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Research Article

CHEMOPREVENTIVE EFFECT OF *ANISOMELES MALABARICA* (L.) WHOLE PLANT EXTRACTS DURING DMBA INDUCED HAMSTER BUCCAL POUCH CARCINOGENESIS

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ABSTRACT

In the present investigation , chemopreventive effect of *Anisomeles malabarica* (L.) whole plants extract during DMBA induced hamster buccal pouch carcinogenesis. Animals were divided into 8 groups. In group 1 animals were painted with a 0.5% solution of DMBA in liquid paraffin on the right buccal pouches three times a week for 14 weeks. In group II and III (Experimental groups), animals were painted with DMBA as in group I. In addition, 500mg/kg body weight of chloroform extract and 500 mg/kg body weight of methanolic extract of *A. malabarica* intragastrically three times a week on days alternate to DMBA application respectively. In group IV(CE control), chloroform extract of *A. malabarica* (500mg/kg body weight) was administered. In group V (ME control), methanolic extract of *A. malabarica* (500 mg/kg body weight)was used. In group VI(untreated control), animals received neither DMBA nor*A. malabarica* extracts. Tumour incidence and histopathlogical changes in hamster. Exophytic tumours induced by DMBA in the oral cavity of hamsters in groups 1 were well differentiated squamous cell carcinomas. The incidence of oral neoplasm in group 1 was 100 percent, whereas in group 2 and 3 only hyperplasia were observed. No malignant neoplasm or permalignant lesion were observed in groups 4,5 and 6. No malignant neoplasm or proneoplastic lesions were observed in other organs in any of the groups. Lipid peroxidation levels in hamsters treated with DMBA alone (group1) was the lowest among the groups and significantly lower than those of untreated controls (group 6). The levels of lipids peroxidation in group 2 and 3 were significantly higher than those in group 1. In group 1, the levels of GHS and the activities of GPx and GST were markedly elevated compared with group 6. In group 2 and 3 enzyme activities and glutathione concentration were found to be higher than in group 4 and 5 and in group 6 (control).

Keywords: *Anisomeles malabarica*, Chemopreventive effect, Buccal pouch carcinogenesis

INTRODUCTION

Anisomeles malabarica (L.) (Malabar catmint) Cogn.Syn. *Nepeta malabarica L.,* (Family: Lamiaceae) is a medicinal plant , has been used as a folkloric medicine to treat amentia, anorexia, fevers, swellings, rheumatism[1]. The herb is reported to possess anti-spasmodic, anti-periodic properties and used in Rheumatoid arthritis[2]. It is used for the traditional treatment of snakebite as antidote[3], and plant leaves are used as carminative, astringent, stomachic, rheumatism and diaphoretic in Coimbatore district[4] and also used as dentifrice to cure various dental problems[5]. The plant *A. malabarica* is used traditionally in the treatment of intermittent fever, colic dyspepsia and catarrhal affections[6]. The paste of stem is mixed with coconut oil and used for curing wounds[7]. Preliminary phytochemical tests were carried out and used for the treatment of various infections[8]. Earlier phytochemical studies on the leaves of *A. malabarica* have shown the presence of ß-sitosterol, ovatodiolide, anisomelic acid, malabaric acid, anisomelol and triterpene betulinic acid[9,10,11]. The present study was designed to investigate the modifying effects of *Anisomeles malabarica* in a hamster buccal pouch carcinogenesis modal using lipid peroxidation, GSH, GPx, GST and GGT as biochemical end points of chemoprevention.

MATERIALS AND METHODS

Chemicals

Heparin, bovine serum albumin, 2-thiobarbuturic acid, trichloroacetic acid (TCA), 1,1,3,3-tetramethoxy propane, 2,4-dinitro phenylhydrazine (DNPH),reduced glutathione (GSH),5,5'-dinitrobenzene (CDNB), 7,12-dimethyl benz (a) anthrance (DMBA) were purchased from Sigma chemical company USA.

