MINI-REVIEW

Piper Betel Linn (Betel Vine), the Maligned Southeast Asian Medicinal Plant Possesses Cancer Preventive Effects: Time to Reconsider the Wronged Opinion

Manoj P Rai[1], Karadka Ramdas Thilakchand[2], Princy L Palatty[3], Prathima Rao[4], Suresh Rao[5], Harshith P Bhat[6] , Manjeshwar Shrinath Baliga[1*]

Abstract

Since antiquity, *Piper betel* Linn (betel vine; family Piperaceae) has been an important medicinal agent in the various traditional and folk systems of medicine in Southeast Asia countries. The leaves are the most valued plant part and in the past were routinely used as a chewing agent to prevent halitosis. The leaves are also supposed to harden the gum, conserve the teeth and to prevent indigestion, bronchitis, constipation, congestion, coughs and asthma. Innumerable scientific studies have validated the ethnomedicinal claims. Betel leaves are an integral component of the betel quid that consists of areca nut (*Areca catechu* Linn.), tobacco (*Nicotiana tabacum* L) and slaked lime; a highly abused agent with carcinogenic properties. Regular chewing of betel quid is associated mainly with oral cancer and detail studies with individual constituents of the quid have shown that both tobacco and areca nut are carcinogenic, while slaked lime is shown to promote the process of carcinogenesis. However unlike other constituents of the betel quid, the betel leaves devoid carcinogenic effects and on the contrary possesses cancer preventive effects including against the carcinogens present in tobacco. This review for the first time provides information on cancer preventive effects and also addresses the various mechanisms which might be involved.

Keywords: Piper betel - betel leaf - betel quid - chemoprevention

Asian Pacific J Cancer Prev, **12**, 2149-2156

Introduction

Piper betel Linn., (family Piperaceae) (Figure 1) commonly known as the betel vine is a important medicinal and recreational plant in Southeast Asia. The most probable place of origin of betel vine is Malaysia but today the plants are also cultivated in India, Srilanka, Bangladesh, Burma and Nepal (Kumar et al., 2010; Guha et al., 2006). The names of beetle vine in various Southeast Asian languages are enlisted in Table 1 (Warrier et al., 1995). Betel leaves are the most important plant part and are of medicinal, religious and ceremonial value in Southeast Asia. In India it is customary to serve betel leaf on various social, cultural and religious occasions and is also offered to guests as a mark of respect (referred to as tambool) (Warrier et al., 1995). Based on the color, size, taste and aroma there are many varieties of betel leaf and some of the most popular Indian varieties are the Magadhi, Venmony, Mysore, Salem, Calcutta, Banarasi, Kauri, Ghanagete and Bagerhati (Satyavati et al., 1987; Warrier

Table 1. Name of Betel in Various Languages

Indian Languages	Names
Sanskrit	Nagavalli, Nagavallari, Nagini
Hindi, Bengal, Gujraji, Urdu	Paan
Kannada	Vilya, Veeleya, Villayadel
Konkani	Phodi paan
Malayalam	Vettila, Vettilakkoti
Marathi	Vidyache pan
Tamil	Vetrilai
Telugu	Tamalapaku
Other Asian Languages	
Vietnamese	Tråu
Arabic	Tanbol
Mon	Plu
Khmer	Maluu
Thai	Plue
Sinhalese	Bulath
Persian	Burg-e-Tanbol
Chamorro	Papulu
Malay	Daun sirih
Kapampangan	Bulung samat

*[1]Research and Development, [2]IInd year MBBS, [3]Department of Pharmacology, Father Muller Medical College, Father Muller Road, Kankanady, [5]Mangalore Institute of Oncology, Mangalore, [6]Research Centre, Maharani Lakshmi Ammani Women's College, Bangalore, Karnataka, [4]Department of Oral Pathology and Microbiology, Century International Institute of Dental Science & Research Center, Poinachi, Kerala, India *For correspondence: msbaliga@gmail.com*

et al., 1995).

Betel vines are one of the highly investigated plants and phytochemical studies show that Piper betel contains a wide variety of biologically active compounds whose concentration depends on the variety of the plant, season and climate. The aroma of betel leaf is due to the presence of essential oils, consisting of phenols and terpenes. The various phytochemicals found in the betel plants are chavibetol, chavicol, hydroxychavicol, estragole, eugenol, methyl eugenol, hydroxycatechol, caryophyllene, eugenol methyl ether, cadinene, γ-lactone, allyl catechol, p-cymene, cepharadione A, dotriacontanoic acid, tritriacontane, p-cymene, terpinene, eucalyptol, carvacrol, sesquiterpenes, cadinene, caryophyllene, dotriacontanoic acid, hentriacontane, pentatriacontane, stearic acid, n-triacontanol, triotnacontane, piperlonguminine, allylpyrocatechol diacetate, isoeugenol, 1, 8-cineol, α-pinene, β-pinene, sitosterol, β-sitosteryl palmitate, γ-sitosterol, stigmasterol, ursolic acid, ursolic acid 3β-acetate (Rastogi and Mehrotra, 1993; Kumar et al., 2010). Some important phytochemicals are depicted in Figure 1.

The leaves and the stalk of the betel vines have been used since time immemorial to treat various ailments in Ayurveda, the traditional Indian system of medicine and also in various folk medicines in Southeast Asia. Chewing betel leaf is supposed to prevent bad breath (halitosis), improve the vocalization, harden the gum, conserves the teeth and sweetens breath. The infusion prepared from the leaves and stems are supposed to be useful in treating indigestion, bronchitis, constipation, congestion, coughs and asthma. The leaf juice is given systemically to treat cough and indigestion in children. The Essential oil isolated from the leaves is supposed to be useful in treating respiratory catarrhs and as an anti-septic (Chopra et al., 1982, Satyavati et al., 1987).

