

RESEARCH COMMUNICATION

Anticarcinogenic Effects of an Aqueous Infusion of Cloves on Skin Carcinogenesis

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Abstract

Spices and flavouring agents are now receiving increasing attention as many of them have been shown to have anticarcinogenic properties. Cloves, sundried unopened flower buds from the plant *Syzygium aromaticum* L, are commonly used as a spice and food flavour. The present study was designed to investigate the chemopreventive action of aqueous infusion of cloves on 9,10-dimethyl benz(a)anthracene (DMBA) and croton oil induced skin carcinogenesis in Swiss mice. The results indicate protection against skin papilloma formation in a dose dependent manner. It has been shown that oral administration of aqueous infusions of clove at a dose of 100µl/ mouse/day not only delays the formation of papilloma but also reduces the incidence of papilloma as well as the cumulative number of papillomas per papilloma bearing mouse. Our observations suggest a promising role for cloves in restriction of the carcinogenesis process.

Key Words: Clove - Skin carcinogenesis - Cancer chemoprevention - Anticarcinogen.

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Introduction

Human epidemiological and animal studies have indicated that cancer risk may be modified by changes in dietary habits or dietary supplements. Experimental studies indicate that phytochemicals with antioxidative and anti-inflammatory properties can inhibit tumour initiation, promotion and progression. Food flavouring spices are known to possess active compounds with medicinal properties. Current focus is on evaluating their anticarcinogenic potential.

Clove, (*Syzygium aromaticum* L.) is used as a spice to add flavor to exotic food preparations. The major chemical constituents of clove include sesquiterpenes, volatile oil (eugenol), caryophyllene, tannins and gum (International Cyber Business Service, 2000; Zheng et al., 1992). Essential oils present in the dried flower buds of clove are eugenol, caryophyllene, alpha-humulene, alpha-terpinyl acetate, eugenyl, methyl eugenol, acetyl eugenol, naphthalene, chavicol, heptanone, sesquiterpenes, methyl salicylate pinene, vanillian (Srivastava and Malhotra, 1991; Zheng et al., 1992; Duke and du Cellier, 1993; International Cyber Business Service, 2000). Among different essential oil eugenol is the principle component, present in amount of 81.1%. Beside this trans-caryophyllene and isoeugenol is present in amount of 7% and 10.1% respectively (Zheng et al., 1992).

Since a number of bacteria and viruses have been implicated in the etiology of cancer and antioxidants have a role on cancer prevention it seemed worthwhile to scrutinize the action of clove, which is known to have antimicrobial and antioxidative properties against cancer. To date the anticarcinogenic possibility of clove has not been explored. Therefore the present study was an endeavour to examine the anticarcinogenic action, if any, of clove using two-stage skin carcinogenesis model in mice, and reports our preliminary observation.

Materials and Methods

Chemicals: 9,10-dimethyl benz(a)anthracene (DMBA) and croton oil were purchased from Sigma Chemicals Co, USA. Acetone was purchased from E. Merck India.

Induction of Skin Carcinogenesis with DMBA-Croton Oil: Female Swiss albino mice in the age group of 5-6 weeks, weighing 20-22 gm were housed in plastic cages with stainless steel wire lid (6 mice per cage). The housing facility was maintained at 30°C.

To induce carcinogenesis the mice received three topical applications of 100 nmol DMBA in 100µl acetone at an interval of 2 days on the shaved skin (initiator) followed by 100µl of 1 gm% croton oil in acetone (promoter) after a week at the same site twice weekly for 8 weeks. The normal

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control group was kept without any carcinogen application.

Preparation of Test Material:

Cloves was purchased from a Govt. shop, Kolkata. It was then powdered, soaked overnight in double distilled water (2gm/100ml) for preparation of an infusion. The infusion was administered at three different doses: 200µl/mouse/day, 100µl/mouse/day, 50µl /mouse/day. Experimental period: 12 weeks

Treatment Groups:

Normal Control Group (NC): This group comprised of healthy normal mice receiving only distilled water every day during treatment of other groups.

