

RESEARCH COMMUNICATION

Protective Effects of Tomato Juice on Mouse Skin Carcinogenesis

Sarmishtha De, Sukta Das

Abstract

The anticarcinogenic effect of tomato juice, a natural source of antioxidants and other chemopreventive / antimutagenic agents, was studied in a skin carcinogenesis model in mice. The possible mode of action was also investigated. Oral administration of tomato juice afforded protection from development of skin tumour and increased life expectancy which may be attributed to the combined action of a number of chemical compounds with cancer chemopreventive properties present in tomato. The protective role may be associated with a decreased level of lipid peroxides noted in the tomato treated group and modulation of host detoxification enzymes. Exposure to the carcinogen resulted in a depression of the liver enzymes- glutathione-S-transferase (GST), glutathione peroxidase (GPx) and superoxide dismutase (SOD). Oral administration of tomato juice resulted in significant activation of all these enzymes ($p < 0.001$). These results suggest a preventive role of tomato juice during carcinogenesis which is mediated possibly by their modulatory effects on biotransformation enzymes and the detoxification system of the host.

Key Words : tomato - skin carcinogenesis - lipid peroxidation - GST, GPx - SOD - CAT - mouse

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Introduction

Tomato (*Lycopersicon esculentus*), a member of Solanaceae, is consumed widely as a vegetable and as processed tomato products (juice, sauce, soup and ketchup). The active compounds isolated from tomatoes that have anticarcinogenic properties include chlorogenic acid, eugenol, quercetin, rutin, kaempferol, naringenin, alpha and beta carotenes, phytoene, neurosporene and lycopene (Beecher, 1995). The latter, a hydrocarbon carotenoid, found almost exclusively in tomatoes and tomato-based food products, possesses exceptionally high antioxidant activity and may be the most effective quencher of singlet oxygen and other oxidizing species, thereby preventing further promotion and replication of neoplastic cells (Khachik et al, 1995). Other carotenoids and flavanols present in tomato are also associated with antioxidative properties (Narisawa et al, 1996). HPLC analysis of flavanols in 20 varieties of tomato have shown the presence of mainly quercetin, kaempferol and rutin and among tomato based products investigated, tomato juice and puree was found to be very rich in these flavanols (Stewart et al, 2000). Experimental studies suggest a protective role of tomato and its components during carcinogenesis. Narisawa et al (1998) reported tomato juice, rich in lycopene, to have a protective

effect against colon carcinogenesis in F344/NSIC rats receiving N-methyl-nitrosourea. Significant reduction both in number and size of DMBA-induced rat mammary tumours was observed following treatment with lycopene-enriched tomato oleoresin (Sharoni et al, 1997). Atanasova-Goranova et al (1997) demonstrated that tomato paste feeding in Wistar rats inhibited endogenous generation of N-nitrosamine from its precursors, amino-pyrene and sodium nitrite.

A recent population study established a close link between dietary intake of tomatoes, a major source of the antioxidant carotenoid lycopene, and lowered risk of cancer (Rao and Agarwal, 1998). Epidemiological studies also suggested beneficial effects of tomato against cancers of pancreas, colon, rectum, esophagus, oral cavity, breast and cervix though it is more effective against prostate, lung and stomach cancers (Giovannucci et al, 1999).

DMBA induced skin carcinogenesis is a useful murine model for screening of new cancer protective agents, because this model displays a pre-neoplastic condition during carcinogenesis in the form of papillomas which are macroscopically visible and can be confirmed histopathologically. Protective effects of tomato juice on skin carcinogenesis have not yet been well documented. The present report is possibly the first of its kind furnishing observations on the effect of oral administration of tomato

Authors for correspondence, Sarmishtha De, Sukta Das, Dept. of Cancer Chemoprevention, Chittaranjan National Cancer Institute, 37, S.P. Mukherjee Road, Calcutta- 7000 26, India, Fax: 91-33-475-7606 Email sukta2001@rediffmail.com

juice during skin papillomagenesis and its possible mode of action.