Several workers have reported on the different biological activities of Piper betel in various in vitro and in vivo test models. Many of these studies have validated the traditional uses, while some experiments have been on exploratory lines and have no traditional use for the performed pharmacological activity. Preclinical experiments have shown that betel leaf possess anti-bacterial, anti-cariogenic, anti-fungal, anti- larval, anti-protozal, anti-filarial, anti-allergic, anti-diabetic, anti-inflammatory, hepatoprotective, anti-ulcer, anti-fertility, cardioprotective, anti-hyperlipidaemic, anti- platelet, vasorelaxation and immunomodulatory effects (Kumar et al., 2010).

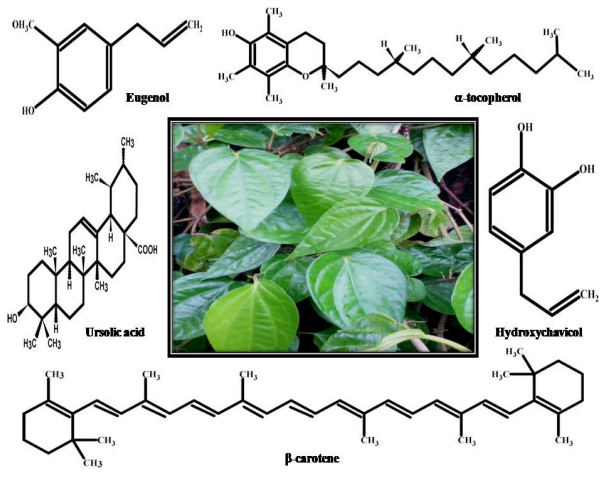

Figure 1. The *Piper betel* Plant with Some Important Phytochemicals Possessing Cancer Preventive Effects

Beetle Quid and Cancer

General

Irrespective of the uses, betel vine is arguably the most maligned plant whose regular consumption is believed to cause cancer of the oral cavity. This infamous accreditation is principally due to the fact that habitual chewing of beetle quid consisting of areca nut or betel nut (*Areca catechu*), betel leaf, catechu, slaked lime, and often tobacco (*Nicotiana tabacum*) causes cancer of the oral cavity (Brunnemann and Hoffmann 1992; IARC, 2004). Innumerable studies have been performed with the individual constituents of the betel quid and observations have conclusively shown that tobacco (Sundqvist et al., 1989; Boetta et al., 2008) and areca nut (Bhide et al., 1979; Canniff and Harvey, 1981; Sundqvist et al., 1989; Jin et al., 1996; Wang and Peng, 1996, Jeng et al., 2000; Wang et al., 2003; IARC, 2004; Lee et al., 2005) are both carcinogenic and slaked lime to promote carcinogenesis (Jeng et al., 1994; Thomas and MacLennan, 1992). However contrary to the accepted belief, scientific studies have shown that betel leaf is devoid of mutagenic and carcinogenic effect. Pioneering studies by Bhide et al., (1979) reported for the first time that aqueous extract of betel leaf failed to induce any tumor in mice in both Swiss mice and C17 mice thereby proving that unlike believed betel leaf was not carcinogenic (Bhide et al., 1979). Subsequent studies by have conclusively shown that the betel leaf and some of its phytochemicals also prevented chemical induced cancers in experimental animals. In the following sections the various chemopreventive effects of betel leaf and its constituents are accordingly addressed.

Betel leaf prevents oral carcinogenesis:

Globally, oral cancer is one of the ten most common cancers with nearly 90% of them being reported from the South East Asia region, where the habits of tobacco chewing and smoking are common (WCRF/AICR, 2007). In one of the earliest studies, Rao (1984) observed that topical application of betel leaf extract inhibited B(a) P-induced oral tumorigenesis in hamsters (Rao, 1984). Subsequent studies have shown that betel leaf was effective in preventing tobacco-specific nitrosamines the N'-nitrosonornicotine and 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone-induced carcinogenesis of tongues against the lower dose of the carcinogen N'-nitrosonornicotine (Padma et al., 1989a). Experiments with Syrian hamsters have also shown that the betel-leaf extract and two of its constituents, β-carotene and α-tocopherol were also observed to be effective in inhibiting the decreasing the incidence, reducing tumor burden, enhancing tumor latency period and to regress the established frank tumors. The combination of betel leaf extract with turmeric was also observed to be effective

suggesting a cooperative effect between the two dietary agents (Azuine and Bhide, 1992).

Prevention of forestomach carcinogenesis:

Globally gastric cancer is the fourth most common cancer and the second most lethal cancer with higher incidence in Asian countries where consumption of betel quid and Helicobacter pylori infection is considerable (WCRF/AICR, 2007). Supplementation of betel leaf extract in drinking water significantly reduced the benzo[a]pyrene-induced forestomach neoplasia in a concentration dependent manner in mice (Bhide et al., 1991a). Subsequent studies with eugenol, hydroxychavicol, β-carotene and α-tocopherol also showed that they were also effective against the benzo pyrene-induced forestomach carcinogenesis in mice (Bhide et al., 1991a). Recently, Manikandan et al. (2010) have also reported that eugenol induces apoptosis and inhibits invasion and angiogenesis in N-methyl-N'-nitro-N-nitrosoguanidine induced gastric carcinogenesis in rats. Preclinical studies have shown that eugenol possess antimicrobial effects on thirty strains of *Helicobacter pylori* indicating its usefulness (Ali et al., 2005). Cell culture studies human gastric epithelial AGS cells have also shown that β-carotene inhibited the Helicobacter pylori-induced expression of inducible nitric oxide synthase and cyclooxygenase-2 known to be important in *H. pylori*-induced gastric diseases (Jang et al., 2009). Together all these observations indicate the usefulness of betel leaf phytochemicals in prevention of gastric cancer.