Carcinogen Control Group (CC): The animals received topical applications of DMBA and croton oil and only distilled water as in case of the NC group.

Group-I: The animals of this group were administered clove infusion topically at a dose of 200µl, 100µl or 50µl /mouse/day for 1 week from the day of application of DMBA and then the treatment was withdrawn.

Group-II: The animals of this group were administered clove infusion topically at a dose of 200µl, 100µl and 50µl /mouse/day started on the same day as the first application of DMBA. Treatment with clove infusion continued upto 8th week along with croton oil application.

Group-III: The animals of this group were administered clove infusion topically starting from the day of first application of DMBA and continued upto 12th week, at a dose of 200µl, 100µl or 50µl /mouse/day.

Group-IV: The animals of this group were administered clove infusion orally starting from the day of first application of DMBA and continued upto 12th week, at a dose of 200µl, 100µl and 50µl /mouse/day.

Number of animals for NC and CC group was 30. In case of Gr I- IV, number of animals for each dose of test material was also 30.

Incidence and Cumulative Number of Papilloma in Different Groups:

Papilloma count in mice Gr NC, Gr CC, Gr I, Gr II, and Gr III, Gr IV were recorded from 5th week of DMBA application at weekly interval upto 12th week.

Statistical Analysis

Data from different experimental groups were analysed and expressed as mean ± SD. In skin papillogenesis the significant level of difference between control and treated values were also statistically analysed using Student's t-test. A value of p < 0.05 was considered to be significant.

Results

All DMBA-croton oil treated mice had a visible rough granular surface on the shaved skin with varying degrees of erythema and sometimes with white plaque like lesion.

Gradually wart (papilloma) or small tumour like outgrowths appeared on the skin of DMBA-croton oil treated mice after 12 weeks. Skin of normal animals remained healthy through out the experimental period.

Onset of Papillomas:

Treatment with aqueous infusion of clove delayed the onset of papillomas in treated groups (GrI, GrII, GrIII, GrIV) as compared to DMBA-croton oil treated carcinogen control group. Treatment was most effective in Gr. IV receiving clove infusion orally.

The onset of papilloma was noted at 5th week in DMBA-croton oil treated mice. At the lowest dose of 50 µl of clove infusion onset of papilloma of all the groups (Gr I, II, III, IV) was same as in CC (Fig1). But at the dose of 100 µl the onset of papilloma was delayed in Gr III and Gr IV by two weeks (Fig2). 200 µl clove infusion also delayed onset of papilloma in Gr III and Gr IV but not to the extent seen with 100 µl (Fig3).

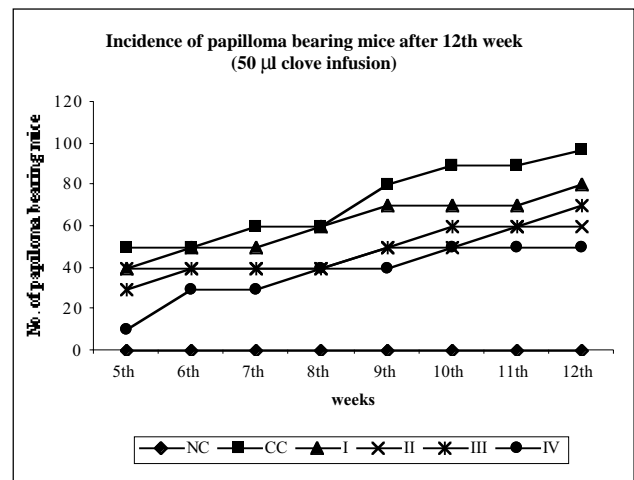


Figure 1. Effect of Aqueous Infusion of Clove at a Dose of 50µl/mouse/day on Incidence of Skin Papilloma at Different Week in Different Groups

