Phytopharmacology **2011, 1(4) 71-81**

Phytopharmacology

Inforesights Publishing

Modulatory effects of *Syzygium aromaticum* (L.) Merr. & Perry and *Cinnamomum tamala* Nees & Ebrem. on toxicity induced by chromium trioxide

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Received: 29 June 2011, **Revised**: 14 July 2011 **Accepted**: 14 July 2011

Abstract

Hexavalent chromium trioxide is known to cause diseases like cancer and specific biological effects on the respiratory system. It induces oxidative stress and lead to formation of stable Cr-DNA adducts that contribute to its cytotoxic and genotoxic effects. In the present study, the antigenotoxic effects of Indian spices viz. *Syzygium aromaticum* (L.) Merr. & Perry and *Cinnamomum tamala* Nees & Ebrem. was evaluated using the *Allium cepa* root chromosomal aberration assay against CrO_3. Roots were given three kinds of treatment. In pre-treatment, roots were first treated with different concentrations of methanol extract of *Syzygium aromaticum* (MSA) and *Cinnamomum tamala* (MCT) (0.1%, 0.50% and 1%) for 2 h followed by chromium trioxide treatment (CrO_3-8 ppm, 2 h). In post-treatment roots were first treated with CrO_3 (8 ppm, 2 h) followed by different concentrations (0.1%, 0.50% and 1%) of MSA and MCT extract for 2 h. In simultaneous treatment, the root tips are treated with CrO_3 (8 ppm) and different concentrations of MSA and MCT extract (0.1%, 0.50% and 1%) simultaneously for 2 h. The treatment of roots with 8 ppm CrO_3 served as positive control. The effects of pre-, post- and simultaneous treatment of MSA and MCT extracts resulted in a dose-dependent decrease in chromosomal aberrations frequency.

Keywords: Chromium trioxide; *Syzygium aromaticum*; *Cinnamomum tamala*; Antigenotoxic.

Introduction

Chromium compounds are prevalent in the industrial areas due to their use in welding, leather manufacturing and metal surface dumping etc. Chromium in environment is introduced mainly because of combustion of fuel, industrial processes. Chromium has many

oxidation states but it is mainly present in hexavalent and trivalent states. Chromium hexavalent compounds are known to cause of various types of cancer (Langard, 1988). Hexavalent chromium exists in the form of an oxyanion at physiological pH. These oxyanions with help of sulfate transport system are transported into the cells leading to their accumulation. Inside the cells, chromium (VI) reacts with numerous reducing agents such as hydrogen peroxide, glutathione, ribonucleotides etc. (Levis and Bianchi, 1982; Snow, 1994).

Plants and plant products are inescapable part of the vegetarian diet and many of them exhibit valuable medicinal properties. In the recent years, there has been an increasing interest in antimutagenesis (Calomme, 1996) and antioxidant activity (Yagi et al., 2002) of dietary components. These components may be useful in preventing cancer and other mutation related diseases by fortifying physiological defense mechanisms (De Flora, 1996). Antimutagenic agents are natural or synthetic compounds capable of lowering the frequency of mutation by diverse mechanisms (De Flora and Ramel, 1988). From olden days, people have recognized the worth of using different spices for preserving foods and for their medicinal value (Jones, 1996).

Spices are added in food stuffs in various forms viz. as whole spices, as ground spices or as isolates from their crude extracts (Schwarz et al., 2001). Human diet containing spices have been known to contain valuable components including polyphenols with antioxidant properties (Croft, 1998). Studies have indicated that diets rich in fruits, herbs and spices are related with a low risk of diseases in humans (Stavric, 1994). Therefore, the regular intake of antimutagens and anticarcinogens is most effective for preventing human cancer and genetic diseases (Irulappan and Natarajan, 2007).

Various genotoxicity tests developed over the past years can be used in a variety of models (prokaryotic, eukaryotic, *in vitro* and *in vivo*) and end points (gene mutation, chromosomal aberrations, DNA damages). In fact, these tests were originally developed to detect genotoxic and carcinogenic substances. But from the last two decades, they were also used for the assessment of antigenotoxic and anticarcinogenic effects of different compounds (De Flora, 1992; Graf, 1998). Higher plants are recognized as excellent genetic models and are frequently used to monitor environmental pollutants (Leme and Marin-Morales, 2009).