Prevention of skin carcinogenesis:

Skin cancers are globally the leading form of cancer and a major health problem in many countries. The basal cell carcinoma accounts for nearly 75%, while squamous cell carcinoma and melanoma makes up 15% and 10% respectively. Of these, melanoma is highly metastatic and more harmful than the other two (WCRF/AICR, 2007). Animal studies have shown that the topical application of betel leaf extract, β-carotene and α-tocopherol was effective reducing the tumor formation significantly in both Swiss mice Swiss bare mice, while hydroxychavicol was highly effective only in Swiss bare mice (Azuine et al., 1991). Detailed studies by other investigators have also shown that eugenol inhibited DMBA croton oil induced skin carcinogenesis in mice (Sukumaran et al., 1994; Pal et al., 2010) and to induce antiproliferative and pro-apoptotic activity of eugenol-related biphenyls on malignant melanoma cells (Pisano et al., 2007). Mechanistic studies have shown that eugenol precludes cutaneous chemical carcinogenesis in mouse by preventing oxidative stress and inflammation and by inducing apoptosis (Kaur et al., 2010) and to downregulate c-Myc and H-ras, and concomitantly activate of p53 dependent apoptosis to eliminate the mutated cells (Pal et al., 2010). Together these observations clearly indicate the usefulness of betel leaf and its phytochemicals in preventing skin carcinogenesis.

Chemoprevention of mammary carcinogenesis:

Globally breast cancer is the second most common cancer and also the second leading cause of cancer related death (after lung cancer) in women (WCRF/AICR, 2007). The aqueous extract of the betel leaf prevented DMBA-induced mammary carcinogenesis in rats, when administered during the initiation phase only. However when fed to the rats already bearing DMBA-induced mammary tumors the extract was ineffective indicating that the betel leaf possessed only chemopreventive effects and did not have anticancer (chemotherapeutic) effects (Rao et al., 1985). Subsequently, Bhide et al., (1994) also observed that administration of betel leaf extract through the drinking water decreased tumor burden and tumor incidence, and delayed the onset of mammary tumors in the DMBA model of rat mammary tumorigenesis (Bhide et al., 1994). Betel leaf was also effective in preventing the mammary tumorigenesis in the genetically predisposed C3H (Jax) mice, where a high incidence of spontaneous mammary tumors is observed. Feeding of betel leaf also caused a significant decrease in tumor incidence and tumor burden (Bhide et al., 1994).

Mechanism Responsible for the Cancer Preventive Effects

General

Carcinogenesis is a multistage process and its prevention or delaying involves myriad process. In the following sections some of the important process involved in the prevention of cancer is addressed (see Figure 2).

Free radical scavenging effects:

Free radicals are constantly produced during the normal cellular metabolism, mainly in the form of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) (Halliwell, 2007). At low concentrations free radical are beneficial while in excess are deleterious and cause cytotoxicity, mutagenesis, and inflammation, steps that initiate/enhance carcinogenesis (Devasagayam et al., 2004). *In vitro* studies have shown that the aqueous extract of the inflorescence of *Piper betel* extract was effective in scavenging H_2O_2, superoxide radical and hydroxyl radical. The extract also prevented the hydroxyl

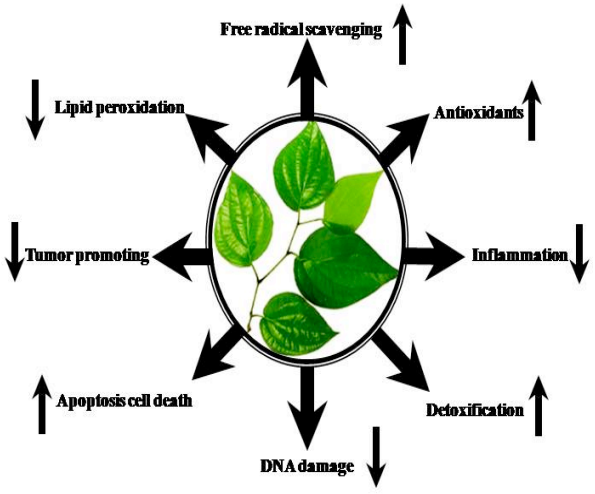

Figure 1. Molecular Targets for Chemoprention of the *Piper betel* Plant and its Constituents

radical-induced DNA strand breaks in the PUC18 plasmid (Lei et al., 2003). The ethanol extracts of Bangla, sweet, and Mysore varieties of betel leaf were effective in scavenging DPPH radicals in vitro, with best effects being observed with the Bangla variety (Rathee et al., 2006). The aqueous extracts of three Indian varieties the Kauri, Ghanagete and Bagerhati were also investigated and observed to be effective in scavenging DPPH, superoxide radical and hydroxyl radical with best effects being observed in the Kauri and lowest in Bagerhati variety (Dasgupta and De, 2004). Recently, Manigauha et al., (2009) have also observed that the methanolic extracts of the betel leaves possess reducing power, DPPH radical, superoxide anion scavenging and deoxyribose degradation activities. Studies have also shown that the hydroalcoholic extract of the betel leaf possess nitrogen oxide scavenging effect in vitro (Jagetia and Baliga, 2004).

Increase in antioxidant effects:

Eukaryotic cells possess antioxidant molecules [like glutathione, vitamin E (α–tocopherol), vitamin A (retinol), vitamin C (ascorbic acid) carotenoids thioredoxin, lipoic acid, and ubiquinol] and the antioxidant enzymes (like SOD, GPx and catalase) to protect against the free radical-induced damage, mutagenesis and carcinogenesis (Devasagayam et al., 2004; Halliwell, 2007). Animal studies by betel leaf extract increased the hepatic levels of vitamin A and vitamin C (Padma et al., 1989a), GSH and SOD (Choudhary and Kale, 2002). The betel leaf constituent's eugenol, hydroxychavicol and alpha-tocopherol are also shown to enhance the levels of GSH in mouse skin (Azuine et al., 1991) and liver (Bhide et al., 1991a). Together all these observations clearly indicate that the betel leaf extracts and some of its constituents increase the cellular antioxidants and mediate the chemopreventive effects at least in part.