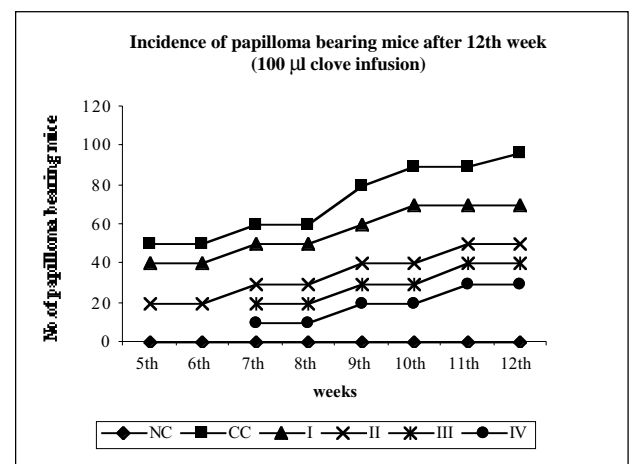


Figure 2. Effect of Aqueous Infusion of Clove at a Dose of 100µl/mouse/day on Incidence of Skin Papillomas at Different Week in Different Groups.

Incidence of Papillomas:

Number of papilloma bearing mice was recorded from 5th weeks onwards since papillomas were observed from this week in CC Gr.

At the end of the experimental period (i.e. after 12 weeks), in DMBA-croton oil treated mice (Gr.CC) incidence of papilloma was 97.33% whereas in DMBA-croton oil + clove infusion treated groups (GrI, GrII, GrIII, Gr IV), different results were obtained at three different doses. Treatment groups revealed lowered incidence of papilloma in comparison to carcinogen control group. At the dose of 50 µl, incidence of papilloma bearing mice in Gr.I, Gr.II, Gr III, Gr.IV were 80%, 60%, 70%, 50% (Fig1) while at 100µl dose percentage of incidence of papilloma bearing mice in treated groups (I, II, III, IV) were 70, 50, 40, 30 respectively (Fig 2) at the end of experiment. In case of dose of 200 µl, incidences of papilloma at 12th week were recorded as 80%, 60%, 60%, and 40% in GrI, GrII, GrIII, and Gr IV respectively (Fig 3). Oral administration was formed to be more effective than topical application in reducing incidence of papilloma. No tumour formation was seen in the normal control animals.

Cumulative Number of Papillomas per Papilloma Bearing Mouse:

The mean number of papilloma/mouse (papilloma burden) was also significantly reduced by treatment with clove infusion in comparison to the carcinogen control group ($p < 0.05$). Most effective result was noted in Gr. IV receiving 100 µl of clove infusion orally. Papilloma burden in carcinogen control group was 3.4 ± 0.31 at 12th week. This was reduced to 2.75 ± 0.31 , 2.33 ± 0.28 , 2.28 ± 0.23 , 2 ± 0.29 in Gr I, Gr II, Gr III, Gr IV respectively after 12 weeks of treatment with 50µl of clove infusion (Fig 4). At a dose of 100µl, papilloma burden was further reduced to 2 ± 0.40 , 1.8 ± 0.48 , 1.5 ± 0.48 , and 1.5 ± 0.44 in GrI, Gr II, Gr III, Gr IV respectively (Fig 5). The same was found to be 2.5 ± 0.30 , 2 ± 0.29 , 2.8 ± 0.32 , 1.75 ± 0.35 in GrI, Gr II, Gr III, Gr IV respectively when the dose used was 200µl (Fig 6).

