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Review

Therapeutic potentials and untoward effects of *Piper betle* and its quid

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SUMMARY

Piper betle Linn. (PB), which belongs to the family Piperaceae, is used traditionally in many Asian countries for treatment of a variety of aliments. It has also been used in Ayurveda and Unani systems of medicine. PB leaves are also used as a masticatory in the form of quid. The basic preparation of PB leaves for chewing purposes (PB quid) is known as Paan in India. It is recommended in ancient scripture of Ayurveda and is closely associated with Indian culture. PB is reported to have several therapeutic potentials as well as to produce some untoward effects. The review deals with phytoconstituents present, therapeutic potentials and untoward effects of PB.

Key words: *Piper betle*; Quid; Traditional uses; Therapeutic potentials; Untoward effects

INTRODUCTION

Piper betle Linn. (PB) belonging to the family Piperaceae, is a tropical, perennial dioecious creeper, probably indigenous to Malaysia. Stems of the plant are semi-woody and it climbs by means of short, adventitious roots. The leaves are 5 - 20 cm long, broadly ovate, yellowish or bright green in color, lightly cordate, shiny on both surfaces and often unequal at the base. The leaves possess acute, entire apex and undulated margin. The petioles are stout and 2.0 - 2.5 cm long. The male spikes are dense, cylindrical while female spikes are 2.5 - 5.0 cm long and pendulous. The fruits are rarely produced and are often sunk in the fleshy spike, forming nodule like structures. Betel leaves have a strong pungent aromatic flavor and are widely used as a masticator. Chewing of PB quid, also known as 'Paan' (betel leaves with various adjuncts), is a common practice in India, Pakistan, countries of South East Asia and South Africa (Anonymous, 2001). Many medicinal properties have been attributed to PB, however some contraindications to its use have also been reported. Hence, this review is directed towards the exploration of the various therapeutic potentials as well as the harmful effects of PB, together with the phytoconstituents present in it.

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TRADITIONAL USES

Almost all parts of PB are used traditionally in most of the Asian countries. In India, the fruit is employed with honey as a remedy for cough and the root is used to prevent child bearing. The juice of the leaves is dropped into the eye for treatment of night - blindness. The essential oil from the

leaves has been successfully used in the treatment of catarrhal disorders and as an antiseptic. In Cambodia, the pounded fresh leaves are used in the preparation of lotion and baths for patients suffering from protracted fever, small pox, enlarged glands and lymphangitis. In the Philippine Islands, the leaves are used eminently for the cure of the disease of children: indigestion, diarrhea, pulmonary catarrh and laryngitis. It is also applied hot to the chest of nursing mothers, as it known to act as a lactagougue (Anonymous, 2001).

According to Ayurveda, PB leaves are carminative, stomachic, anthelminthic, tonic, aphrodisiac and laxative. They are useful in vata (malfunction of the central and autonomic nervous system) and kapha (malfunction of thermotaxis and in the formation of preservative fluids e.g. mucus, sinovial fluid etc.), foul smell in the mouth, bronchitis and elephantiasis of the leg. The leaves are also thought to be helpful in treating poisoning, thirst, alcoholism, asthma and loss of consciousness. Finally, they are used to improve appetite and as a treatment for leprosy.

According to the Unani system of medicine, PB leaf improves taste and appetite; acts as a tonic to the brain, heart, and liver, strengthens the teeth, lessens thirst, and clears the throat. It is also used as a vulnerary and styptic (Kirtikar and Basu, 1987).

PB leaves are also used as a masticatory in the form of quid. The basic preparation of PB for chewing purposes is known as Paan in India. Paan is recommended in ancient scripture of Ayurveda and is closely associated with Indian culture. In its simplest form it consists of PB leaf smeared with hydrated lime and eaten with scrapings of betel nut (*Areca catechu*). This traditional combination is considered ideal but many other flavourings such as coconut shavings, clove, cardamom, fennel, powdered liquorice, nutmeg and tobacco are used according to one's taste (Badami *et al.*, 2004). According to Ayurveda, PB quid is useful as a post prandial digestive stimulant, oral deodorant, natural antiseptic, astringent, diuretic, mood elevator, aphrodisiac and nerve tonic. It relaxes the mind, creates a feeling of well being, and improves the vocal cords (Bakhru, 1993; Seth, 1996). PB quid is used in social and cultural functions, as a primary means of initiating and promoting interpersonal relationships and for various ceremonial purposes (Personal communication).