Induction of detoxification enzymes:

Induction of phase II drug metabolizing enzymes especially the GST is a very important mechanism for protecting cells against the electrophilic insults induced by mutagens, carcinogens and the carcinogenic metabolites. GST conjugates the toxic metabolites and facilitates its excretion (Percival 1997). Accordingly, agents that activate phase II enzymes are considered to be extremely important as chemopreventive agents. Animal studies have shown that the intraperitoneal administration of the betel leaf extract and its constituent's β-carotene, α-tocopherol and hydroxychavicol significantly increased the levels of GST in mouse skin (Azuine et al., 1991) and liver (Bhide et al., 1991a). However eugenol the other important constituent was effective in increasing GST only in the skin and not in the liver indicating an organ specific action (Bhide et al., 1991a). Together these observations clearly indicate that by increasing GST levels, betel leaf extract and its phytochemicals mediate the chemopreventive effects at least in part.

Inhibition of lipid peroxidation:

The polyunsaturated fatty acids present in cell membranes are highly vulnerable to peroxidative attack by free radicals. This damage consequently leads to loss of fluidity, decrease in membrane potential, increase in permeability for protons and calcium ions and eventually the loss of cell membrane function (Devasagayam et al., 2004). Additionally the aldehydic end product of lipid peroxidation especially malondialdehyde is mutagenic and may contribute to the process of carcinogenesis. In vitro studies with rat liver mitochondria have shown that the alcoholic extract of the betel leaf inhibited the radiation induced lipid peroxidation (Bhattacharya et al., 2005). The aqueous extracts of Kauri, Ghanagete and Bagerhati variety of betel leaves were also effective in inhibiting the FeSO4-induced lipid peroxidation in egg yolk (Dasgupta and De, 2004). With regard to phytochemicals studies have shown that chevibetol, allylpyrocatechol and their respective glucosides were effective in preventing lipid peroxidation induced by Fe(II) in both liposomes and rat brain homogenates in vitro with best effects being observed with allylpyrocatechol (Rathee et al., 2006). Animal studies have also shown that betel leaf extract and its phytochemical allylpyrocatechol inhibited/reduced indomethacin-induced gastric ulceration and decreased the levels of lipid peroxidation (Bhattacharya et al., 2007a, b). A similar mechanism might have contributed to the cancer preventive effects of betel leaf extract in gastric carcinogenesis induced by benzo (a) pyrene and other carcinogens.

Anti-inflammatory effects:

Laboratory and clinical studies have confirmed that chronic inflammation initiates/aggravates many human diseases, including cancer (Halliwell, 2007). The betel leaf is used as a common household remedy for inflammation in the oral cavity (Satyavati et al., 1987). Scientific studies have shown that the ethanolic extract of betel leaf has been reported to possess anti-inflammatory activities at non toxic concentrations in the complete Freund's adjuvant-induced model of arthritis in rats. Mechanistic studies showed a concentration dependent decrease in the extracellular production of nitric oxide in murine peritoneal macrophages. This decrease in the generation of reactive nitrogen species was mediated by the down-regulating transcription of inducible nitric oxide synthase in macrophages with concomitant decrease in the expression of interleukin-12 p40. This study indicates the ability of betel leaf to down-regulate T-helper 1 pro-inflammatory responses (Ganguly et al., 2007).

Eugenol, one of the principal constituent of betel leaf has also been shown to possess anti-inflammatory effects in various animal models of studies with various inflamogens (Dohi et al., 1989; Lee et al., 2007). Mechanistic studies with in vitro systems showed that eugenol blocked the release of the bone resorbing mediators, including IL-1β, TNF-α, and PGE2 from of LPS-stimulated human macrophages by suppressing the messenger RNA expression of LPS-induced IL-1β, TNF-α and COX-2 in macrophages (Lee et al., 2007b). Eugenol suppressed the COX-2 gene expression in LPS-stimulated mouse macrophage cells (Kim et al., 2003). Allylpyrocatechol has also been demonstrated to possess anti-inflammatory effects in an animal model

of inflammation and mechanistic studies suggest the allylpyrocatechol targets the inflammatory response of macrophages via inhibition of iNOS, COX-2 and IL-12 p40 through down regulation of the NF-ϰB pathway (Sarkar et al., 2008). Hydroxychavicol isolated from the chloroform extraction of aqueous extract of leaves are also shown to possess potent antioxidant and anti-inflammatory activities (Sharma et al., 2009).

Antimutagenic effects:

Infliction of DNA damage and the fixation of the induced mutation is a cardinal event in the process of carcinogenesis. Oxidative and nitrative occurs during stress and damage the cellular DNA and cause mutations (Waris and Ahsan, 2006; Devasagayam et al., 2004). The changes in DNA such as base modification, rearrangement of DNA sequence, miscoding of DNA lesion, gene duplication and the activation of oncogenes play an important role in the initiation and progression of multistage carcinogenesis in various organs (Halliwell, 2007; Weisburger, 2001).

Multiple studies have shown that the betel leaf is devoid of mutagenic activities in both prokaryotic and eukaryotic assay systems (Umezawa et al 1981; Shirname et al., 1983; Bhattacharya et al., 2005) and also to possess antimutagenic (Shirname et al., 1983) and anticlastogenic effects (Bhattacharya et al., 2005). In vitro studies with cultured cells have shown that betel leaves did not cause any morphological transformation of the hamster embryo cells or induce sister chromatid exchanges in both virally transformed cells and PHA-stimulated human lymphocytes (Umezawa et al., 1981). Additionally, the ethanolic extract of betel leaf is also reported to possess γ-ray induced clastogenesis in plasmids (Bhattacharya et al., 2005).

With respect to the antimutagenic effects, seminal studies by Bhide and her co investigators have shown that the betel leaf was effective against different mutagens of environmental significance in Ames test with and without S9 mix (Shirname et al., 1983; Nagabhushan et al., 1987). Betel leaf extracts suppressed the mutagenicity of N'-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, benzo(a)pyrene and dimethyl benzanthracene in a dose dependent manner. The acetone extract was more potent than water extract in inhibiting mutagenicity of environmental mutagens (Nagabhushan et al., 1987; Padma et al., 1989b; Bhide et al., 1991b).