Syzygium aromaticum (L.) Merr. & Perry. (Family Myrtaceae), commonly known as clove. It is commonly used as spice in Indian food delicacies. In Ayurvedic system of medicine, the *Syzygium aromaticum* (clove) is popularly known for its aphrodisiac property and used to treat male sexual disorders (Sharma, 2001, Tajuddin et al., 2003). *Cinnamomum tamala* Nees & Eberm commonly known as Tej pat or Indian cassia (Family Lauraceae). Leaves and bark of plant have aromatic, astringent, stimulant and carminative properties and commonly used in the treatment of rheumatism, colic, diarrhea, nausea and vomiting etc.

The *Allium cepa* has been considered as an efficient test organism to indicate the presence of mutagenic chemicals (Fiskesjo, 1985), due to its sensitivity, low cost and correlation with mammalian test system (Cabrera and Rodriguez, 1999; Yi and Meng, 2002). In the present study, we have used *Allium cepa* root chromosomal aberration assay to evaluate anti-genotoxic potential of spices viz. *Syzygium aromaticum* (L.) Merr. & Perry and *Cinnamomum tamala* Nees & Ebrem. against chromium trioxide induced genotoxicity.

Material and Methods

Collection and Extraction

Flower buds of *Syzygium aromaticum* Linn and leaves of *Cinnamomum tamala* Nees & Eberm. were purchased from the local market at Amritsar, Punjab, India. These were ground to fine powder. The finely ground powder of each spice was extracted with methanol for 24 h using maceration method. The supernatant was filtered using Whatman no.1 filter paper and concentrated using vacuum rotary evaporator to obtain methanol extract of *Syzygium aromaticum* (MSA) extract and *Cinnamomum tamala* (MCT) extract respectively.

Phytochemical analysis

The MSA and MCT extracts were analyzed for the presence of different phytoconsitituents viz anthraquinones, glycosides, saponins, flavonoids and Tannins (Trease and Evans, 1996; Wagner and Baldt, 1996; Bot et al., 2007).

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Antigenotoxic effect of spices

Spices were evaluated for their protective effect against the genotoxicity of chromium trioxide(CrO_3 – 8 ppm) by using the *Allium cepa* chromosomal aberration assay. Onion bulbs of *Allium cepa* were obtained from local market. The loose outer scales were carefully removed and the bottoms were scraped to expose root primordia. Onion bulbs were placed on coupling jars filled with distilled water until the roots emerged and grew to average length of 0.5-1cm. Roots were given three modes of treatment. In pre-treatment, roots were first treated with different concentrations of MSA and MCT extracts (0.1%, 0.50% and 1%) for 2 h followed by chromium trioxide treatment (CrO_3-8 ppm, 2 h). In post-treatment roots were first treated with CrO_3 (8 ppm, 2 h) followed by different concentrations (0.1%, 0.50% and 1%) of MSA and MCT extract for 2 h. In simultaneous treatment, the root tips are treated with CrO_3 (8 ppm) and different concentrations of MSA and MCT extracts (0.1%, 0.50% and 1%) simultaneously for 2 h. The treatment of roots with 8 ppm CrO_3 and distilled water served as positive and negative control respectively. After the treatment, the root tips were fixed in farmer′s fluid for 24 h followed by transfer to 70% alcohol and stored at 4°C. The slides were prepared by hydrolyzing the root tips in 1N HCl with intermittent heating for 1 min. and transferred to watch glass containing a mixture of 1N HCl and aceto orcein stain (2% orcein in 45% glacial acetic acid in the ratio of 1:9). The watch glass with root tips was warmed intermittently for 2-3 min. covered and kept aside for 30 min. The root tips were then squashed in a drop of 45% of acetic acid by tapping with match stick and mounted with DPX. The cells were scored at different stages of mitosis. Different types of chromosomal aberrations were noticed. About 600-700 dividing cells from 6-8 root tips were scored for each treatment**.**

The antigenotoxic potential of methanol extract of spices was calculated using given formula. Inhibitory activity (%) = a-b/a-c×100, Where a = number of aberrant cells induced by Chromium trioxide (8ppm) (positive control), b= the number of aberrant cells induced by Chromium trioxide in presence of MSA/MCT extracts, c = number of aberrant cells induced in the negative control (distilled water).

