al. 2015). The pretreatment with eugenol effectively inhibits nuclear factor kappa-light-chain (NF-kB), an enhancer of activated B cells, ornithine decarboxylase (ODC) activity, and expression of nitric oxide synthase (iNOS) and COX-2 and decreases the levels of proinflammatory cytokines (Baliga et al. 2013). Primary melanoma cells established from patient tissues have shown that eugenol causes a concentration-based suppression of the cell growth. Apigenin also inhibits the DMBA-initiated tumorigenesis and reduces UV-B-induced increase of COX-2 levels in both mouse and human keratinocyte cell lines and prevents UV-B-induced skin cancer in mice. Likewise, luteolin present in tulsi has the potential to increase the survival of normal human keratinocytes (NHKs) after UV-B irradiation. However, malignant cells were not affected and remained unchanged. Luteolin was reported to attenuate UV-B-induced cell death by delaying or inhibiting intrinsic pathways of apoptotic signals and enhancing antioxidant activities (Verschooten et al. 2010; Baliga et al. 2013).

18.4.3 Role of Tulsi in Lung Cancer

Lung cancer is another leading death causing cancers which is estimated to a total of 1.5 million deaths worldwide annually. The lung cancer is only diagnosed at a later stage due to local invasion or distal metastases (Perlikos et al. 2013). Carcinogens with the ability to cause mutagenesis induce the activation of oncogenes and lead to lung carcinogenesis. Lung cancer metastasis is due to transition of epithelial cells to mesenchymal cell type which occur though the activation of numerous cell signaling pathways including Akt/GSK3β, MEK-ERK, and Fas (Perlikos et al. 2013).

The extract of tulsi induces apoptosis in A549 (lung cancer) cells and suppresses lung cancer development in C57BL/6 mice (Magesh et al. 2009). The molecular mechanism by which tulsi prevent lung carcinoma is through the phosphorylation of survival genes (Akt and ERK), increasing the levels of cytochrome C, and reducing the expression of Bcl-2, an anti-apoptotic protein (Bhattacharyya and Bishayee 2013). Treatment of tulsi plant extracts to animals with carcinoma was shown to inhibit the tumor growth in a dose-dependent manner (Bhattacharyya and Bishayee 2013).

It is also reported that the leaf extract of tulsi inhibits cell adhesion and decreases matrix metallopeptidase 9 (MMP-9) and increases antioxidant enzyme levels (Nangia-Makker et al. 2004). The administration of the extract to mice relatively reduced the formation of tumor lumps and significantly reduced the weight of lung. Luteolin, one of the major component of tulsi induces the arrest of G2 phase of the cell cycle, induces apoptosis and suppresses the growth and migration of adenocarcinomic A549 (human alveolar basal epithelial) cells and helps in preventing the lung cancer (Magesh et al. 2009; Baliga et al. 2013; Nana-Sinkam and Powell 2013).

18.4.4 Role of Tulsi in Liver Cancer

According to the recent report of the American Cancer Society, liver cancer is the fifth and eighth most common cancer in men and women, respectively, worldwide (Torre et al. 2015; Siegel et al. 2016). The major risk factors associated with liver cancer are chronic infections of HBV (hepatitis B virus) and HCV (hepatitis C virus) and high alcohol consumption (Baliga et al. 2013). There are numerous phytomedicines which are being used for preventing and treating different liver disorders or hepatotoxicity (Ashfaq and Idrees 2014). Tulsi is also one of them. Scientific studies have revealed that the extracts of tulsi and its oil significantly elevated the function of cytochrome b5, cytochrome P450, and glutathione S-transferase in the liver; all of these play an important role in detoxifying carcinogens and mutagens (Siegel et al. 2016).