Betel leaf extract also suppressed the mutagenic effects of these nitrosamines in both Ames Salmonella/microsome assay (with TA100 +S9) and also in mice bone marrow micronucleus test (Padma et al., 1989b). The tobacco-specific N-nitrosamines [N'-nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and 4-(N-nitroso methyl amino)-1-(3-pyridyl)-1-butanone] are carcinogens present in unburnt forms of tobacco, including chewing tobacco and occupies important role in tobacco carcinogenesis (Padma et al., 1989b). Further in a study that has immense social relevance and significance, Trivedi et al., (1994) observed that the aqueous extract of betel leaf protected against the genotoxic effect of pan masala (with or without tobacco) extract in the Chinese hamster ovary (CHO) cells. The authors observed that the leaf extract decreased the pan masala-induced chromosome aberration and sister chromatid exchange when compared with the pan masala only (Trivedi et al., 1994).

With regard to the betel phytochemicals Amonkar et al., (1986; 1991) have observed that neither eugenol nor hydroxychavicol was mutagenic when tested in various strains of Salmonella typhimurium with or without metabolic activation. Both compounds exhibited dose-dependent suppression of dimethylbenzanthracene-induced mutagenesis in the S. typhimurium strain TA98 with metabolic activation. Hydroxychavicol was observed to be more potent than eugenol (Amonkar et al., 1986, 1991) and that it was effective against the mutagenicity of the N'-nitrosonornicotine and 4-(nitrosomethylamino)-1-(3-pyridyl)-1-butanone in both Ames Salmonella/microsome assay and the micronucleus test in mice (Amonkar et al., 1989).

Studies have shown that both hydroxychavicol and eugenol exhibited dose-dependent suppression of nitrosation in vitro in the Salmonella typhimurium strain TA100 and TA1535 (devoid of S9 mix) without affecting the bacterial survival. Structure based studies showed that the blocking of hydroxy group(s) in the benzene ring by acetylation abolished the anti-nitrosating activity of the molecule (s) indicating that these groups are important for the antimutagenic effects. The authors suggest that the nitrosation inhibition by hydroxychavicol is through scavenging of nitrite ions (Amonkar et al., 1989). Sukumaran and Kuttan (1995) have also observed that eugenol significantly inhibited tobacco-induced mutagenicity and nitrosation of methylurea in a dose-dependent manner.

In another important study, Yokota et al. (1986) observed that in the Ames test, direct addition of eugenol into the assay mixtures could not prevent the mutagenic activity of benzo (a) pyrene while the S9 fraction prepared from the livers of rats pretreated with eugenol suppressed mutagenesis. This indicates that eugenol inhibits the activation of the cytochrome P-450, which metabolizes benzo (a) pyrene to ultimate mutagens. The mutagenicity of benzo (a) pyrene catalyzed by microsomes from rats fed ad libitum on a diet containing 5% eugenol in the Ames test was significantly decreased (Yokota et al., 1986). Mutagenicity was also suppressed in the Ames test when the liver microsomes were used instead of the S9 fraction. Quantitative assays showed that in eugenol-treated microsomes the levels of cytochrome P-450, arylhydrocarbon hydroxylase and total benzo (a) pyrene hydroxylase activities were decreased and this had contributed for a reverse correlation between mutagenicity of benzo (a) pyrene with the dose of eugenol fed to animals (Yokota et al., 1986). Taken together, all these studies accentuate that the betel leaf and its constituent's eugenol and hydroxychavicol are good antimutagens, are effective against a range of environmentally important carcinogens and effective in both prokaryotes and eukaryotes.

Antitumor-promoting activities

Carcinogenesis is a multi stepped process and

innumerable studies have conclusively shown that tumor promoters that are agents which can enhance the effects of carcinogens without being carcinogenic themselves enhance carcinogenesis by allowing illegitimate reinitiation of replication within a single cell cycle. One such tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) is widely used in promoting skin cancer in laboratory animals. At the biochemical levels TPA enhance the generation of ROS, decreases ROS detoxification enzymes and bind and activate protein kinase C, causing a wide range of effects in cells and tissues (Mechali et al., 1983). Exploratory studies by Murakami et al., (2000) using the 12-O-hexadecanoyl phorbol-13-acetate-induced Epstein-Barr virus activation in Raji cells have shown that the betel leaf extract was effective as an antitumor-promoting agent. These studies clearly suggest that betel leaf could develop as a potential chemopreventive agent for preventing cancers of different histological origins and that it could develop as a novel agent.

Induction of selective apoptosis and cell death of neoplastic cells:

Apoptosis, a form of programmed cell death, plays a fundamental role in the maintenance of tissues and organ systems by providing a controlled cell deletion to balanced cell proliferation. Studies have confirmed that many dietary chemopreventive agents can preferentially inhibit the growth of mutated, preneoplastic and tumor cells by targeting one or more signaling intermediates leading to induction of apoptosis (De Flora and Ferguson, 2005). Recently Wagh et al., (2011) have shown that NPB001-05, a standardized extract of Piper betle (500 mg/kg once or twice a day for two weeks) was observed to be effective in inhibiting the growth of human chronic myelogenous leukemia in xenograft models. The extract was observed to be more effective than imatinib and with no relevant toxicity in animal models during the study period. At a molecular level, the extract reduced the tyrosine kinase activity, decreased Bcr-Abl protein levels and increased apoptosis. Microarray based transcription profiling studies also showed that NPB001-05 dysregulated ER stress, PI3K/AKT, MAPK pathways genes (Wagh et al., 2011). Studies have also shown that the vital phytochemicals hydroxychavicol (Chang et al., 2002; Jeng et al., 2004), ursolic acid (Kassi et al., 2007; Yamai et al., 2009; Yu et al., 2010; Shao et al., 2011), chlorogenic acid (Rakshit et al., 2010), eugenol (Ghosh et al., 2005; Pisano et al., 2007; Manikandan et al., 2010; Jaganathan et al., 2011) also induce apoptosis in tumor cells of different histological origins, thereby clearly indicating the possibility of a possible cooperative activities between the various phytochemicals to mediate the chemopreventive effect.