Statistical analysis

Results are presented as the average and standard error of two independent experiments. The data were analyzed for statistical analysis of variance (one way ANOVA) and the difference among means was compared by high-range statistical domain (HSD) using Tukey′s test. The term significant had been used to indicate differences for which $p \leq 0.05$.

Results

Phytochemical analysis

The phytochemical analysis demonstrated that MSA was found to be rich in glycosides, flavonoids, saponins and tannins while MCT showed the presence of glycosides, flavonoids and saponins (Table-3).

Antigenotoxicity

The genotoxic effects of different concentrations of CrO_3 (2-10 ppm) were first evaluated. CrO_3 (8 ppm) induced chromosomal aberrations such as physiological aberrations (74.59%) included C- mitosis, delayed anaphase/s, laggard/s, stickiness, vagrant/s

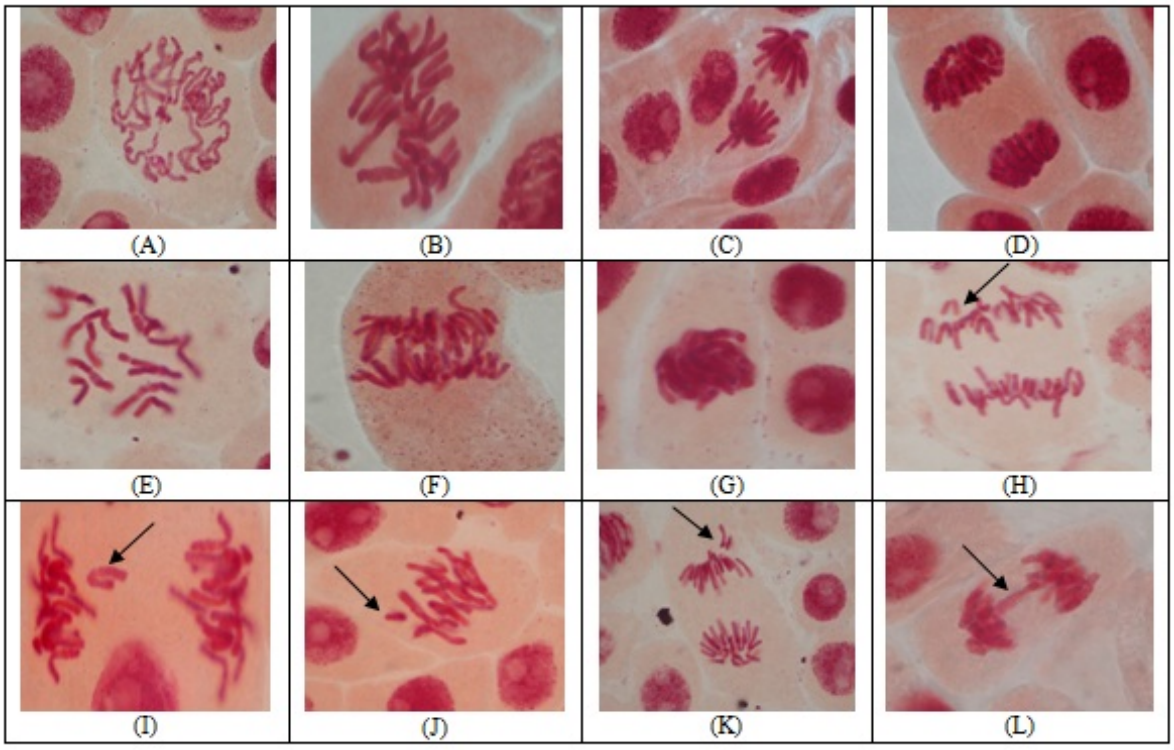

Figure 1. Control stages of mitosis (A-D) (A) Prophase (B) Metaphase (C) Anaphase (D) Telophase: Disturbed stages of mitosis in root tip cells of *A. cepa* treated with Chromium trioxide (E-L) (E) C-mitosis (F) Delayed anaphase (G) Stickiness (H) Vagrant (I) Laggard (J) Chromosomal break at metaphase (K) Chromosomal break at anaphase (L) Chromatin bridge.