Collection of plant material and preparation of extract

The whole plant of *Anisomeles malabarica* (Linn.) R.Br, were collected from Aadiveeraganallur near srimushnam in Cuddalore District of Tamil Nadu, India. The plant was botanically authenticated. A voucher specimen was deposited in the Department of Botany, Annamalai University. The whole plant of *Anisomeles malabarica(Linn.) R.Br* were dried in shade and powdered. The powdered plant materials were successively extracted with methanol (80ºC) by hot continuous percolation method in Soxhlet apparatus[12] for 24 hrs. The solvent from the extracts was recovered under reduced pressure using rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Animals and diets

All the experiments were carried out with male Syrian hamsters,aged 8-10 weeks, weighing 85-90g,obtained from the central animal house, Rajah Muthiah Medical Collage and Research institute,Annamalai University, India. They were housed six to a polypropelene cage and provided food and water ad *libitum*. The animals were maintained in a controlled environment under standard condition of temperature and humidity with an alternating 12 hours light/dark cycles.All animals were fed standared pellet diet (Mysore snack feed Ltd, Mysore).

Treatment schedule

The hamsters were randomized into experimental and control groups and divided into four groups of six animal each. In group (Tumour control group), the right buccal pouches were painted three times per week with a 0.5% solution of DMBA in liquid paraffin with a number 4 brush. Each application leaves approximately 0.4mg DMBA[13.] In group II and III (Experimental groups), animals were painted with DMBA as in group I. In addition, 500mg/kg body weight of chloroform extract and 500 mg/kg body weight of methanolic extract of *A. malabarica* intragastrically three times a week on days alternate to DMBA application respectively. In group IV(CE control), chloroform extract of *A. malabarica* (500mg/kg body weight) was administered. In group V (ME control), methanolic extract of *A. malabarica* (500 mg/kg body weight)was used. In group VI(untreated control), animals received neither DMBA nor *A. malabarica* extracts.

The experiment was terminated at the end of 14 weeks and all animals were sacrificed by cervical dislocation after an overnight fast. Fresh buccal pouch tissues were used for estimations.

Biochemical assays

Fresh homogenized tissues were used for biochemical estimation. Lipid peroxidation was estimated as evidenced substances formation of thiobarbituric acid- reactive substances (TBARS). TBARS were assayed in tissues according to the method described by Ohkawa et al.,[14], GSH was determined by the method of

Anderson[15] based on the development of yellow color, when 5,5′ - dithiobis (2- nitrobenzoic acid) is added to compounds containing sulfhydryl groups. Glutathione peroxidase (GPx) activity was assayed by following the utilization of hydrogen peroxide according to the method of Rotruk et al.,[16] with minor modifications. Glutathione -S- transferase was assayed by the method of Habig et al, [17]. GGT was assayed by using γ - glutamyl p – nitroanilide as substrate by the method of Fiala et al., [18]. The protein content was estimated by the method of Lowry et al.,[19].

Statistical analysis

The values are expressed as mean ± SD. Body weight were analysed using Student's *t* test. The data for lipid peroxides and antioxidants were analysed using ANOVA and the group means were compared by LSD test. The results were considered statistically significant at P<0.05.

RESULTS

Body weight: Mean body weight at the end of the study was indicated in table 1. The mean body weight of hamsters in group 1 was significantly lower than that of group 6 (control). The mean body weight of hamsters in group 2 and 3 was significantly higher than that of group 6 (control).

Table 1: Body weight of hamsters in each group (mean±SD; n=6).

Group	Treatment	Body weight	
		Inital	Final
1.	DMBA	106.28 ± 0.36	81.17 ± 0.36
2.	DMBA+CE	97.88 ± 0.36	117.07 ± 0.26
3.	DMBA+ME	99.01 ± 0.27	121.05 ± 0.26
4.	CE control	98.83 ± 0.18	123.72 ± 0.19
5.	ME control	100.88 ± 0.38	125.72 ± 0.23
6.	control	96.29 ± 0.35	123.26 ± 0.35

Significantly different from group 6 by student's *t* test P<0.05

Table 2 summarizes the incidence of oral neoplasm and preneoplastic lesions in different groups. Exophytic tumours induced by DMBA in the oral cavity of hamsters in groups 1 werewell differentiated squamous cell carcinomas. The incidence of oral neoplasm in group 1 was 100 percent, whereas in group 2 and 3 only hyperplasia were observed. No malignant neoplasm or permalignant lesion were observed in groups 4,5 and 6. No malignant neoplasm or proneoplastic lesions were observed in other organs in any of the groups.