Conclusion

Scientific studies in the past have demonstrated unequivocally that betel leaf and some of its compounds possess chemopreventive effects against cancers of different histological origins. Several mechanisms are likely to account for the observed pharmacological effects, the most important being the free radical scavenging, antioxidant, antimutagenic, anti-inflammatory and possible induction of selective apoptosis in the neoplastic cells are the most important. Further studies should be aimed at understanding the efficacy and the mechanism of action of the standardized extracts of the betel leaves and the important phytochemicals like hydroxychavicol. Due to its abundance, low cost and safety in consumption, Betel leaf remains a species with tremendous potential and countless possibilities for further investigation.

Acknowledgements

The authors are grateful to Rev. Fr. Patrick Rodrigus (Director), Rev. Fr. Denis D'Sa (Administrator) and Dr Jaya Prakash Alva (Dean), Father Muller Medical College for their support. Mr Harshith P Bhat and Dr MS Baliga are also grateful to Prof. TL Shantha (Director) and Prof MB Nagaveni (Head of Biotechnology), Maharani Lakshmi Ammani Women's College, for their unstinted support. The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

References

Ali SM, Khan AA, Ahmed I, et al (2005). Antimicrobial activities of Eugenol and Cinnamaldehyde against the human gastric pathogen *Helicobacter pylori*. *Ann Clin Microbiol Antimicrob*, **4**, 20.

Amonkar AJ, Nagabhushan M, D'Souza AV, Bhide SV (1986). Hydroxychavicol: a new phenolic antimutagen from betel leaf. *Food Chem Toxicol*, **24**, 1321–4.

Amonkar AJ, Nagabhushan M, D'Souza AV, Bhide SV (1991). Hydroxychavicol: a new phenolic antimutagen from betel leaf. *IARC Scientific Publications,* **105**, 520-4

Amonkar AJ, Padma PR, Bhide SV (1989). Protective effect of hydroxychavicol, a phenolic component of betel leaf, against the tobacco-specific carcinogens. *Mutat Res,* **210**, 249–53.

Azuine MA, Amonkar AJ, Bhide SV (1991). Chemopreventive efficacy of betel leaf extract and its constituents on 7,12-dimethylbenz(a)anthracene induced carcinogenesis and their effect on drug detoxification system in mouse skin. *Indian J Exp Biol*, **29**, 346-51.

Azuine MA, Bhide SV (1992). Protective single/combined treatment with betel leaf and turmeric against methyl (acetoxymethyl) nitrosamine-induced hamster oral carcinogenesis. *Int J Cancer,* **51**, 412-5.

Bhattacharya S, Banerjee D, Bauri AK, Chattopadhyay S, Bandyopadhyay SK (2007b). Healing property of the *Piper betel* phenol, allylpyrocatechol against indomethacin-induced stomach ulceration and mechanism of action. *World J Gastroenterol,* **13**, 3705-13.

Bhattacharya S, Chaudhuri SR, Chattopadhyay S, Bandyopadhyay SK (2007a). Healing properties of some Indian medicinal plants against indomethacin-induced gastric ulceration of rats. *J Clin Biochem Nutr,* **41**, 106-14.

Bhattacharya S, Subramanian M, Roychowdhury S, et al (2005). Radioprotective property of the ethanolic extract of *Piper betel* Leaf. *J Radiat Res,* **46**, 165-71

Bhide SV, Shivapurkar NM, Gothoskar SV, Ranadive KJ (1979). Carcinogenicity of betel quid ingredients: feeding mice with aqueous extract and the polyphenol fraction of betel nut. *Br J Cancer,* **40**, 922-6.

Bhide SV, Zariwala MB, Amonkar AJ, Azuine MA (1991a). Chemopreventive efficacy of a betel leaf extract against benzo[a]pyrene-induced forestomach tumors in mice. *J Ethnopharmacol*, **34**, 207-13.

Bhide SV, Padma PR, Amonkar AJ (1991b). Antimutagenic and anticarcinogenic effects of betel leaf extract against the tobacco-specific nitrosamine 4-(N-nitroso methyllamino)-1-(3-pyridyl)-1-butanone (NNK). *IARC Scientific Publications,* **105**, 520-4.

Bhide SV, Azuine MA, Lahiri M, Telang NT (1994). Chemoprevention of mammary tumor virus-induced and chemical carcinogen-induced rodent mammary tumors by natural plant products. *Breast Cancer Res Treat,* **30**, 233-42.

Boffetta P, Hecht S, Gray N, Gupta P, Straif K (2008) Smokeless tobacco and cancer. *Lancet Oncol*, **9**, 667–75

Brunnemann KD, Hoffmann D (1992). Chemical composition of smokeless tobacco products. In: Smokeless Tobacco or Health. An International Perspective (Smoking and Tobacco Control Monograph No.2), Bethesda (MD): National Cancer Institute.

Canniff JP, Harvey W (1981). The aetiology of oral submucous fibrosis: the stimulation of collagen synthesis by extracts of areca nut. *Int J Oral Surg*, **10**, 163-7.

Chang MC, Uang BJ, Wu HL, et al (2002). Inducing the cell cycle arrest and apoptosis of oral KB carcinoma cells by hydroxychavicol: roles of glutathione and reactive oxygen species. *Br J Pharmacol*, **135**, 619-30.

Chopra RN, Chopra IC, Handa KL, Kapur LD (1982). Chopra's Indigenous Drugs of India; 2nd ed. AP: New Delhi, Calcutta.

Choudhary D, Kale RK (2002). Antioxidant and non-toxic properties of *Piper betel* leaf extract: in vitro and in vivo studies. *Phytother Res,* **16**, 461-6.

Dasgupta N, De B (2004). Antioxidant activity of *Piper betle* L. leaf extract in vitro. *Food Chemistry*, **88**, 219-24

De Flora S, Ferguson LR (2005). Overview of mechanisms of cancer chemopreventive agents. *Mutat Res*, **591**, 8-15.

Devasagayam TP, Tilak JC, Boloor KK, et al (2004). Free radicals and antioxidants in human health: current status and future prospects. *J Assoc Phys India*, **52**, 794-804.