PHYTOCONSTITUENTS

The important phytoconstituents of the leaf are the essential oils and the sugars (shown in Fig. 1). Depending on the variety, leaves consist of various essential oils such as eugenol, anethole, cis-caryophyllene, α-thujene, trans-β-ocimene, terpinolene, allo-ocimene, D-cadinene, terpenen-1-ol, α-costol, D-cadinol, methyl-2-hexadecane-1-ol, geraniol etc. The essential oil content of different Indian types varies from 0.7 to 2.6 per cent. The oil content increases with the maturity of the leaves but declines in over-ripe leaves. Oil of betel is a bright yellow to dark brown liquid possessing an aromatic, somewhat creosote-like odour resembling that of tea, and with a pungent, sharp taste. The oil consists of phenols and terpenes, their relative proportions varying with the origin of the leaves. The higher the proportion of phenols in the oil, the better the quality. An isomer of eugenol named chavibetol (4-allyl-2-hydroxy-1-methoxy benzene; $C_{10}H_{12}O_2$) is considered to be the characteristic constituent of betel oil. Betel oils of Indian types contain eugenol as the predominant phenolic constituent. PB leaves consist of reducing sugars such as glucose and non-reducing sugars such as sucrose, polysaccharide such as starch. In addition to essential oils & sugars, compounds like hexadecanoic acid, methyl benzoate, tannin, protein, fat, fiber and mineral matter such as calcium, phosphorus, iron, ionisable iron, vitamins such as carotene (vitamin A), thiamine, riboflavin, nicotinic acid and vitamin C. PB leaves also contain iodine and a high content of potassium nitrate, the amount depending upon the position of the leaf on the vine. The leaves contain significant amounts of all the essential