Materials and Methods

Preparation of tomato juice

Tomatoes were obtained fresh from local market and juiced in an electrically operated mixer-grinder with water (1:2).

Dose and route of administration

Fresh tomato juice was orally administered to mice daily, using feeding needle, at a dose of 10l, 50 ul, 100l and 200l.

Experimental animals

Swiss albino female mice weighing 18-20 gms in the age group of 5-6 weeks were supplied by the animal colony of our institute. They were caged in groups of 5 per cage and kept under alternating periods of light and dark conditions of 12 hours each. Standard animal food pellets (Lipton India Ltd.) and water were provided ad libitum. Number of animals per group was 25.

Treatment groups

Group 1: Normal control - mice receiving distilled water only in place of tomato juice; Group 2: Normal mice receiving oral administration of tomato juice; Group 3: Carcinogen control - mice receiving 3 topical applications of 1% DMBA on alternate days followed by croton oil (1%) applied twice weekly. This group received water in place of tomato juice; Group 4: Mice receiving tomato juice daily, simultaneously with carcinogen administration.

Detection of papillomas

Animals in group 3 and 4 were continuously examined for detection of papillomas. Morphological observation was confirmed by histopathology.

Biochemical assays

For biochemical study eight mice from each group were sacrificed after four weeks and the liver collected and processed for further analysis.

Glutathione-S-transferase (GST) activity was measured in the liver cytosol following the method of Habig et al (1974). The enzyme activity was determined from the increase in absorbance at 340nm with 1-chloro-2-4-dinitrobenzene (CDNB) as the substrate and specific activity of the enzyme expressed as formation of 1-chloro-2-4-dinitrobenzene (CDNB) -GSH conjugate per minute per mg of protein. Glutathione peroxidase (GPx) activity was determined in the post mitochondrial fraction by the method of Paglia and Valentine (1967). The reaction mixture contained NADPH and glutathione reductase. The decrease in absorbance following addition of H₂O₂ was recorded at 340 nm. Enzyme activity was expressed as moles of NADPH utilized per minute per mg protein using molar extinction co-efficient at 340 nm as 6200 m⁻¹cm⁻¹. Activity of catalase (CAT) in liver was estimated by the method of Luck (1963). The

enzyme activity was determined spectrophotometrically at 250 nm and expressed as unit/mg protein where the unit is the amount of enzyme that liberates half the peroxide oxygen from H₂O₂ in 100 seconds at 25°C. Superoxide dismutase (SOD) activity was determined by quantification of pyrogallol auto oxidation inhibition by the method of Marklund and Marklund (1974) and expressed as unit/mg protein. One unit of enzyme activity is defined as the amount of enzyme necessary for inhibiting the reaction by 50%. Auto oxidation of pyrogallol in Tris-HCL buffer (50mM, pH 7.5) is measured by increase in absorbance at 420 nm. Lipid peroxidation was estimated in liver microsomal fraction by using the method of Okahawa et al (1979). The levels of lipid peroxides formed was measured using thiobarbituric acid and expressed as thiobarbituric acid reactive substance (TBARS) formed per mg protein using an extinction coefficient of 1.56 x 10⁵ M⁻¹cm⁻¹. Protein was estimated by the method of Lowry (1951).

Chemicals

7,12-Dimethylbenz(a)anthracene (DMBA), 1-chloro-2-4-dinitrobenzene (CDNB), glutathione (GSH), glutathione reductase (GR), -nicotinamide adenine dinucleotide phosphate, reduced form (-NADPH), pyrogallol, diethylenetriamine penta acetic acid (DTPA) and thiobarbituric acid (TBA) were purchased from Sigma Chemical Co., St Louis, MO, USA. Sodium dodecyl sulphate (SDS) was purchased from Gibco BRL, USA. Hydrogen peroxide solution (30%) was obtained from E. Merck (India).