Dohi T, Terada H, Anamura S, Okamoto H, Tsujimoto A (1989) The anti-inflammatory effects of phenolic dental medicaments as determined by mouse ear edema assay. *Jap J Pharmacol,* **49**, 535-9.

Ganguly S, Mula S, Chattopadhyay S, Chatterjee M (2007). An ethanol extract of *Piper betle* Linn. mediates its anti-inflammatory activity via down regulation of nitric oxide. *J Pharma Pharmacol,* **59**, 711-8.

Ghosh R, Nadiminty N, Fitzpatrick JE, et al (2005). Eugenol causes melanoma growth suppression through inhibition of E2F1 transcriptional activity. *J Biol Chem*, **280**, 5812-9.

Guha P (2006). Betel leaf: The neglected green gold of India. *J Hum Ecol*, **19**, 87-93.

Halliwell B (2007). Biochemistry of oxidative stress. *Biochem Soc Trans,* **35**, 1147-50.

IARC (2004). Betel-quid and areca nut chewing and some areca-nut derived nitrosamines, In IARC Monographs vol. 85, International Agency for Research on Cancer, WHO, Lyon, France.

Jaganathan SK, Mazumdar A, Mondhe D, Mandal M (2011). Apoptotic effect of eugenol in human colon cancer cell lines. *Cell Biol Int*, **35**, 607-15.

Jagetia GC, Baliga MS (2004). The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants in vitro: a preliminary study. *J Med Food,* **7**, 343 – 48.

Jang SH, Lim JW, Kim H (2009). Beta-carotene inhibits Helicobacter pylori-induced expression of inducible nitric oxide synthase and cyclooxygenase-2 in human gastric epithelial AGS cells. *J Physiol Pharmacol*, **60**, 131-7.

Jeng JH, Ho YS, Chan CP, et al (2000). Areca nut extract up-regulates prostaglandin production, cyclooxygenase-2 mRNA and protein expression of human oral keratinocytes. *Carcinogenesis*, **21**, 1365-70.

Jeng JH, Kuo ML, Hahn LJ, Kuo MY (1994). Genotoxic and non-genotoxic effects of betel quid ingredients on oral mucosal fibroblasts in vitro. *J Dent Res*, **73**, 1043-9.

Jeng JH, Wang YJ, Chang WH, et al (2004). Reactive oxygen species are crucial for hydroxychavicol toxicity toward KB epithelial cells. *Cell Mol Life Sci*, **61**, 83-96.

Jin YT, Tsai ST, Wong TY, Chen FF, Chen RM (1996). Studies on promoting activity of Taiwan betel quid ingredients in hamster buccal pouch carcinogenesis. *Eur J Cancer B Oral Oncol*, **32B**, 343-6.

Kassi E, Papoutsi Z, Pratsinis H, et al (2007). Ursolic acid. A naturally occurring triterpenoid, demonstrates anticancer activity on human prostate cancer cells. *J Cancer Res Clin Oncol*, **133**, 493-500.

Kaur G, Athar M, Alam MS (2010). Eugenol precludes cutaneous chemical carcinogenesis in mouse by preventing oxidative stress and inflammation and by inducing apoptosis. *Mol Carcinog*, **49**, 290-301.

Kim SS, Oh OJ, et al (2003). Eugenol suppresses cyclooxygenase-2 expression in lipopolysaccharide-stimulated mouse macrophage RAW264.7 cells. *Life Science*, **73**, 337-48.

Kumar N, Misra P, Dube A, et al (2010). *Piper betle* Linn. a maligned Pan-Asiatic plant with an array of pharmacological activities and prospects for drug discovery. *Curr Sci*, **99**, 922-32

Lee CH, Lee JM, Wu DC, et al (2005). Independent and combined effects of alcohol intake, tobacco smoking and betel quid chewing on the risk of esophageal cancer in Taiwan. *Int J Cancer,* **113**, 475–82.

Lee YY, Hung SL, Pai SF, Lee YH, Yang SF (2007). Eugenol suppressed the expression of lipopolysaccharide-induced proinflammatory mediators in human macrophages. *J Endod*, **33**, 698-702.

Lei D, Chan CP, Wang YJ, et al (2003). Antioxidative and antiplatelet effects of aqueous inflorescence *Piper betle* extract. *J Agric Food Chem*, **26**, 51:2083-8.

Manigauha A, Ali H, Maheshwari MU (2009). Antioxidant activity of ethanolic extract of Piper betel leaves. *J Pharm Res*, **2**, 491-4

Manikandan P, Murugan RS, Priyadarsini RV, Vinothini G, Nagini S (2010). Eugenol induces apoptosis and inhibits invasion and angiogenesis in a rat model of gastric carcinogenesis induced by MNNG. *Life Sci*, **86**, 936-41.

Mechali M, Mechali F, Laskey RA (1983). Tumor promoter TPA increases initiation of replication on DNA injected into *Xenopus* eggs. *Cell,* **35**, 63-9

Murakami A, Ali AM, Mat-Salleh K, Koshimizu K, Ohigashi H (2000). Screening for the in vitro anti-tumor-promoting activities of edible plants from Malaysia. *Bioscience Biotech Biochem,* **64**, 9-16.

Nagabhushan M, Amonkar AJ, D'Souza AV, Bhide SV (1989). Hydroxycahvicol: a new anti-nitrosating phenolic compound from betel leaf. *Mutagenesis,* **4**, 200–4.

Nagabhushan M, Amonkar AJ, D'Souza AV, Bhide SV (1987). Nonmutagenicity of betel leaf and its antimutagenic action against environmental mutagens. *Neoplasma*, **34**, 159-67.