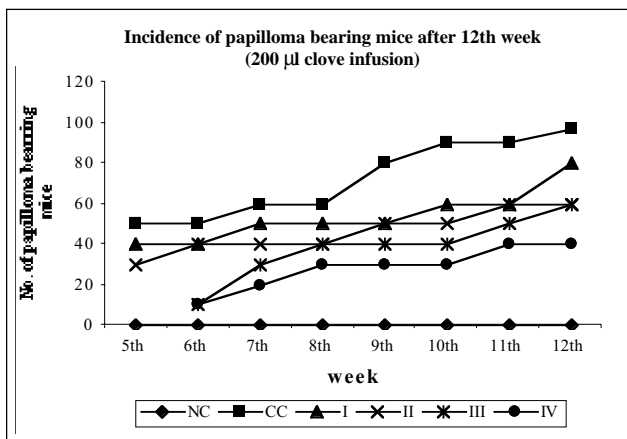


Figure 3. Effect of Aqueous Infusion of Clove at a Dose of 200µl/mouse/day on Incidence of Skin Papillomas at Different Week in Different Groups

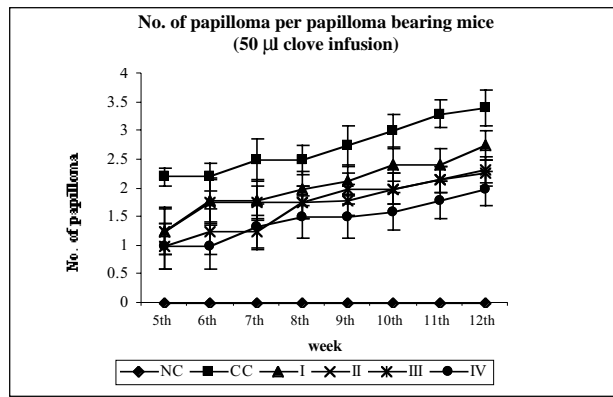


Figure 4. Role of Clove Infusion at a Dose of 50µl/mouse/day on Cumulative Number of Papilloma per Papilloma Bearing Mouse at Different Week in Different Groups. Data represented as Mean±SD. Comparisons are made between Gr.CC and Gr.I; Gr.CC and Gr.II; Gr.CC and Gr.III; Gr.CC and Gr.IV. $P < 0.05$.

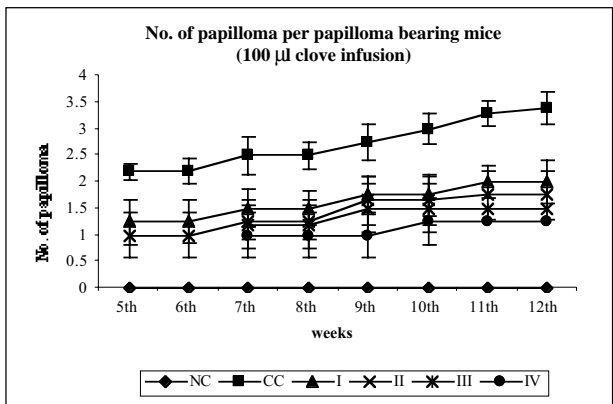


Figure 5. Role of Clove Infusion at a Dose of 100µl/mouse/day on Cumulative Number of Papilloma per Papilloma Bearing Mouse at Different Week in Different Groups. Data represented as Mean±SD. Comparisons are made between Gr.CC and Gr.I; Gr.CC and Gr.II; Gr.CC and Gr.III; Gr.CC and Gr.IV. $P < 0.05$.

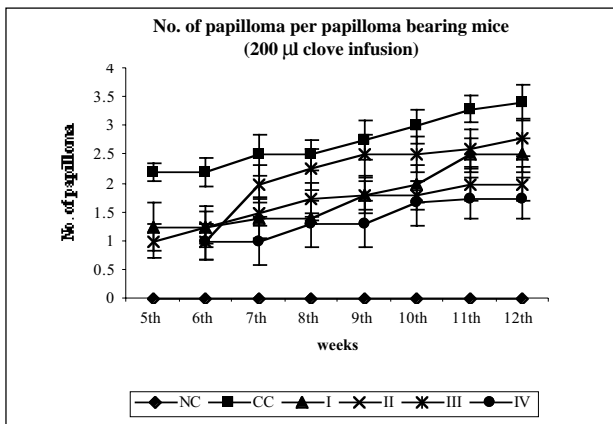


Figure 6. Role of Clove Infusion at a Dose of 200µl/mouse/day on Cumulative Number of Papilloma per Papilloma Bearing Mouse at Different Week in Different Groups. Data represented as Mean ±SD. Comparisons are made between Gr.CC and Gr.I; Gr.CC and Gr.II; Gr.CC and Gr.III; Gr.CC and Gr.IV. $P < 0.05$.

