RESEARCH COMMUNICATION

Anticarcinogenic Effects of *Solanum lycopersicum* Fruit Extract on Swiss Albino and C57 Bl Mice

RC Agrawal[1]*, Rachana Jain[2], Wasim Raja[1], M Ovais[3]

Abstract

In the present studies, the effect of *Solanum lycopersicum* extract on DMBA induced skin papillomas and B6 F10 melanomas was studied. Topical single application of DMBA at the dose of 4 mg/kg b.wt. followed by 1 % croton oil for 16 weeks produced a 100% incidence of skin papillomas which started appearing from the 6th week onwards. The mice which additionally received *S. lycopersicum* extract at 0.6 g/kg 2 day/week for 16 weeks showed a significant decrease in the number and incidence of tumors ($p < 0.05$), with a delay in their appearance to week 10. Histopathological examination showed well and poorly differentiated squamous cell carcinomas in the group which received DMBA + Croton oil treatment whereas hyperkeratosis and hyperplasia were more prevalent in DMBA + Croton oil + Lycopersicum extract treated animals. In a second experiment the effect of cyclophosphamide alone and in combination with *S. lycopersicum* extract was studied in B16F10 melanoma tumour bearing mice. The inhibition rate was 25.9% in the cyclophosphamide treated group but this increased to 37.7%with *S. lycopersicum*. The life span of tumour bearing animals was also increased. Thus in two models, *S. lycopersicum* extract exerted protective potential against skin tumors.

Key Words: Chemoprevention - *Solanum lycopersicum* - skin papillomas - melanomas

Asian Pacific J Cancer Prev, **10**, 379-382

Introduction

In recent years, the role of the diet in preventing the occurrence of cancer has been a popular and important area of research. The botanical name of tomato is *Solanum lycopersicum* Linn which belongs to Solanacae family. Epidemiological studies suggest that diets rich in tomato may account for a reduction in the risk of several different types of cancer (Franceschi et al., 1994; Giovannucci and Clinton, 1998; Okajima et al., 1998; Michaud et al., 2000). Initial studies have suggested that cooked tomatoes (i.e., tomato sauce or paste) are a better source of available lycopene than raw tomato juice because the heating action allows the body to quickly absorb the lycopene and it is reported as a powerful antioxidant (Rao and Agarwal, 1998). A population-based case-control study found that *Solanum lycopersicum*-based foods was associated with a small reduction in risk for prostate cancer (Giovannucci and Clinton, 1998). High concentration of lycopene in prostate tissues resulted in a nearly three-fold increase in programmed cell damage among cancer cells. In animal studies the antitumour effect of lycopene was reported in S180 tumors (Pan et al., 2004).

The antitumor effect may be related to its immune function and antioxidative effect. Pre-treatment with lycopene had significantly reduced the frequency of MNNG-induced bone marrow micronuclei and chromosomal aberrations (Velmurugan et al., 2004). Lycopene did not caused direct maternal or developmental toxicity in rats or rabbits at dosages as high as 2000 or 3000 mg/kg/day (Christian et al., 2003).

We have therefore undertaken to study the anticarcinogenic effects in Swiss albino mice in skin papillomas and B16F10 melanoma cell line in C57 BL hybrid mice.

Materials and Methods

Animals

The study was conducted on random bred, 6-7 weeks old and 24- 28 gm body weight male Swiss albino mice. and C57 BL hybrid mice. Animals were maintained under controlled conditions of temperature and light (light: dark, 12 hrs: 12 hrs.). They were provided standard mice feed and water ad libitum. The study protocol was approved by the Institutional Animal Ethical Committee. (IAEC, Ref. No.-2157/225/2006)

Chemicals

The chemicals, 7, 12-dimethylbenz (a) anthracine (DMBA) and croton oil were procured from Sigma Chemicals Co., St. Louis, USA. DMBA was dissolved at a concentration of 100 µg/100 µl in acetone. Croton oil was mixed in acetone to give a solution of 1% dilution.

*[1]Department of Research, [2]Department of Pathology, Jawaharlal Nehru Cancer Hospital and Research Centre, [3]Department of Bioscience, Barkatullah University, Bhopal, India *For Correspondence: rcagrawal60@yahoo.com*

Skin Bioassay Protocol

The animals were randomly divided in to 7 groups. Each group comprises of 6 animals. The skin of the mice were shaved in 2cm2 area with the help of hair removing cream in interscapular region initially and after every 2 weeks hair were removed with the help of scissors. The treatments were provided topically on shaved area up to 16 weeks using the following protocol.