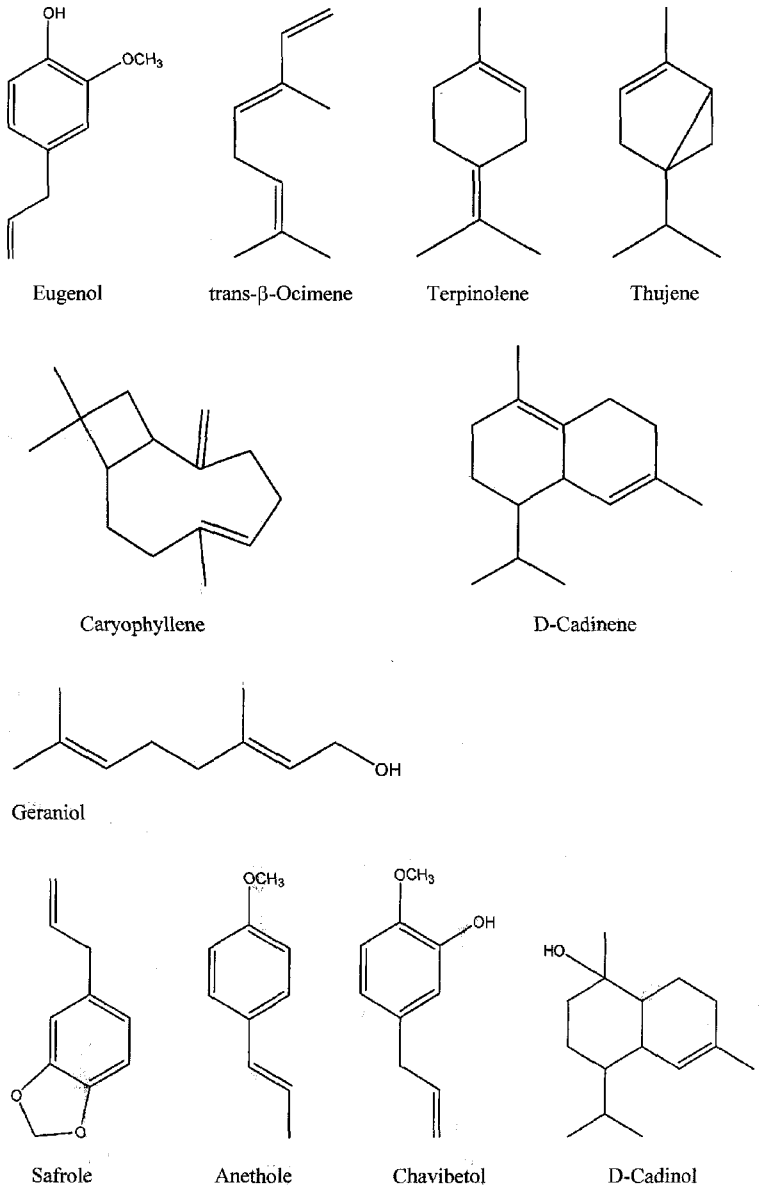

Fig. 1. Phytoconstituents of *Piper betle*.

amino acids except lysine, histidine and arginine, which occur in traces. Large concentrations of asparagine are present, while glycine (in a combined state) and proline occur in good amounts; ornithine is present in traces (Anonymous, 2001).

THERAPEUTIC POTENTIALS

Vasorelaxatory effect

PB leaf possesses potential health benefits because of its vasorelaxatory effect. This vascular effect is

Table 1. Therapeutic potentials of *Piper betle*

Sl. No.	Test	Activity	References
1	PB leaf	Vasorelaxant	Runnie *et al.*, 2004
2	PB leaf extract	Antioxidative	Lim and Mohamed, 1999; Lei *et al.*, 2003; Badami *et al.*, 2004
3	PB quid	Antiplatelet	Jeng *et al.*, 2002; Lei *et al.*, 2003
4	PB leaf stalk	Antifertility	Tewari *et al.*, 1970
5	PB leaf	Anticarcinogenic	Rao *et al.*, 1985; Anonymous, 2001
6	PB leaf	Antimutagenic	Nagabhushan *et al.*, 1987; Nagabhushan *et al.*, 1989
7	PB leaf	Digestive	Prabhu *et al.*, 1995
8	PB leaf extracts	Antifungal	Chou and Yu, 1984; Lim and Mohamed, 1999
9	Oils of PB leaf	Antibacterial	Anonymous, 2001
10	Oils of PB leaf	Anthelmintic	Anonymous, 2001
11	Oils of PB leaf	Larvicidal	Kumarasinghe *et al.*, 2002
12	PB quid	Wound healing	Sachs *et al.*, 2002
13	Oils of PB leaf	Carminative	Anonymous, 2001
14	Oils of PB leaf	Antispasmodic	Anonymous, 2001
15	PB quid	Psychostimulant	Chu, 2001; Wang and Hwang, 1997

mainly endothelium-dependent, and mediated by nitric oxide as supported by the inhibition of action in the presence of N -ω- nitro-L-arginine, an nitric oxide synthase inhibitor, or by the removal of endothelium. In contrast, vasodilatory action of PB leaves in resistance vessels appear to involve several biochemical mediators, including nitric oxide, prostanoids, and endothelium-dependent hyperpolarizing factors (Runnie, 2004).

Antioxidant activity

PB leaves and inflorescence are reported to possess potent antioxidant property. 50% methanol extract of leaves is found to be a good scavenger of DPPH (2, 2, diphenyl picryl hydrazyl) free radical (Badami *et al.*, 2004). Aqueous extract of inflorescence of PB extract was shown to be a scavenger of H_2O_2 (hydrogen peroxide), superoxide radical and hydroxyl radical. PB inflorescence extract also prevented the hydroxyl radical induced plasmid DNA breaks (Lei *et al.*, 2003). The anti-oxidant action of the leaves may be due to the presence of phenols, particularly hydroxy-chavicol (4-allyl pyrocatechol). The ascorbic acid in the leaves probably acts as a synergist to the phenols present in them (Anonymous, 2001). There are other reports available on antioxidant potential of PB (Lim and Mohamed, 1999).