It has been reported that tulsi-treated lung cancer cell lines, NCI-H460, exhibit anticancer activity by increasing intracellular ROS, by decreasing cell proliferation, and by altering the mitochondrial membrane potential (Das et al. 2006). Apigenin, a phytochemical from tulsi, has been shown to inhibit the phenobarbital (PB)-promoted and N-nitrosodiethylamine (NDEA)-induced experimental hepatocellular cancer initiation (Sridevi et al. 2016). Similarly, the ethanolic extract of tulsi leaves at a dose of 400 and 800 mg/kg body weights was shown to modulate aryl hydrocarbon hydroxylase and cytochrome P450 enzymes that are known to metabolize carcinogens. In vivo studies have shown that the ethanolic leaf extract of tulsi can alleviate damages in the liver due to antituberculosis drugs in rats (Ubaid et al. 2003). In their study, it has been revealed that tulsi exhibits the protective effects by reducing the levels of protein and lipid oxidation, by decreasing phase I enzymes, and by enhancing phase II and antioxidant enzymes.

In vitro studies with the primary hepatocytes in rats have revealed that feeding the leaf extract (ethanolic) of tulsi (20–500 μg/ml) before DMBA treatment (10 or 50 μg) cause the decrease the levels of DMBA-DNA adducts significantly. It suggests the capability of tulsi extract in preventing the DMBA-induced tumorigenesis in the early stages (Prashar et al. 1998). Further, they have suggested that leaf extract of tulsi blocks the events that are linked to chemical tumorigenesis by suppressing the metabolic activation of carcinogenic substances (Aggarwal and Mali 2015).

Experiments have shown that the ethanolic extract of tulsi leaves administered orally at a dose of 200 mg/kg in male Wistar albino rats gave protection against the liver injury induced by paracetamol (Chattopadhyay et al. 1992). Likewise, the cold water extract (3 g/100 g, administered orally for 6 days) of tulsi was found to be highly effective against carbon tetrachloride (0.2 ml/100 g, subcutaneously)-induced liver damage in experimental rats (Seethalakshmi et al. 1982).

Various investigations have shown that phytochemicals of tulsi prevent hepatocarcinoma in rats and the protective effects are achieved through mitigating the oxidative stress. A pentacyclic triterpene acid, ursolic acid isolated from tulsi, has been stated to inhibit the activities of NF-κB triggered by carcinogenic substances including tumor necrosis factor, phorbol ester, hydrogen peroxide, okadaic acid, and tobacco smoke (Srinivas et al. 2016). According to them, ursolic acid suppresses the degradation and phosphorylation of IκBα, activation of IκB kinase, phosphorylation of NF-kB-p65 subunit, nuclear translocation of p65 subunits, and NF-κB-dependent reporter gene expression. In addition, ursolic acid suppresses the rate of cell proliferation and promotes apoptotic events and arrests the cell cycle phases (Srinivas et al. 2016). Moreover, the in vitro cell culture assays have revealed that treating ursolic acid induces cytotoxicity and apoptosis in various cell lines including HA22T (Gayathri et al. 2009), HepG2 (Tang et al. 2009), Huh (Yang et al. 2010), and H22 (Wang et al. 2011). It also has some antiangiogenic effects and decreases the expression of VEGF and maintains GSH levels and reduces the cell invasion and migration in HA22T and Hua7 cells (Lin et al. 2011).

The phytoconstituent, apigenin (a flavone), present in tulsi has been documented with antioxidant, anti-inflammatory, and anticancer potential (Shukla and Gupta 2010). Cell culture studies have shown that it causes apoptosis mediated through the generation of ROS (Choi et al. 2007). Furthermore, apigenin has been shown to inhibit human hepatoma cancer cell (Huh7) growth by inducing apoptosis mediated via altered expression of several regulatory genes and inhibiting PI3K/Akt/mTOR signaling pathways (Cai et al. 2011; Tong and Pelling 2013). Likewise, luteolin is a type of flavonoid most often found in tulsi leaves that induces apoptosis in HepG2 cells mediated by activating the release of cytochrome C enzymes and mitochondrial translocation of Bak and Bax apoptogenic proteins (Lee et al. 2010). Myrtenal, which is a monoterpene present in tulsi, has been found very effective in preventing diethylnitrosamine (DEN)-induced hepatocellular carcinoma in rats (Babu et al. 2012). Its administration corrected the modified levels of enzymes, involved in metabolism of carbohydrate, as compared to the carcinogen alone treated groups (Lingaiah et al. 2012). It also reduced the levels and activities of phase I enzymes (cyto b5, cyto P450 CPR, CBR) and concomitantly increased the phase II enzymes (GST, UGT). It modulated the levels of p53, TNF-α, and caspase-3.