Group 1 (Untreated control) No treatment

Group 2 (Vehicle control) 100 µl acetone 2 times/week up to 16 weeks

Group 3 (DMBA Alone): 104 µg DMBA was dissolved in 100 µl acetone and a single application was given

Group 4 DMBA + Croton Oil : DMBA followed by 1 % Croton oil applied on skin 2 times a week up to 16 weeks

Group 5 DMBA + Croton Oil + *Solenum lycopersicum* (tomato) fruit extract, 100µl dose one hour before the each application of 1 % croton oil

Group 6 Croton oil alone

Group 7 Tomato Extract alone

Melanoma Skin Bioassay

Melanoma cell line were obtained from National Cell science centre, Pune and maintained in our laboratory. The C57 BL hybrid mice of both sexes of the mean weight of 25 gm and 6-7 weeks old were obtained from the animal colony of our institute. They were housed in good laboratory condition and were given standard mouse pellet diet and water ad Libitum. All the mice were kept at controlled light and temperature condition. Cell suspension having total 500,000 cells / animal were injected for implantation of the melanoma tumour. The animal was kept under observation and experiment was started after 10 days when the palpable tumours were seen. The treatment was given orally for 30 days and tumour volume and survival time of each animal were recorded.

The following groups were maintained.

Control Group: This group consisted of four mice. The melanoma cell line (B6F10) were injected subcutaneously (S.C.) in all four mice.

Test Group: This group was divided into two sub groups. Each group consisted of four animals. The melanoma cell line was injected By S.C. route. The tumour bearing mice were orally given dose of 500 and 1000 mg/kg body weight in aqueous extract of tomato.

The animals of all groups were kept under observation for gross and microscopic changes in skin. Papillomas appearing on the dorsal skin of mice were recorded at weekly intervals. For final evaluation of the data, only those papillomas which persisted for two weeks or more (diameter < 1 mm) were taken into consideration. The differences in the incidence of tumors among different groups were considered to be significant at the 5% significance level ($p<0.05$) when evaluated by Student's 't' test.

Results

The results are summarized in Table 1. Topical single application of DMBA at the dose of 4 mg/kg b.wt. followed by 1 % croton oil for 16 weeks produced skin Papillomas which started appearing from 6th week onwards. The mice which received S. lycopersicum extract showed a significant decrease in the number and incidence of tumor appearance as compared with that of the DMBA + croton oil group. When S. lycopersicum extract was topically applied at the dose of 0.6 g/kg 2 day/week for 16 weeks on the skin of animals, the tumor incidence was found to be 50% and the values for cumulative number of papillomas, and tumor yield were significantly ($p< 0.05$) lower than group-5. The first tumor appeared in week 10.

Table 1. Effects of *Solanum lycopersicum* Fruit Extract on DMBA-induced Papillomas in Swiss albino Mice

Groups	Body weight		No. of Papillomas	Tumour Yield	Inc*
	Initial	Final			
No Treatment	26.8+1.2	30.9+1.1	0	0.0	0/6
Vehicle alone	26.7+1.6	30.3+1.9	0	0.0	0/6
DMBA alone	25.0+1.3	30.0+1.3	0	0.0	0/6
Croton oil alone	24.7+2.2	28.8+2.8	0	0.0	0/6
DMBA+ Croton	26.0+1.4	30.9+1.4	17	2.8	6/6
DMBA+ S. lycopersicum + Croton oil	25.7+2.1	29.5+2.6	6	1.0	3/6
S. lycopersicum ext. alone	26.1+1.8	30.1+2.4	0	0.0	0/6

*Inc, Incidence

With DMBA + Croton oil infiltrating nests of neoplastic squamous epithelium were observed . Tumour cells exhibited a high nuclear/cytoplasmic ratio. Moderate cytoplasm and dense clumped chromatin were also seen. Adjacent epithelium showed marked hyperkeratosis. This is suggestive of kerating squamous cell carcinoma grade II.

With theDMBA + Croton oil + Lycopersicum extract most of the sections showed hyperkeratosis and hyperplasia. One section showed mild dysplasia but no malignancy was observed.

Table 2 shows the effects of cyclophosphamide alone, *S. lycopersicum* extract + Cyclophosphamide and S. lycopersicum alone on B16F10 melanoma tumour bearing mice. Tumour growth inhibition was evident with increase in life span in the *S. lycopersicum* extract + cyclophosphamide group as compared to cyclophosphamide alone and *S. lycopersicum* extract alone groups.

Discussion

The present study demonstrates that when *S. lycopersicum* extract was given one hour before the each

Table 2. Effects of *Solanum lycopersicum* Fruit Extract on B16F10 Melanoma Tumors

Group	Volume	VDT (days)	IR%	ILS%
Control	2.55 ± 0.49	3.5 ± 1.2	-	23.5
CP alone	0.71 ± 0.05	5.5 ± 2.1	25.9	28.2
Ext. + CP	0.60 ± 0.13	10.0 ± 0.8	37.7	54.7
Ext. alone	0.85 ± 0.13	3.8 ± 1.8	11.6	32.1

VDT, volume doubling time; IR, inhibition rate; ILS, increase in life span

treatment of croton oil , the incidence and the number of skin papillomas were significantly decreased. The appearance time of papillomas was also prolonged in the *S. lycopersicum* experimental groups in comparison to the carcinogen treated animals. The reduction in tumor counts may be due to effect in the promotional phase of tumorigenesis which prevent the reduction of free redicals (Huachen and Krystyn, 1991).