Results

The following observations concern the effects of only one dose i.e. 100l only because no influence was noted at the lower doses and at 200l the result was similar to that for 100l.

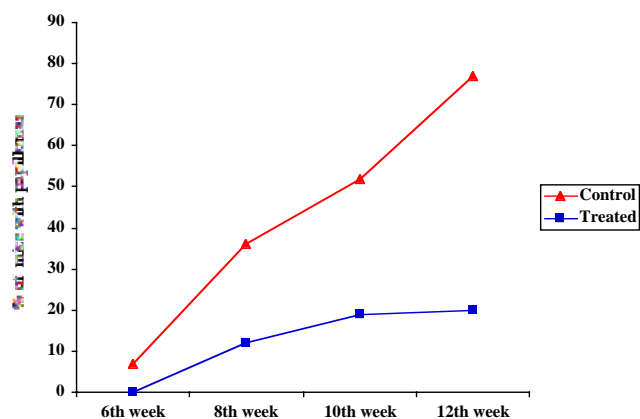


Figure 1. Incidence of Skin Papillomas Noted in Mice receiving Oral Administration of Tomato Juice as Compared to the Control Group following Topical Application of DMBA as Initiator and Croton Oil as Promoter.

Effect of tomato juice on development of papillomas

Topical application of DMBA followed by croton oil produced skin papilloma which started appearing from the 6th week onwards. The number of papilloma per mouse varied from 3-8 and incidence of papilloma increased from 5.88% in the 6th week to 76.47% in the 12th week after which many of the tumours were histologically found to be squamous cell carcinomas. Therefore incidence of papilloma and effect of tomato on this pre-cancer condition was recorded after 12 weeks.

Treatment with tomato juice (100l/mouse/day) delayed the onset of papilloma and also reduced the incidence. The results of this group is presented in Figure 1 showing sharp decrease in incidence of papilloma where the reduction was more than 50% on the 12th week. The number of papillomas per mouse however was similar to that noted in the control group. The median survival of the host in untreated group was 119 days which increased to 130 days in the treated group i.e., there was 9.24% increase in life span.

Effect of tomato juice on Glutathione-S-Transferase, Glutathione Peroxidase, Catalase and Superoxide Dismutase.

GST activity in liver was found to be decreased following exposure to the carcinogen, in comparison to that of normal values ($P < 0.001$). Treatment with tomato juice restored and enhanced the activity of the enzyme significantly ($P < 0.001$). Increase in activity of GST following treatment was also noted in normal animals (Figure 2).

Significant decrease in GPx activity was also noted during carcinogenesis as compared to normal animals ($P < 0.001$). Concomitant treatment with tomato juice resulted in an increased activity of this enzyme ($P < 0.001$, Figure 3). CAT activity decreased significantly ($P < 0.001$) in the carcinogen treated group but tomato juice treatment did not influence activity of this enzyme (Figure 4). Tomato juice

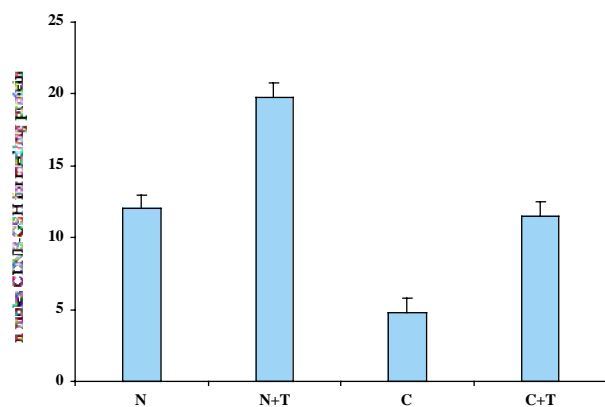


Figure 2. Glutathione-S-transferase Activity in Liver. Normal untreated mice (N), normal mice treated with tomato juice (N+T), carcinogen treated mice (C) and mice receiving carcinogen and tomato juice (C+T). Data are means \pm SE.

consumption was found to increase the level of SOD significantly ($P < 0.001$) both in normal condition and during chemical carcinogenesis (Figure 5).