Antiplatelet activity

Aqueous extract of PB is a potential reactive oxygen species scavenger and may prevent the platelet aggregation possibly via scavenging reactive oxygen species, as reactive oxygen species are crucial for platelet aggregation. It has been reported that PB extract inhibits the arachidonic acid induced and collagen-induced platelet aggregation (Lei *et al.*, 2003). PB extract also inhibits the arachidonic acid, collagen and thrombin induced thromboxane B_2 (TXB_2) production. However, PB extract shows little effect on thrombin-induced aggregation (Jeng *et al.*, 2003). Thus, PB has therapeutic potential in thromboembolic disorders.

Antifertility activity

Alcohol extract of PB leaf stalk, when fed to rats in diestrus phase, did not alter the estrus cycle, nor was any estrogenic or antiestrogenic effect noted. A mild progestational activity was found in immature estrogen-primed rabbits but some follicle depressant type action was noted (Tewari *et al.*, 1970). The

crude extract of PB leaf exhibits antimotility or inhibitory effects on washed human spermatozoa. The alcoholic crude extract of leaf stalk was reported to exhibit antigonadal activities in albino rats and a strong non-steroidal pregnancy interceptive agent in rats, thus showing the possibility of generation of a new class of antifertility agents (Anonymous, 2001).

Anticarcinogenic activity

The leaf extract and its constituents are reported to exhibit anticarcinogenic activity against benzo[a]pyrene induced tumors in mouse models. The leaf constituents hydroxychavicol and β-carotene were found to reduce the number of papillomas per mouse. Aqueous extract of the leaves of PB, when given orally during the initiation phase of 7, 12-dimethylbenz [a] anthracene-induced mammary carcinogenesis in rats, causes inhibition of emergence of tumors. β-carotene and α-tocopherol present in the betel leaf are reported to significantly inhibit the 7, 12-dimethylbenz [a] anthracene induced skin tumour formation in mice. In regards to eugenol, no appreciable degree of inhibition of tumor growth has been reported in case of rats bearing 7, 12-dimethylbenz [a] anthracene induced mammary tumors for 8 weeks (Rao *et al.*, 1985).

Antimutagenic activity

PB leaf aqueous and acetone extract are nonmutagenic in *S. typhimurium* strains. Both extracts suppress the mutagenicity of PB quid mutagens in a dose dependent manner. Moreover both the extracts of PB leaf reduce the mutagenicity of benzo[a] pyrene and dimethylbenzanthracene. Acetone extract was more potent than aqueous extract in inhibiting mutagenicity of environmental mutagens (Nagabhushan *et al.*, 1987). PB leaf extract when administered simultaneously with mutagenic tobacco specific nitrosamines (present in the extract of chewed tobacco), viz. nitrosonornicotine and 4-(methylnitrosaminol)-1-(3-pyridyl)-1-butanone, was reported to suppress the mutagenic effects and reduce the tumour incidence in mice (Anonymous, 2001). Nitrosation of methylurea results in the formation of mutagenic N-nitrosomethylurea. Hydroxychavicol and eugenol, the phenolic compounds of PB leaf, modulate nitrosation of methylurea by sodium nitrite at pH 3.6 and 30°C. The formation of mutagenic N-nitrosomethylurea has been monitored by checking the mutagenicity of reaction mixture in *Salmonella typhimurium* strain TA100 and TA1535. Hydroxychavicol and eugenol exhibit dose-dependent suppression of nitrosation in vitro without affecting the survival of the bacteria. Pre- or post-treatment of bacterial cells from *S. typhimurium* strains TA100 and TA1535 with phenolics does not modify the mutagenicity of nitrosomethylurea. The blocking of hydroxy group(s) in the benzene ring by acetylation abolishes the anti-nitrosating activity of the molecule(s). The nitrosation inhibition by hydroxychavicol is through scavenging of nitrite ions in the media, thus making them non-available for the nitrosation of methylurea (Nagabhushan *et al.*, 1989).