Antitumour effects of lycopene, an active component of *S. lycopersicum* (tomato) fruit extract was also reported is S180 transplanted tumors (Pan et al., 2004). Antioxidant and antimutagenic effect of lycopene have also been reported (Velmurugan et al., 2004). Topical application of TPA (active constituent of croton oil) has been reported to increase production of free radicals (Huachen and Krystyn, 1991). This is perhaps due to the free radical oxidative stress that has been implicated in the pathogenesis of a wide variety of clinical disorders (Hursting et al., 1999; Das, 2002; Kausar et al., 2003). The exact mechanism of anticarcinogenicity of *S. lycopersicum* extract is not fully understood but it may be possible the free radical scavenging activity of lycopene which is reported to powerful antioxidant This result is important because the tomato is an important vegetable in Indian diet and considerable important has been given for the role of tomato and lycopene in prevention of prostate and other type of cancers.

References

Christian MS, Schulte S, Hellwig J (2003) Developmental embryo-fetal toxicity/teratogenicity toxicity studies of synthetic crystalline lycopene in rats and rabbits. *Food Chem Toxicol*, **41**, 773-833.

Das UN (2002). A radical approach to cancer. *Med Sci Monit*, **8**, 79-92.

Franceschi S, Bidoli E, La Vecchia C, et al (1994). Tomatoes and risk of digestive-tract cancers. *Int J Cancer*, **59**, 181-4.

Freudenheim JL, Marshall JR, Vena JE, et al (1996). Premenopausal breast cancer risk and intake of vegetable, fruits and related nutrients. *J Natl Cancer Inst*, **88**, 340-8.

Giovannucci E, Clinton SK. (1998). Tomatoes, lycopene, and prostate cancer. *Proc Soc Exp Biol Med*, **218**, 129-39.

Huachen W, Krystyna F (1991). In vivo formation of oxidized DNA base in tumor promoter-treated mouse skin. *Cancer Res*, **51**, 4443.

Hursting SD, Slaga TJ, Fischer SF, DiGiovanni J, Phang JM (1999). Mechanism-based cancer preventing approaches: targets, examples and the use of transgenic mice. *J Natl Cancer Inst*, **91**, 215-25.

Kausar H, Bhasin G, Zargar MA, Athar M (2003). Palm oil alleviates 12-O tetradecanoylphorbol-13-acetate-induced tumor promotion response in murine skin. *Cancer Lett*, **192**, 151-60.

London SJ, Stein EA, Handerson IC, et al (1992). Carotenoids, retinol and risk of proliferative begin breast cancer disease & breast cancer. *Cancer Causes Control*, **3**, 503-72.

Mayne ST, Cartmel B, Lin H, Zheng T, Goodwin WJ, (2004) Low plasma lycopene concentration is associated with increased mortality in a cohort of patients with prior oral, pharynx or larynx cancers. *J Am Coll Nutr*, **23**, 34-42.

Michaud DS, Feskanich D, Rimm EB, et al (2000). Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. *Am J Clin Nutr*, **72**, 990-7.

Nagasawa H, Mitamura T, Sakamoto S, Yamamoto K (1995). Effects of lycopene on spontaneous mammary tumour development in SHN virgin mice. *Anticancer Res*, **15**, 1173-8.

Norrish AE, Jackson RT, Sharpe SJ, Skeaff CM (2000). Prostate cancer and dietary carotenoids. *Am J Epidemiol*, **151**, 119-23.

Okajima E, Tsutsumi M, Ozono S (1998). Inhibitory effect of tomato juice on rat urinary bladder carcinogenesis after N-butyl-N-(4-hydroxybutyl)nitrosamine initiation. *Jpn J Cancer Res*, **89**, 22-6.

Pan H, Jiang X, Wan L, Na L, Wang J (2004) Experimental studies of lycopene in inhibiting tumor growth in S180-bearing mice. Wei Sheng Wan Jiu, 33, 456-7 (in Chinese).

Rao AV, Agarwal S (1998). Bioavailability and in vivo antioxidant properties of lycopene from tomato products and their possible role in the prevention of cancer. *Nutr Cancer*, **31**, 199-203.

Velmurugan B, Santhiya ST, Nagini S (2004) Protective effect of S-allylcysteine and lycopene in combination against N-methyl-N'-nitro-N-nitrosoguanidine-induced genotoxicity. *Pol J Pharmacol*, **56**, 241-5.