Influence of tomato juice on Lipid Peroxidation

Considerable elevation of lipid peroxides was noted during skin carcinogenesis ($P < 0.001$). Oral intake of tomato juice significantly reduced lipid peroxidation ($P < 0.001$) both in normal and carcinogen treated groups (Figure 6).

Discussion

Considering the existing knowledge in carcinogenesis and anticarcinogens and in view of the growing interest in investigating the ability of diets rich in fresh fruits and vegetables in reducing the risk of cancer in several organs, the present study was undertaken to experimentally evaluate the role of tomato juice, which is a component of human food, in prevention of chemically induced skin carcinogenesis and to understand their mechanism of action. The concept of dietary prevention of cancer is based on the mechanism of action of the chemical compounds and/or micronutrients present in food, their toxicity and the chemopreventive efficacy (Beecher and Khachik, 1989). The skin carcinogenesis model in experimental animals has been found to be a very useful system for investigating the influence of dietary chemopreventors both mechanistically and operationally (Morse and Stoner, 1993).

In the present study the chemopreventive efficacy of tomato juice, which is a combination of many antioxidants, as well as other potential chemopreventive compounds was assessed during skin carcinogenesis. Oral administration of tomato juice was found to reduce the incidence of chemically induced skin papilloma and at the same time improve the life expectancy of the host.

Along with reduced tumour incidence, tomato juice also induced activities of hepatic GST an enzyme which plays

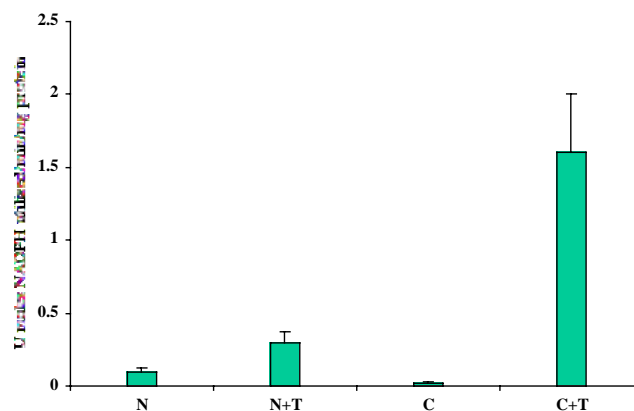


Figure 3. Effect of Tomato Juice on Glutathione Peroxidase Activity in Liver. N- normal mice, N+T- normal mice treated with tomato, C- carcinogen treated mice, C+T- Carcinogen + tomato juice treated mice. Data are means \pm SE.

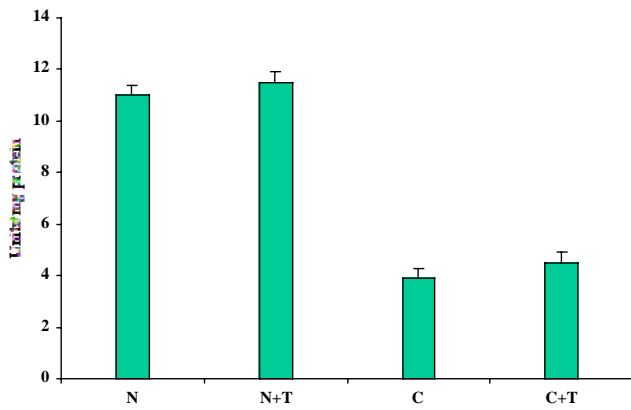


Figure 4. Effect of Tomato Juice on Catalase Activity in Liver N- normal mice, N+T- normal mice treated with tomato, C- carcinogen treated mice, C+T- Carcinogen + tomato juice treated mice. Data are means±SE.