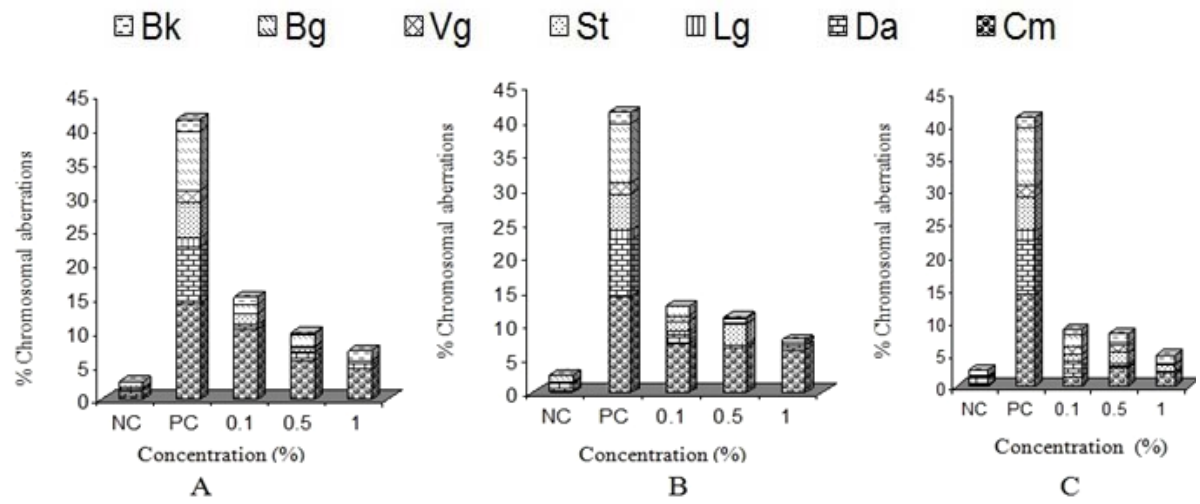

Figure 2. Effect of MSA extract on % clastogenic and physiological chromosomal aberrations induced by CrO_3: A- Pre-treatment; B- Post treatment; C- Simultaneous treatment

clastogenic aberrations (25.40%) included chromatin bridge/s, chromosomal break/s (Figure 1). It induced more physiological aberrations than clastogenic aberrations. C-mitosis was the most frequent kind of aberration in dividing cells. The effects of pre-, post- and simultaneous treatment of MSA and MCT extracts resulted in a dose-dependent decrease in chromosomal aberrations frequency (Figure 2, 3, 4). The inhibitory percentage ranges from 88.70% to 94.35% (MSA extract) and 85.22% to 86.96% (MCT extract) at the maximum tested dose of 1% (Table 1, 2). All the three types of treatment viz pre-, post- and simultaneous were found to be equally effective. The simultaneous treatment of MSA extract was found to be very effective and resulted in a significant decrease in the physiological aberrations (3.24 % at the highest dose tested) and post treatment of MSA extract found to reduce clastogenic aberrations up to 1.15% at the highest dose tested. On other hand post-treatment of MCT