Digestive property

PB leaves do not influence bile secretion and composition but they have a significant stimulatory influence on pancreatic lipase activity. In addition, the PB leaf has a positive stimulatory influence on intestinal digestive enzymes, especially lipase, amylase and disaccharides. However, a slight lowering in the activity of these intestinal enzymes and a negative effect on pancreatic amylase has been seen in case of the Mysore variety of PB leaf (Prabhu *et al.*, 1995).

Antifungal activity

The whole plant extract is reported to exhibit antifungal activity against collar-rot disease of *Phaseolus aureus* Roxb. caused by *Thanatephorus cucumeris* (Frank) Donk and three fungal pathogens of Rice, *Pyricularia oryzae, Cochliobolus miyabeanus* and *Rhizoctonia solani*. The essential oil obtained from the leaves of PB has been found *in vitro* to be highly active against the growth of keratinophilic

fungi, *Arthroderma benhamiae, Microsporum gypseum, Trichophyton mentagrophytes, Ctenomyces serratus* and pathogenic *Aspergilli* (Anonymous, 2001). PB leaf exerts a complete inhibitory effect on the growth and aflatoxin production by *Aspergillus parasiticus*. Among the solvent extracts, chloroform and ethanol extracts of betel leaf prepared from a single solvent extraction showed antifungal activity. The ethanol extract of PB leaf can eliminate *Aspergillus parasiticus* growth and aflatoxin production. The antimycotic activity of the ethanol extract was most pronounced at pH 4 (Chou and Yu, 1984; Lim and Mohamed, 1999).

Antibacterial activity

PB leaf oil possesses strong antibacterial activity and is toxic against gram-positive bacteria *Bacillus subtilis, Bacillus pumilus, Staphylococcus aureus, Salmonella typhi, Vibrio cholerae* and several other pathogenic micro-organisms (Anonymous, 2001).

Anthelmintic activity

The essential oils of PB have been found to be more effective against tape worms (*Taenia solium*) and hookworms (*Bunostomum trigonocephalum*) than the synthetic anthelmintics such as piperazine phosphate and hexyl resorcinol (Anonymous, 2001).

Larvicidal activity

Aqueous extracts, ethanolic extracts and essential oils of PB leaf have been reported to exhibit larvicidal effect on *Chrysomya* larvae, which is responsible for cutaneous myiasis. Hence, this natural product may be effective in the treatment of wound myiasis (Kumarasinghe *et al.*, 2002).

Psychostimulant activity

Studies on the physiological effects of chewing PB leaves have shown that initially chewing of PB quid containing areca nut and other adjuncts causes excitation of the salivary glands and a mild degree of stimulation resulting in a sensation of warmth and well-being, besides imparting a pleasant odor. The active principle responsible for the stimulating effects upon the central nervous system is the alkaloid, arecoline present in areca nut; lime helps in liberation of the alkaloid. The essential oil in the leaf is reported to enhance the effects of areca nut and to act synergistically upon the central nervous system In an *in vitro* study investigating the secretion of catecholamines from chromaffin cells, both arecoline and arecaidine increased secretion in a dose-response fashion. The data suggest that arecoline and arecaidine may have sympathomimetic actions. Similarly, hydroxychavicol, isoeugenol and eugenol, which are phenolic compounds in PB flower or leaf, have also been found to stimulate the release of catecholamines from chromaffin cells, and thus may also contribute to the psychostimulating effects of PB quid chewing (Wang and Hwang, 1997). More interesting was the *in vitro* secretion of catecholamines by different salivas collected from volunteers who were asked to chew various combinations of areca nut, PB flower and red lime. The saliva collected from chewing a mixture of areca nut and red lime released more catecholamines than the saliva from chewing areca nut only or a mixture of PB flower and red lime. The saliva from chewing a mixture of areca nut and PB flower had an even stronger effect. These data seem to suggest that both areca nut and PB flower have sympathomimetic effects *in vitro* (Chu, 2001). In another study, a sympathetic model system of adrenal chromaffin cells and sensory evaluation were used to examine the physiological effects of PB quid and the interaction of all the ingredients (areca nut, PB inflorescence and red time paste) in PB quid. It was evident that the responses in the sympathetic model system were closely correlated with the physiological feeling of well being. The inhibitory effects of all the chewing juices on catecholamine secretion evoked by carbachol and a high concentration of potassium ion showed that they perhaps affected the calcium ion influx through voltage-sensitive channels or the steps