Table 2: Tumer incidence and histopathlogical changes in hamster(n=6)

Group	Treatment	Tumour incidence	keratosis	Hyperplasia	Dysplasia	Inflammatory exudates	Squamous cell carcinoma
1	DMBA	6/6 (100)	+ to + + +	+ to + + +	+ + +	-	+ + + +
2	DMBA + CE	0/6 (0)	+ +	+	-	+	-
3	DMBA + ME	0/6 (0)	-	+	-	-	-
4	CE Control	0/6 (0)	-	+	-	-	-
5	CE Control	0/6 (0)	-	+	-	-	-
6	Control	0/6 (0)	-	-	-	-	-

+ = mild; + + =moderate; + + + =severe; + + + + = Well differentiated Parantheses percentage of lesions.

Biochemical findings

Lipid peroxidation (TBARs) in the buccal pouch mucosa of control and experimental animals in each group is given in table 3. Lipid peroxidation levels in hamsters treated with DMBA alone (group1) was the lowest among the groups and significantly lower than those of untreated controls (group 6). The levels of lipids peroxidation in group 2 and 3 were significantly higher than those in group 1.

Table 3. Effect on lipid peroxidation in the buccal pouch mucosa of hamsters (mean±SD; n=6)

Group	Treatment	TBARs(nmoles/100mg protein)
1.	DMBA	67.98 ± 3.73^{b}
2.	DMBA+CE	77.67 ± 3.57^{c}
3.	DMBA+ME	84.01 ± 2.83^{d}
4.	CE control	92.87 ± 3.56
5.	ME control	94.32 ± 2.64
6.	control	93.98 ± 5.42^{a}

Values not sharing a common superscript letter differ significantly at P<0.05

Table 4 shows the activities of GPx and GST and the levels of glutathione in the buccal pouch mucosa of control and experimental animals. In group 1, the levels of GHS and the activities of GPx and GST were markedly elevated compared with group 6. In group 2 and 3 enzyme activities and glutathione concentration were found to be higher than enzyme studied were significantly higher in group 4 and 5 and in group 6 (control).

Table 4: Effect of *Anisomeles malabarica* on GSH levels and activities of GSH-dependent enzymes in hamster buccal pouch mucosa (mean±SD; n=6)

Group	Treatment	GSH(mg/g tissue)	GPx(UA/g protein)	GST(UA/mg protein)	GGT(UB/g tissue)
1.	DMBA	0.23 ± 0.01^{b}	7.52 ± 0.69^{b}	1.53 ± 0.14^{b}	25.57 ± 1.12^{b}
2.	DMBA+CE	0.34 ± 0.01^{c}	5.54 ± 0.24^{ac}	1.83 ± 0.06^{c}	17.74 ± 0.94^{c}
3.	DMBA+ME	0.33 ± 0.01^{a}	6.58 ± 0.23^{c}	1.65 ± 0.11^{d}	12.59 ± 0.85^{d}
4.	CE	0.29 ± 0.01	5.46 ± 0.42	0.74 ± 0.02	10.24 ± 0.92
5.	ME	0.32 ± 0.01	5.47 ± 0.64	0.74 ± 0.06	11.02 ± 0.11
6.	control	0.31 ± 0.01^{a}	5.45 ± 0.23^{a}	0.75 ± 0.07^{a}	10.67 ± 0.67^{a}

Values not sharing a common superscript letter differ significantly at P<0.05

A- µ mole of glutathione utilized/min.

B- µ mole of CDNB-GSH conjugate formed/ min.