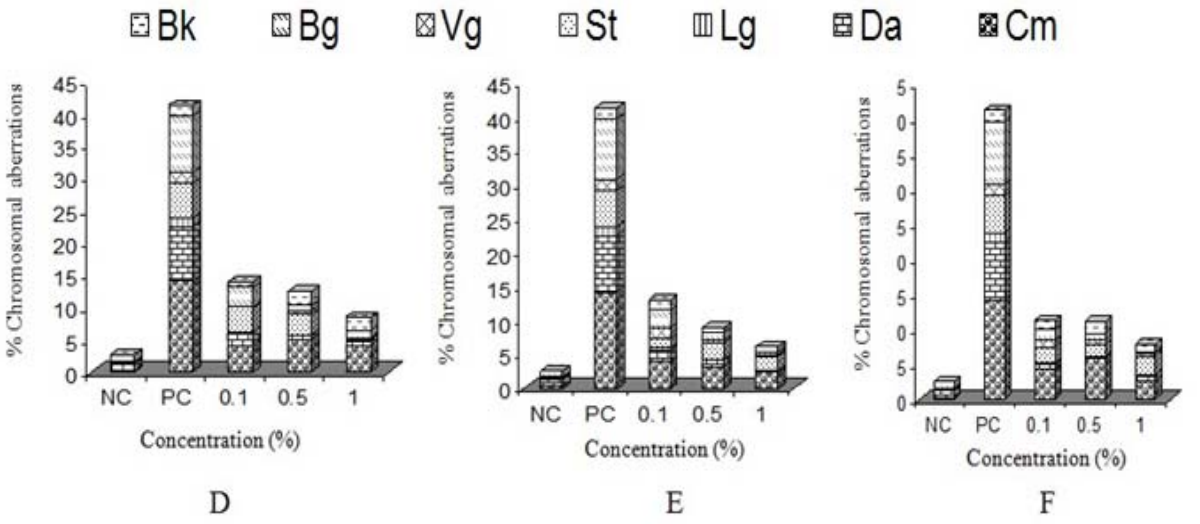

Figure 3. Effect of MCT extract on % clastogenic and physiological chromosomal aberrations induced by CrO_3: D- Pre-treatment; E- Post treatment; F- Simultaneous treatment.

Table 1: Effect of methanol extract of *Syzygium aromaticum* (MSA extract) on genotoxicity of Chromium trioxide (8 ppm)

Treatment	Concentrations (%)	No. of cells	Physiological Aberration(PA)					Clastogenic Aberration(CA)		Total Aberrant Cells	Percentage Aberrant Cells±SE	% inhibition
			Cm	Da	Lg	St	Vg	Bg	Bk			
NC	-	700	02	07	-	01	01	07	-	18	02.92±0.05	-
PC	-	600	85	50	08	32	10	53	10	248	41.33±0.67[a]	-
Pre	0.1	600	61	05	-	10	-	08	06	90	15.00±0.33[a]	68.70
	0.50	600	34	07	01	04	01	10	02	59	09.83±0.50[b]	82.17
	1	611	28	03	-	03	-	-	10	44	07.20±1.98[c]	88.70
Post	0.1	601	43	08	03	09	05	09	02	79	13.14±0.81[b]	73.48
	0.50	600	40	02	-	18	01	08	02	71	11.83±0.50[c]	76.96
	1	608	38	04	01	02	-	07	-	52	8.55±0.86[d]	85.21
Simultaneous	0.1	648	10	15	-	08	06	12	06	57	7.45±1.96[b]	83.04
	0.50	600	17	02	02	10	06	03	09	49	8.16±1.16[bc]	86.52
	1	647	13	02	-	06	-	02	08	31	04.79±0.24[d]	94.35

PC= Positive control (CrO_3-8 ppm); NC-Negative control (distilled water); Cm-C-mitosis; Da- Delayed anaphase/s; Lg-Laggard/s; St-Stickiness; Vg-Vagrant/s; Bg- Chromatin bridge/s; Bk-Chromosomal break/s

One-way ANOVA
Pre-treatment: (F ratio = 125.63*; HSD = 5.52)
Post-treatment: (F ratio = 111.94*; HSD = 5.71)
Simultaneous-treatment: (F ratio = 178.44*; HSD = 5.07)
Different letters (a-d) within the row indicates that concentrations are significantly differs from each other (*$p \leq 0.05$).

Table 2. Effect of methanol extract of *Cinnamomum tamala* (MCT extract) on genotoxicity of Chromium trioxide (8ppm)