involved in secretion after calcium ion entry to stimulate basal catecholamine secretion from chromaffin cells. Thus PB quid chewing produces a psychostimulant effect by stimulating the release of catecholamines from chromaffin cells (Wang and Hwang, 1997).

Miscellaneous

Oil of PB has been used in the treatment of various respiratory catarrhs, as a local application for wound management (Sachs *et al.*, 2002) and in diphtheria. It also possesses carminative and antispasmodic properties (Anonymous, 2001).

UNTOWARD EFFECTS

Oral submucous fibrosis is a chronic disabling disease developing in up to 0.5% of the estimated 500 million habitual chewers of the PB quid. PB quid chewing shows a strong correlation to the incidence of oral submucous fibrosis and leukoplakia. Hydroxychavicol (4-allyl-catechol), a major phenolic compound in PB leaf may be responsible for the same since it has shown to induce oxidative stress, glutathione depletion and cell cycle deregulation. Hydroxychavicol induced apoptosis and cell cycle arrest are associated with mitochondrial membrane potential depolarization as revealed by a decrease in rhodamine fluorescence. These results indicate that Hydroxychavicol consumption may be associated with betel quid-chewing-related oral mucosal diseases via glutathione depletion, reactive oxygen species production, mitochondrial dysfunction, cell cycle disturbance and the induction of apoptosis. These events are related to the production of superoxide radicals and hydrogen peroxide (Jeng *et al.*, 2004). One of the probable reasons for this is the use of areca nut (*Areca catechu*) with the PB quid. Epidemiological and animal studies have pointed to a close association of oral submucous fibrosis with the prolonged usage of areca nuts (Canniff and Harvey, 1981). The pathobiological effects of aqueous extracts of three PB quid constituents (areca nut, inflorescence of PB and lime), one areca nut alkaloid (arecoline), and one areca nut polyphenol ((+)-catechin) on cultured oral mucosal fibroblasts were studied. Extracts of areca nut and inflorescence of PB induced DNA strand break formation in a dose-dependent manner. Extracts of areca nut and inflorescence of PB, (+)-catechin, and arecoline decreased cell survival and proliferation in a dose-dependent manner. However, aqueous extract of lime increased cell proliferation by 20-40%. These results indicate that PB quid contains not only genotoxic and cytotoxic agents, but also compounds, which stimulate cell proliferation. These compounds may act synergistically in the pathogenesis of oral submucous fibrosis and oral cancer in PB quid chewers (Jeng *et al.*, 2004).

Various studies have been conducted to verify the tumorogenic potential of PB. In one of the studies, the tumor-promoting neoplastic transformation effect of PB quid was examined on JB6 cells. *Microculture tetrazolium* test showed that the extract of PB quid was toxic to JB6 cells. With a long-term treatment (approximately 30 days) of low doses of PB quid, the production of H_2O_2 and the activity of myeloperoxidase and ornithine decarboxylase were increased in JB6 cells (Lin *et al.*, 2003, 2004). In a study, the tumor promoting effects of PB quid and additives in PB quid on epidermal hyperplasia in CD-1 mouse skin were investigated. It was found that PB quid and additives caused significant induction of hyperplasia, but only additives caused an increase of epidermal ornithine decarboxylase. Treatment of mouse skin with additives caused remarkable increases in the production of H_2O_2 as well as marked increases of myeloperoxidase. Application of additives or PB quid also caused induction of protein kinase, additives exhibited more significant effect than BQ. These results indicated that PB has the potential of being a promoting agent, and that additives should play a major role in increasing the effects of PB quid-caused skin hyperplasia and inflammation (Lee *et al.*, 2002).