18.4.5 Role of Tulsi in Gastric Cancer

Gastric cancer is another more frequently diagnosed cancer types in worldwide population. According to the epidemiological survey report on cancers worldwide, nearly 22,220 cases of gastric cancer patients are detected annually in the United States of America alone. Among them, about 10,990 patients are expected to die (Siegel et al. 2016), and based on the 2009–2013 data, the number of new cases of gastric cancer was 7.4 per 100,000 patients per year which accounts for 8% of the total cases (Jemal et al. 2011). Nowadays, many herbs have been investigated for bioactive compounds, and modern research studies are mainly aimed to understand their antitumor activity against various cancers, and the results are found positive to a certain extent (Ovadje et al. 2015). Tulsi is a rich source of phytochemical compounds and thus exhibits innumerable pharmacological effects including anticancer activity. A dose of 400 and 800 mg/kg of tulsi leaf extract has been found to modulate the carcinogen-metabolizing enzymes such as GST, hydrocarbon hydroxylase, and cytochrome P450 that play a role in detoxifying mutagens and carcinogens (Banerjee et al. 1996).

Tulsi plant leaves administered orally to benzo[a]pyrene-induced gastric cancer in mice and N-methyl-N^1-nitro-N-nitrosoguanidine (MNNG)-induced gastric cancer in rats effectively prevented the tumorigenesis (Aruna and Sivaramakrishnan 1992). In their preclinical experiments, tulsi leaves were incorporated into diet (200 mg/g) before and after tumor induction to prevent forestomach tumorigenesis. The animals fed with the leaves of tulsi showed very low incidences (29%) of tumorigenesis suggesting the chemopreventive role of tulsi (Aruna and Sivaramakrishnan 1992). Likewise, 70% ethanolic leaf extract when administered to MNNG-induced gastric cancer rats significantly reduced the incidences of carcinogenesis (Manikandan et al. 2007a).

Mechanistic investigations of tulsi revealed that its extract selectively induces apoptosis in MNNG-induced gastric tumors. However, it does not affect the normal tissues of the stomach. Also, tulsi extract influences the molecular cascades and mechanisms that are involved in cancer progression (Manikandan et al. 2007b). Tulsi extract when administered in animals has shown that it decreases the levels of cytokeratin, proliferating cell nuclear antigen (PCNA), VEGF (angiogenesis), proteins involved in proliferation (GST-pi), and anti-apoptotic protein (Bcl-2) with a synchronized rise in the levels of proapoptotic proteins such as cytochrome C, Bax, and caspase-3 (Manikandan et al. 2007b, 2010, 2011). Administration of tulsi methanolic leaf extract (100 mg/kg) showed a significant ulcer protection against ethanol and pyloric ligation-induced gastric ulcers in animal models (Goel et al. 2005). Besides that, the leaf extract significantly inhibited the secretion of offensive acid-pepsin and increased the mucin secretion, gastric defensive factors, and cellular

mucus. Eugenol is one of the most abundant essential oils present in the leaves of tulsi that has an anticancer capability (Udupa et al. 2006). Preclinical studies have revealed that it also has an effective activity against MNNG-induced gastric carcinogenesis in rats and benzo[a]pyrene-induced forestomach tumors in mice (Bhide et al. 1991; Manikandan et al. 2010).