Padma PR, Lalitha VS, Amonkar AJ, Bhide SV (1989a). Anticarcinogenic effect of betel leaf extract against tobacco

carcinogens. *Cancer Lett,* **45**, 195-202.
Padma PR, Amonkar AJ, Bhide SV (1989b). Antimutagenic effects of betel leaf extract against the mutagenicity of two tobacco-specific N-nitrosamines. *Mutagenesis*, **4**, 154-6.
Pal D, Banerjee S, Mukherjee S, et al(2010). Eugenol restricts DMBA croton oil induced skin carcinogenesis in mice: downregulation of c-Myc and H-ras, and activation of p53 dependent apoptotic pathway. *J Dermatol Sci*, **59**, 31-9.
Percival M (1997). Phytonutrients and detoxification. *Clinical nutrition insights*, **5**, 1-3
Pisano M, Pagnan G, Loi M, et al (2007). Antiproliferative and pro-apoptotic activity of eugenol-related biphenyls on malignant melanoma cells. *Mol Cancer*, **6**, 8.
Pisano M, Pagnan G, Loi M, et al (2007). Antiproliferative and pro-apoptotic activity of eugenol-related biphenyls on malignant melanoma cells. *Mol Cancer***, 6**, 8.
Rakshit S, Mandal L, Pal BC, et al (2010). Involvement of ROS in chlorogenic acid-induced apoptosis of Bcr-Abl+ CML cells. *Biochem Pharmacol*, **80**, 1662-75.
Rao AR (1984). Modifying influences of betel quid ingredients on B(a)P-induced carcinogenesis in the buccal pouch of hamster. *Int J Cancer,* **15**, 581-6.
Rao AR, Sinha A, Selvan RS (1985). Inhibitory action of Piper betel on the initiation of 7,12-dimethyl benz[a]anthracene-induced mammary carcinogenesis in rats. *Cancer Lett,* **26**, 207-14.
Rastogi RP, Mehrotra BN (1993). Compendium of Indian Medicinal Plants, Vol. 3; Publications and Information Directorate: *New Delhi*, 502-3.
Sarkar D, Saha P, Gamre S, et al (2008). Anti-inflammatory effect of allylpyrocatechol in LPS-induced macrophages is mediated by suppression of iNOS and COX-2 via the NF-kappaB pathway. *Int Immunopharmacol*, **8**, 1264-71
Satyavati GV, Raina MK, Sharma M (1987). Medicinal Plants of India. Vol. 1. New Delhi: Indian Council of Medical Research, New Delhi, India.
Shao JW, Dai YC, Xue JP, et al (2011). In vitro and in vivo anticancer activity evaluation of ursolic acid derivatives. *Eur J Med Chem*, **46**, 2652-61
Sharma S, Khan IA, Ali I, et al (2009). Evaluation of the antimicrobial, antioxidant, and anti-inflammatory activities of hydroxychavicol for its potential use as an oral care agent. *Antimicrob Agents Chemother*, **53**, 216-22
Shirname LP, Menon MM, Nair J, Bhide SV (1983). Correlation of mutagenicity and tumorigenicity of betel quid and its ingredients. *Nutrition and Cancer,* **5**, 87-91
Sukumaran K, Unnikrishnan MC, Kuttan R (1994). Inhibition of tumour promotion in mice by eugenol. *Ind J Physiol Pharmacol*, **38**, 306-8.
Sukumaran K, Kuttan R (1995). Inhibition of tobacco-induced mutagenesis by eugenol and plant extracts. *Mutat Res,* **343**, 25-30.
Sundqvist K, Liu Y, Nair J, et al (1989). Cytotoxic and genotoxic effects of areca nut-related compounds in cultured human buccal epithelial cells. *Cancer Res*, **49**, 5294-8.
Thomas SJ, MacLennan R (1992). Slaked lime and betel nut cancer in Papua New Guinea. *Lancet*, **340**, 577-8.
Trivedi AH, Patel RK, Rawal UM, Adhvaryu SG, Balar DB (1994). Evaluation of chemopreventive effects of betel leaf on the genotoxicity of pan masala. *Neoplasma,* **41**, 177-81.
Umezawa K, Fujie S, Sawamura M, et al (1981). Morphological transformation, sister chromatid exchange and mutagenesis assay of betel constituents. *Toxicol Lett*, **8**, 17-22.
Wagh V, Mishra P, Thakkar A, et al (2011). Antitumor activity of NPB001-05, an orally active inhibitor of Bcr-Abl tyrosine kinase. *Front Biosci*, **3**, 1349-64.
Wang CK, Peng CH (1996). The mutagenicities of alkaloids and N-nitrosoguvacoline from betel quid. *Mutat Res,* **360**, 165-71.
Wang LY, You SL, Lu SN, et al (2003) Risk of hepatocellular carcinoma and habits of alcohol drinking, betel quid chewing and cigarette smoking: a cohort of 2416 HBsAg-seropositive and 9421 HBsAg-seronegative male residents in Taiwan. *Cancer Causes Control,* **14**, 241–50.
Waris G, Ahsan H (2006). Reactive oxygen species: role in the development of cancer and various chronic conditions. *J Carcinog*, **5**, 14.
Warrier PK, Nambair VPK, Ramankutty C (1995). Indian Medicinal Plants: A Compendium of 500 Species. Arya Vaidya Sala, Kottakal, Kerala; Orient Longman, India.
Weisburger JH (2001). Antimutagenesis and anticarcinogenesis, from the past to the future. *Mutat Res*, *480-481*, 23-35.
World Cancer Research Fund / American Institute for Cancer Research (2007). Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. Washington DC: AICR.
Yamai H, Sawada N, Yoshida T, et al (2009). Triterpenes augment the inhibitory effects of anticancer drugs on growth of human esophageal carcinoma cells in vitro and suppress experimental metastasis *in vivo*. *Int J Cancer*, **125**, 952-60.
Yokota H, Hoshino J, Yuasa A (1986). Suppressed mutagenicity of benzo[a]pyrene by the liver S9 fraction and microsomes from eugenol-treated rats. *Mutat Res*, **172**, 231-6.
Yu YX, Gu ZL, Yin JL, et al (2010). Ursolic acid induces human hepatoma cell line SMMC-7721 apoptosis via p53-dependent pathway. *Chin Med J*, **123**, 1915-23.