Discussion

Humans are exposed to large numbers of carcinogenic chemicals and other carcinogenic stimuli in their daily life. Small amount of many naturally occurring mutagens and carcinogens are also ingested through our normal diet. Besides such carcinogens and mutagens our diet also provide a large number of naturally occurring anti-mutagens anti-carcinogens (Ames, 1983; Beecher, 1995). Epidemiology and animal model studies do indicate that cancer risk may be influenced by dietary factors (Kraemer et al., 1988; Doll, 1992; Wattenberg, 1992). It is now believed a wide variety of naturally occurring substances from plant food offer protection from carcinogenic exposure (Das, 2004a). There has been a growing realization that spices possess anticarcinogenic properties which is supported by experimental evidences (Unnikrishnan and Kuttan, 1990; Das, 2004b; Das et al., 2004; Sengupta et al., 2005)

Clove, used for food flavoring, was used by the traditional Ayurvedic healers of India since ancient times to treat respiratory and digestive ailments. Clove is the sundried unopened flower buds with a short stock of the plant *Syzygium aromaticum* L. (syn. *Eugenia caryophyllus*, *E. aromatica*, *Caryophyllus aromaticus*), belonging to the family Myrtaceae. Aqueous clove infusion was found to inhibit growth of germinated spores of *Bacillus subtilis* (El Hag et al., 1999), and inhibit the pathogens *Campylobacter jejuni*, *Salmonella enterides*, *S.aureus* and *Escherichia coli* (Al-Khayant and Blank, 1985). Clove may therefore be considered an effective antimicrobial agent and may be used as an antiseptic.

There are several reports to suggest antiviral activity of clove and its components. An anti-herpes virus compound eugenin was purified from clove, which could inhibit viral DNA synthesis (Montes-Belmont and Carvajal, 1998). Water extracts of clove have inhibitory effect on hepatitis C virus protease (Shiraki et al., 1998). In gastric cancer *Helicobacter pylori* (HP) is well recognized as being the primary etiological agent for gastric cancer and clove has been shown to inhibit the growth of HP and thereby reduce the risk of gastric cancer (Bhamarapavati et al., 2003). Eugenol, the principle component of clove has been shown to prevent lipid peroxidation (Hussein et al., 2000) and also known as strong scavengers of active oxygen radicals (Oya et al., 1997) Observation from our study on mouse skin model strongly suggests that aqueous infusion of clove can restrict carcinogenesis. Aqueous infusion of clove was found to produce a remarkable reduction in the incidence of mouse skin papilloma, which is a pre-neoplastic benign growth and also the tumour multiplicity in a dose dependent manner. Out of three doses used (50 µl, 100 µl, 200 µl), 100µl of clove infusion/mouse/day administered orally was considered to be the most effective dose for chemopreventive intervention. This dose (100µl of clove infusion/mouse/day orally given) has been found to delay the onset of papilloma by two weeks, markedly reduced incidence of papilloma and multiplicity of tumour than the other two doses (50 µl

& 200 µl).

No adverse effect or toxic effect of this most effective dose (100 µl/mouse/day, orally administered) of clove infusion was noted to ascertained on normal mice for one month. This was based on comparison of body weight, skin texture, food intake and general behavior observed between untreated and clove treated mice, no adverse response was noted in this selected dose.

This study is important in that it is the first of its kind to provide experimental support to suggest that clove may be considered as a potential cancer chemopreventive agent. Further studies are in process to understand the mechanism of anticarcinogenic action of aqueous clove extract and to identify the active compounds.

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