Treatment	Concentration	No. of cells	Physiological Aberration					Clastogenic Aberration		Total Aberrant Cells	Percentage Aberrant Cells±SE	% inhibition
			Cm	Da	Lg	St	Vg	Bg	Bk			
NC	-	700	02	07	-	01	01	07	-	18	02.92±0.05	-
PC	-	600	85	50	08	32	10	53	10	248	41.33±0.67[a]	-
Pre	0.1	612	25	13	-	23	01	18	05	85	13.88±0.40[b]	70.87
	0.50	612	30	05	-	20	04	07	14	80	13.08±0.50[c]	73.04
	1.00	612	25	04	-	02	01	8	12	52	08.49±0.24[d]	85.22
Post	0.1	603	24	10	02	08	10	16	09	79	13.1±0.44[b]	73.48
	0.50	613	20	06	-	15	02	07	05	55	08.97±0.56[c]	83.91
	1.00	600	14	01	-	13	02	02	05	37	06.17±0.17[d]	91.74
Simultaneous	0.1	602	26	04	01	14	06	09	08	68	11.63±1.04[b]	78.26
	0.50	604	36	01	-	10	05	05	10	67	11.09±0.17[c]	78.70
	1.00	613	16	05	01	16	02	02	06	48	07.82±0.29[d]	86.96

PC= Positive control (CrO_3-8 ppm); NC-Negative control (distilled water); Cm-C-mitosis; Da- Delayed anaphase/s; Lg-Laggard/s; St-Stickiness; Vg-Vagrant/s; Bg- Chromatin bridge/s; Bk-Chromosomal break/s

One-way ANOVA
Pre-treatment: (F ratio = 86.07*; HSD = 5.31)
Post-treatment: (F ratio = 152.49*; HSD = 5.19)
Simultaneous-treatment: (F ratio = 162.95*; HSD = 4.84)
Different letters (a-d) within the row indicates that concentrations are significantly differs from each other (*p≤0.05).