PB quid chewing has been associated with an increased risk of oral squamous cell carcinoma. PB inflorescence, which contains 0.015% w/w safrole, is a unique ingredient of PB quid. Chewing such prepared PB quid may contribute to safrole exposure in human beings. In a study, using a ^{32}P-post-labeling method a high frequency of safrole-like DNA adducts in PB quid associated oral squamous cell carcinoma and non-cancerous matched tissue have been found. Safrole forms stable safrole-DNA adducts in human oral tissue following PB quid chewing, which may contribute to oral carcinogenesis (Chen *et al.*, 1999). In a study conducted by Chen *et al.* (2002), the hydroxyl radical formation, as represented by the presence of o- and m-tyrosine in saliva from volunteers who chewed PB quid containing phenylalanine was measured. Their saliva contained significantly higher amounts of o- and m-tyrosine as compared to the controls. In addition, chewing PB quid containing PB inflorescence generated higher amounts of m-tyrosine, but not o-tyrosine, in saliva than did chewing PB quid without containing PB inflorescence. The effects of areca nut and PB inflorescence extracts and arecoline on the growth, total DNA synthesis and unscheduled DNA synthesis of cultured human gingival keratinocytes were investigated in a study. Arecoline and areca nut extract suppressed the growth of gingival keratinocytes over 5 days of incubation in a dose-dependent fashion. Arecoline was also toxic to gingival keratinocytes, but did not induce intracellular vacuolization. Simultaneous exposure of confluent gingival keratinocytes to areca nut extract, PB inflorescence extract and arecoline for 1 to 5 days led to different degrees of cytotoxicity that was dose and time-dependent. These results indicate that areca nut, PB inflorescence and arecoline take part in the pathogenesis of PB quid chewing-related oral mucosal lesions, possibly through both genotoxic and non-genotoxic mechanisms (Jeng *et al.*, 1999). PB quid is a promoter rather than an initiator during the carcinogenesis of hamster buccal pouch carcinoma. The maximum promoting activity was demonstrated 24 weeks after PB quid implantation, following an initial application of 7, 12-dimethyl benzanthracene three times per week for 4 weeks. The incidence of tumours in the hamster buccal pouch was significantly higher in groups exposed to dry areca nut fiber and cold aqueous extract of PB quid. The results indicate that betel nut fiber and cold aqueous extract of PB quid may promote carcinogenesis in the hamster buccal pouch (Jin *et al.*, 1996).

Powdered slaked lime applied to the chewed areca nut with PB inflorescence at the corner of the mouth causes the mean pH to rise to 10, at which reactive oxygen species are generated from betel quid ingredients *in vitro*. Reactive oxygen species, together with sustained lime-induced cell proliferation, suggest a possible mechanism of carcinogenesis (Thomas and MacLennan, 1992). In a study in Pakistan where PB quid is usually eaten along with tobacco, it was found that people using PB quid without tobacco were 9.9 times more likely and those using PB quid with tobacco were 8.4 times more likely to develop oral cancer as compared with non-users. Thus, there is an independent effect of PB quid without tobacco in the causation of oral cancer (Merchant *et al.*, 2000).

PB quid chewing has been related to mutagenesis by epidemiological study. Areca nut, one of the common additives of PB quid contains some alkaloids, of which arecoline is the major one. N-nitrosoguvacoline, one of the N-nitrosation products of arecoline, is the only one N-nitrosamine found in the saliva of PB quid chewers. The mutagenic studies in *Ames Salmonella* microsome test showed that crude alkaloid extracts of areca nut and arecoline were active in *Salmonella typhimurium* TA100, and N-nitrosoguvacoline was weakly active in TA98 and TA100. Nitrite was significantly consumed during the N-nitrosation of arecoline and sodium nitrite at acidic condition (pH 3), whereas the formation of N-nitrosoguvacoline was favored at neutral condition (pH 7). Crude phenolic

extracts of leaf and inflorescence of PB inhibited the formation of N-nitrosoguvacoline by blocking the nitrite. However, a high amount of crude phenolic extracts of areca nut enhanced the formation of N-nitrosoguvacoline. Thus the additives used along with PB leaves in PB quid are more responsible for mutagenesis as found in a study (Wang and Peng, 1996).