Manikandan et al. (2011) has investigated and invented that eugenol exerts its protective effects by the suppression of NF-κB activation and altered expression of NF-kB target genes that inhibit or promote the survival and proliferation of cells. Likewise, eugenol treatment was shown to increase apoptosis by the modulation of Apaf-1, caspases, Bcl-2 family proteins, and cytochrome C (Bhide et al. 1991). In a study, eugenol was shown to promote apoptosis of AGS (human gastric cancer) cells, mediated by both intrinsic and extrinsic cell signaling apoptotic pathways. During the course of gastric carcinoma development, alterations in p53 occur. Eugenol inhibited the cell proliferation rate as evidenced from the decreased population of cells at the S-phase which corresponds to a decreased level of proliferating cell nuclear antigen (PCNA) expression with its treatment. Thus, the antiproliferative potential of eugenol was found to be dependent on p53 (Sarkar et al. 2015). Also, in vitro studies have shown that luteolin, another chemical component of tulsi, initiates cell death and apoptosis in AGS tumor cells (Wu et al. 2008). Furthermore, apigenin (Zhang et al. 2006) and β-sitosterol (Zhao et al. 2009) are also reported to induce apoptosis and cause cell death. Additionally, ursolic acid treatments of BGC-803 cells have also shown to arrest cell cycle at G0/G1 stage and enhance apoptosis by enhancing the expression of caspase-8 and caspase-3 genes (Wang et al. 2011). Likewise, ursolic acid from tulsi has been shown to inhibit the activities of NF-κB activation induced by several carcinogens that include phorbol ester, hydrogen peroxide, tumor necrosis factor, and cigarette smoke. Finally, it inhibits multiplication of cells, prompts apoptosis, and arrests the cell cycle (Aggarwal et al. 2011).

18.4.6 Role of Tulsi in Oral Cancer

Oral cancer is common in the world with approximately 90% of them being reported from the regions of Southeast Asia, where the people are more inclined toward smoking and tobacco chewing. Mouth ulcers and other infections related to the mouth are very effectively reduced by leaves of tulsi, and it has been found that eugenol extracted from tulsi prevents early events of DMBA-induced buccal pouch tumorigenesis (Karthikeyan et al. 1999). The oral administration of the ethanolic and aqueous tulsi leaf extracts and tropical application of its leaf paste decreased the occurrence of squamous cell carcinomas (Shivpuje et al. 2015).

Phytochemical studies have shown that the flavonoid, apigenin, exhibits chemopreventive effects against the DMBA-induced oral carcinogenesis in buccal pouches of golden Syrian hamsters. It has been also found that a natural benzenediol abietane diterpene, carnosic acid, and a phenolic compound, rosmarinic acid, are equally efficient in inhibiting DMBA-induced oral carcinogenesis in hamsters. Both

compounds mediated the protective effects by enhancing antioxidant and detoxification enzymes and decreasing the levels of lipid peroxidation (Manoharan et al. 2010; Anusuya and Manoharan 2011).

18.5 Conclusions and Future Prospects

Information accrued from preclinical studies suggests that tulsi is useful in cancer prevention. Tulsi plant possesses several bioactive compounds belonging to different classes of phytocompounds including polyphenols, alkaloids, terpenes, steroids, etc. Some of the major active principles of tulsi include eugenol, apigenin, ursolic acid, luteolin, carvacrol, linalool, rosmarinic acid, β-sitosterol, and stigmasterol. Both plant extracts and individual compounds of tulsi were proved to be effective in preventing several types of cancers as evidenced from the in vitro and in vivo study models. Some of the molecular events that are involved in the prevention of tumorigenesis include the induction of apoptosis mediated by activating the release of cytochrome C enzymes and mitochondrial translocation of Bak and Bax apoptogenic proteins, p53, TNF-α and caspases, Apaf-1, and Bcl-2 family proteins, decreasing the expression of VEGF, maintaining GSH levels, reducing the cell invasion, phosphorylation of IκBα, activation of IκB kinase, phosphorylation of NF-kB-p65 subunit, nuclear translocation of p65 subunits, and NF-κB-dependent reporter gene expression. Additionally, studies have also confirmed that tulsi has a high margin of drug safety and does not possess toxic effects. However, additional studies are required to aim for a better understanding of the mechanisms of action of the tulsi leaf phytoconstituents, and also to determine their efficacy in animal models. Only a few isolated compounds, such as eugenol, luteolin, and apigenin from tulsi have been proved to have antiproliferative activity. Therefore, future research should focus on isolating more anticancer compounds from tulsi plant and extensively evaluate to understand their role in the cytotoxicity events. Moreover, combination therapy involving tulsi plant compounds should be considered for a safe and effective cancer treatment. With wide distribution, easy availability, and safety in the consumption, tulsi plant has a tremendous therapeutic potential. However, its myriad prospects require additional investigations to understand its cancer preventive effects in humans.

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