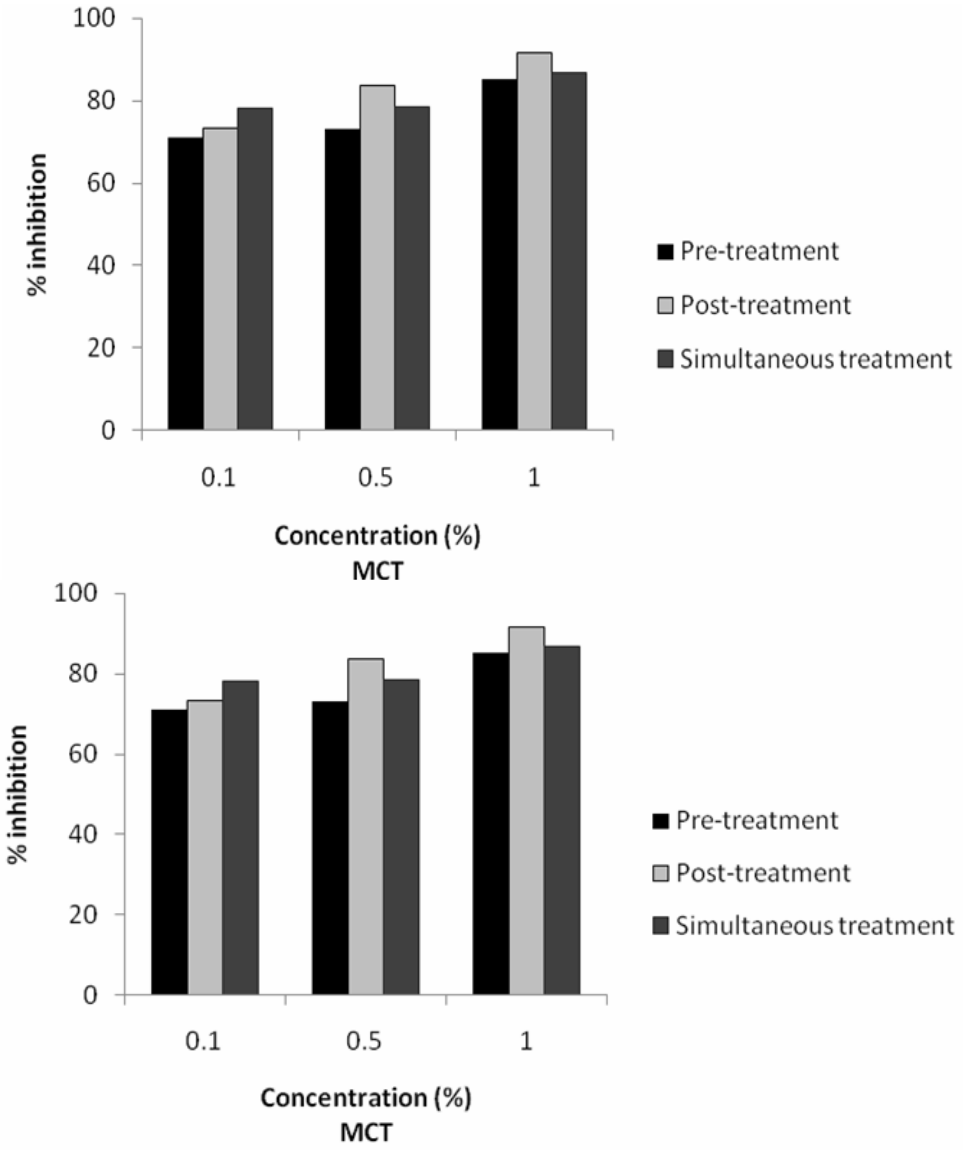

Figure 4. Antigenotoxic potential of spices against Chromium trioxide (8 ppm)

extract caused significant reduction in both physiological aberrations (5.00%) and clastogenic aberrations (1.16%) at the highest dose tested.

Discussion

Results obtained from this study showed that both spices reduced all types of aberrations significantly (Figure 2). In case of MSA extract of *Syzygium aromaticum*, antigenotoxicity i.e. percent inhibition of the chromosomal aberrations was found to be 88.70%, 85.21% and 94.35% in pre-, post- and simultaneous mode of treatments respectively. This antigenotoxic potential of the MSA extract may in part be a contribution of phytoconstituents as shown by phytochemical analysis (Table 3). The main inhibitory component of *S. aromaticum* is eugenol. Eugenol (2-methoxy-4-(2-propenyl) phenol) is naturally occurring phenolic compound in *S. aromaticum* oil. Eugenol has been shown to possess many medicinal properties such as antispasmodic (Wagner et al., 1979), antipyretic

Table 3: Phytochemical analysis data of MSA and MCT extracts.

Phytochemicals	***Syzygium aromaticum* Linn. (MSA)**	***Cinnamomum tamala* Nees & Ebern. (MCT)**
Glycosides	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	-
Anthraquinones	-	-

(Feng and Lipton, 1987), anti-inflammatory (Hume, 1983) and antibacterial (Moleyar and Narasimham, 1992). *S. aromaticum* has been reported to show anticarcinogenic effect against 7,12-dimethylbenz[a]anthracene induced carcinogenesis in Swiss mice (Banerjee and Das, 2005). The aqueous extract of clove and eugenol significantly inhibited 5-Lox enzyme activity in human polymorphonuclear leukocytes cells (Raghavenra et al., 2006). Clove has also been reported to provide protection against peroxynitrite-mediated tyrosine nitration and lipid peroxidation (Ho et al., 2008; Chericoni et al., 2008). MCT extract of *Cinnamomum tamala* was also found to be quite effective in inhibiting the genotoxicity of CrO_3 (8 ppm). The inhibitory percentage was 85.22%, 91.74% and 86.96% at the maximum tested dose (1%) during pre-, post- and simultaneous mode of treatments. The protective effect of *C. tamala* may be due to the presence of various active components including cinnamaldehyde, cinnamyl acetate and cinnamyl alcohol and various volatile substances (Matan et al., 2006). Thus presence of polyphenolic compounds in the *C. tamala* may be responsible for antimutagenicity of extracts. *C. tamala* constituents possess antioxidant potential and also prevent free radical damage (Lee and Shibamoto, 2002; Dragland et al., 2003; Jayaprakasha et al., 2003). Al-Attar (Al-Attar, 2007) reported the protective role of *C. tamala* against hepatotoxicity induced by CCl_4 in frog. Bavani Eshwaran *et al.* (2010) reported that *C. tamala* possess significant gastroprotective activity, may be due to its free radical scavenging activity. The present study suggests that the methanol extracts of *Syzygium aromaticum* and *Cinnamomum tamala* possess marked antimutagenic potential. Further studies are required to isolate and characterize these antimutagenic factors for their further use as chemotherapeutic agents.

Acknowledgements

The authors are thankful to the Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab (India) for providing research facilities to carry out the work.

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