an important role in initiating detoxification (Sedlack and Lindsay, 1968) by catalyzing the conjugation of GSH to the electrophilic foreign compounds for their elimination from the system. Tomato juice feeding could also augment GPx and SOD activity in normal condition as well as during DMBA initiated skin papillomagenesis. To protect cells from damages, radical and non-radical reactive oxygen species including peroxides and superoxides need to be inactivated enzymatically by CAT, SOD and GPx (Vang et al, 1997). By increasing the GPx and SOD activity, that removes the peroxides and superoxides produced both in normal condition and also in large amount during carcinogen metabolism, tomato juice may prevent the accumulation of reactive species by trapping them. These modulations in the antioxidant enzymes system which upregulate the host detoxification process may be associated with reduced incidence of skin papilloma. Lipid peroxides which also

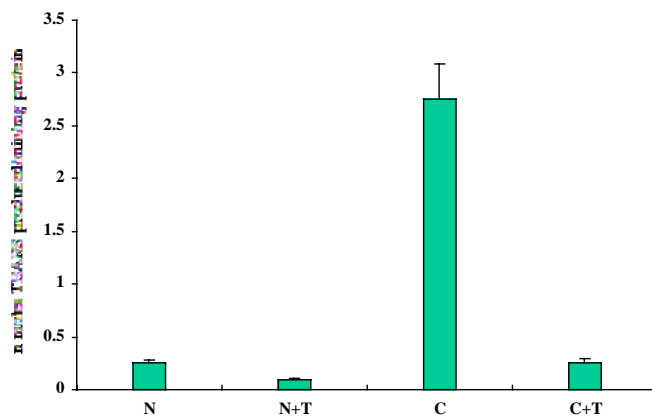


Figure 6. Effect of Tomato Juice on Lipid Peroxidation in Liver. N- normal mice, N+T- normal mice treated with tomato, C- carcinogen treated mice, C+T- Carcinogen + tomato juice treated mice. Data are means±SE.

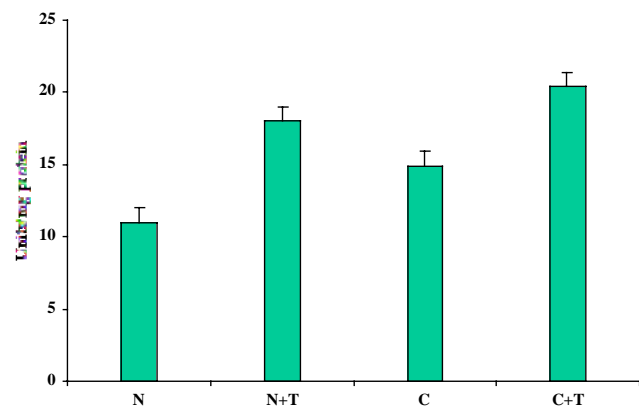


Figure 5. Superoxide Dismutase Activity in Liver. N- normal mice, N+T- normal mice treated with tomato, C- carcinogen treated mice, C+T- Carcinogen + tomato juice treated mice. Data are means±SE.

cause damage to cellular macromolecules by generation of reactive species (Chung et al, 1996) is considered to enhance carcinogenesis. DMBA induced increase in lipid peroxides noted in our study could be significantly reduced by tomato juice (De and Das, 2000). This effect is most likely a consequence of the modulatory influence of the juices on the biotransformation enzymes of detoxification. Rao and Agarwal (1998) had reported of reduced serum thiobarbituric acid reactive substances by dietary supplementation of lycopene through tomato juice, spaghetti sauce and tomato oleoresin. Lycopene was also shown to enhance gap junctional communication and inhibit lipid peroxidation (Zhang et al, 1991).

It had been reported earlier that the juice and whole fruit of tomato consumed at a rate of 14.5 gm per person per day was antimutagenic and such action was suggested to be due to the presence of chlorogenic acid, kaempferol, lanosterol, naringenin, quercetin and rutin (Beecher, 1995). The anticarcinogenic activity as well as the modulatory influence on host detoxification enzymes of tomato juice noted in the present study may be attributed to the combined action of the different chemopreventive agents present in tomato.

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