Chen *et al.* (1984) studied the mutagenic components in the aqueous extracts of PB quid ingredients. Only nitrite-treated aqueous extract of PB fruits, leaves or rhizoma were demonstrated to exhibit a mutagenic response, using *Salmonella typhimurium* strains TA100 and TA1535 in the *Ames* test. When the aqueous extract of the fruit was nitrosated, the greatest number of mutagenic substances was formed at pH 3. The formation of mutagens was enhanced by increasing the temperature from 5 to 95°C. Maximum production of the mutagens occurred within 15 min when nitrosation was conducted at 35°C. The mutagenic components in nitrite-treated aqueous extract of PB fruit were found to be N-nitrosopiperidine, N-nitrosopyrrolidine, N-nitrosomorpholine, and other compounds, as determined by gas chromatography-thermal energy analyzer.

In another study, the mutagenic potential of hydroxychavicol, the major phenolic components of PB inflorescence in *Salmonella typhimurium* TA97, TA98, TA100 and TA102 was tested. The results showed that hydroxychavicol was positive in *Salmonella typhimurium* TA102 without metabolic activation. In Chinese hamster ovary K1 cells, hydroxychavicol-induced chromosome aberrations were observed in a dose-dependent manner and the majority were chromosome-type aberrations. In addition, hydroxychavicol dose-dependently induced copper-dependent strand breaks in plasmid DNA. The oxidative DNA damage potential of hydroxychavicol was determined by measuring 8-hydroxydeoxyguanosine formation in Chinese hamster ovary K1 cells and it was found that hydroxychavicol induced increase in 8-hydroxydeoxyguanosine levels dose-dependently (Lee-Chen *et al.*, 1996).

PB inflorescence extracts contain eugenol (6.2%) and safrole (78.9%). Intravenous injections of aqueous extracts of PB inflorescence, eugenol and safrole in rats induce hypotensive and bradycardiac effects, whereas both intraarterial and intrathecal injections of aqueous extracts of PB inflorescence, eugenol and safrole results in hypotensive and tachycardiac effects. Eugenol and safrole induce the same pattern on blood pressure and heart rate changes as aqueous extracts of PB inflorescence in rats after various treatments. Thus it can be suggested that acute administration of PB inflorescence extracts by different routes may activate C-fiber-evoked parasympathetic and sympathetic cardiovascular reflexes in rats resulting in arrythmia (Chen *et al.*, 1995).

CONCLUSION

For ages PB has been used for the treatment of various ailments in many Asian countries. Its use has also been mentioned in Ayurveda and Unani systems of medicine. PB leaf is popularly taken as a masticatory in the form of quid. PB leaf consists of essential oils, sugars, tannin, protein, vitamins, fat, fibre and mineral matter. Of the essential oils eugenol is the predominant constituent and chavibetol is the characteristic constituent of PB. Various therapeutic potentials of PB such as vasorelaxatory, antioxidative, antiplatelet, antifertility, anticarcinogenic, antimutagenic, digestive, antifungal, antibacterial, anthelmintic, larvicidal, wound healing, carminative, antispasmodic and psychostimulant activities have been reported. Together with this, some untoward effects of PB have also been reported. Several authors have claimed PB as a causative factor for oral submucous fibrosis and oral squamous cell carcinoma; it is also reported as a tumorigenic.

These effects have been correlated with PB quid chewing and additive ingredients in it, like tobacco, areca nut and slaked lime. Some of the reported therapeutic and untoward effects are

contra-indicatory to each other e.g. reported carcinogenic and anticarcinogenic potential of PB. As a result of such anomalous reports, detailed study on PB is essential to establish the actual facts, especially in regards to the untoward effects. It is necessary to establish the phytoconstituents responsible for the therapeutic potential or untoward effects of PB.

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