DISCUSSION

The present study has revealed an intriguing aspect of tumour biochemistry. The decreased susceptilbility of HBP carcinoma the extent of lipid peroxidation was found to be low.Lipidperoxides play an important role in the control of cell division[20]. Low concentration of oxygen free radicals have been reported to found and stimulate cell proliferation, whereas high levels include toxicity and cell death[21]. An inverse relationship has been observed between lipid peroxidation and the rate of cell proliferation with highly proliferating tumours showing low levels of lipid peroxidation. The decreased susceptibility of DMBA induced oral cancers to lipid peroxidation, therefore appears to be related to cell proliferation occurring in oral carcinogenesis. The decline in peroxidation DMBA induced HBP cancer was associated with enhanced levels of GSH,GPX and GST protect cells against cytotoxic and carcinogenic chemical by conjugating potentially mutagenic electrophiles with GSH. The ultimate carcinogenic from of DMBA is electrophilic[22]. Induction of the glutathione redox cycle by DMBA may eliminate electrophiles and genotoxic adducts. The increased detoxification capacity reflects an adaptive mechanism by which tumour cell gain a selective advantage over their surrounding normal cells.

GSH has regularly affects on cell proliferation. Over expression of GSH and the GSH dependent enzymes GPx and GST has been reported in malignant tumours and cell lines[20]. It has been suggested that over expression of GST is a potential marker in oral carcinogenesis[23]. The enhanced levels of GSH, GPx and GST may serve as markers of cell proliferation and account for the diminished lipid peroxidation in tumour. Our findings are consistent with Slater's hypothesis that a decrease in peroxidation is associated with an increase in antioxidant capacities, conferring a selective growth advantage on cancer cells[24].

Administration of *Anisomeles malabarica* whole plant extracts reversed the decreased susceptibility of oral mucosa to lipid peroxidation.Indicating that *Anisomeles malabarica* whole plants has a suppressing effect on cell proliferation in the target organ. It is possible that *Anisomele malabarica* whole plants mediates its chemopreventive effects by increasing the susceptibility of the oral mucosa to inhibition of carcinogenesis are reported to have an enhancing effect on GSTs. A mutigene family of detoxifying enzyme that are involved in the detoxification of electrophilic ultimate carcinogens[25]. Plant products that induces GST are regarded as potential chemopreventive agents. Induction of GST activity by *Anisomeles malabarica* whole plant extracts observed in the present study may be of significance in protection against neoplastic transformation of the oral cavity. Traditionally, *Anisomeles malabarica* whole plants extracts have been used in india for treating inflammation, viral infection, hypertension, fever and skin disorders. We have demonstrated that the chloroform and methanolic extracts of *Anisomeles malabarica* whole plants extracts effectively suppresses DMBA-induced oral cancer as revealed by the reduced incidence of carcinomas. The present data suggest the *Anisomele malabarica* extracts has potential anticarcinogenesis properties in experimental animals. In general, the beneficial effects of plants products such as *Anisomele malabarica* may be attributable to one or more phytochemical including antioxidants, terpenes, flavonids and other substances. Although quantitation and characterization of individual components was not carried out, this preliminary study lends support to the chemoprotective nature of *Anisomeles malabarica* whole plant extracts based on specific biochemical markers. It is likely peroxidation, antioxidation and detoxification systems. Due to lack of toxicity and ubiquitous distribution in nature, *Anisomeles malabarica* whole plant extracts may be regarded as a valuable plant source for use in traditional medicine and modern drug development.

CONCLUSION

Several mechanism may be involved in the chemopreventive action of *Anisomeles malabarica* whole plant extracts were found to reduced the binding of dihydrodiol epoxide of DMBA to mammary cell DNA, inhibiting DNA adduct formation and tumorogenesis. Although multiple mechanisms may interplay in tumour inhibition by *Anisomeles malabarica* whole plant extracts ,we speculate that modulation of lipid peroxidation, enhancement of GSH and GPx together with elevation of carcinogen detoxification system such as GST are important mechanism by which *Anisomeles malabarica* whole plants extracts exerts its chemopreventive effect.

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