INHIBITORY EFFECT OF TANNIC ACID FROM NUTGALL ON IRON-DEXTRAN AUGMENTED 7,12-DIMETHYL BENZ(A)ANTHRACENE-INITIATED AND CROTON OIL-PROMOTED SKIN CARCINOGENESIS

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ABSTRACT

Tannic acid (TA) is naturally occurring polyphenols present in fruits and vegetables. In this study, inhibition of the carcinogenic potential of croton oil in normal and iron overloaded mice skin by TA is reported. Albino Swiss mice were given iron-dextran for two weeks and were pretreated with a single topical application of tannic acid. After one hour tumors were initiated by a single dose of 7,12-dimethylbenz(a)anthracene (DMBA) the promoting agent croton oil was applied twice a week for 30 weeks. The appearance, number and percent tumor incidences were recorded. When compared to control groups, the pretreated groups showed a significant high inhibition of tumors incidences. Biochemical studies in mice skin tissues were based on the measurement of lipid peroxidation (LPO). TA diminished cutaneous LPO level in mice skin as compared to the untreated groups. This study showed that TA inhibits the augmentation potentials of croton oil and iron dextran significantly. A depletion in LPO levels in TA pretreated groups indicates that excessive generated oxidants in the mice skin tissues may be quenched by TA because of chelation of redox active iron and its faster elimination from the body. It is supposed that inhibition of iron mediated oxidative stress by TA may be responsible for diminishment of cutaneous tumorigenesis.

Key words: Tannic acid, Iron overload, 7,12-Dimethylbenz(a)anthracene, Croton oil, Skin cancer.

INTRODUCTION

There is increasing interest in identification of naturally occurring substances that may be capable in diminishing tumorigenicity of the environmental carcinogens. Tannic acid (TA) is naturally occurring plant phenol present in fruits and vegetables (1). In addition of its use as an additive in medicinal products for human, it has been used for treatment of burns, diarrhea and chemical antidotes in poisoning and as a local astringent (2). It has been shown to be anti-oxidant and it is a potent antagonist of the mutagenicity of polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene (BP) (3,4). According to the extensive experimental data it is now known that PAHs must be metabolically activated by peroxo radical dependent pathway and the electrophilic bay-region diol-epoxides act as the ultimate carcinogenic metabolites of PAH (5). Consistent with this hypothesis during the past several years, naturally occurring plant phenols, including TA have shown to inhibit the mutagenicity of (+)-7â,8â-dihydroxy-9,10-â-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDE-2), an ultimate mutagenic and carcinogenic metabolite of BP. It has been shown that TA possesses exceptionally high antimutagenic activity as compared to hydroxylated anthraquinones and cinnamic derivatives against BPDE-2 mutagenesis (4,6). In addition, pretreatment of animals with tannic acid inhibits DMBA, benzo(a)pyrene, 3-methyl cholanthrene and N-methyl-N-nitrosourea-induced tumorigenesis in mice. Besides of its effect on cytochrome P-450 dependent mono-oxygenase activity, it also potenitates quenching free radicals and inhibits lipoygenase activity induced by tumor promoters (7,8). In a subsequent study, it has been reported that tannic acid as well as other polyphenols including quercetin and green tea polyphenols inhibit skin tumorigenesis (9). When is applied topically, injected, or added to the diet or drinking water it can decrease the risk of tumorigenesity in the skin and other organs (10). Based on these studies, it appears that TA acts on different sites by different mechanisms and inhibits the manifestation of carcinogenesis. Previously we have reported that iron acts as a mild tumor promoter in skin as well as augments the effect of
benzyl peroxide mediated cutaneous tumori-
genesis (11,12). It is possible that antioxidant
potential of TA may be due to the quenching of
the excessively generated oxidants and inhibition
tumor promoting response. In this study, the
inhibitory effect of TA on croton oil promoted in
both normal and iron overload mice was
investigated.

MATERIALS AND METHODS

Chemicals
Iron dextran (imferon) was purchased from Rallis
India Ltd. (pharmaceutical Div., Konnagar India),
7,12- Dimethylbenz(a)anthracene and croton oil
were purchased from Sigma Chemical Co. USA.
Nutgall was procured from local market. All other
chemicals and biochemicals used in this study were
either of analytical grades or of highest
purity grades which were commercially available.

Animals
Swiss albino female mice were procured from
Central Animal House Facility of Tabriz
University of Medical Sciences. Animals were
housed in an air conditioned room in polypropy-
lene cages. For long term chronic experiments,
usually 20 mice and for acute experiment, 6 mice
were housed in each group. They had free access
to pellet diet and water ad libitum. The animals
were kept at room temperature of 24°C (±2°C)
and were exposed to alternate cycles of 12 hours
light and darkness. The dorsal skin of the mice
were shaved with an electric clipper followed by
applying hair removing cream, at least two days
prior to the treatment. Only mice that did not
show signs of hair re-growth were used for
experiments.

Experimental protocol
Preparation of Tannic acid from Nutgall: The
Nutgall was ground and extracted by acetone.
The extract was evaporated through Rotary
evaporator. The obtained residue was 69% of the
initial Nutgall powder. The amount of tannic acid
was determined quantitatively by colorimetric
method at 379 nm by the use of nitrous acid reagent.
The tannic acid (Obtained from Sigma
Chemical Co.) was used for preparation of
standard curve. The purity of tannic acid obtained
by this method was 97%.

Tissue preparation for biochemical assays: Tissue
samples were prepared by the Method Mohandas
et al (13). After the desired time period, control
and treated animals were killed by cervical dislocation. The animals were immediately
dissected to remove their skins which were
washed in ice-cold saline (0.9% NaCl) and the
extraneous material was removed. All subsequent
operations were carried out on ice at 4°C. For
biochemical studies, a known amount of tissue
was minced and homogenized in a polytron
homogenizer. For biochemical estimation, post
mitochondrial supernatant, were used on the same
day that animals were killed.

Assay of lipid peroxidation: The assay for
microsomal lipid peroxidation was performed by
the method of Wright et al (14). The reaction
mixture in a total volume of 1.0 ml contained
0.58 ml phosphate buffer (0.1 M, pH 7.4), 0.2 ml
microsome obtained from the homogenate (10% w/v), 0.2 ml ascorbic acid (100 mM), 0.02 ml
ferric chloride (100 mM). The reaction mixture
was incubated at 37°C in a shaking water bath for
one hour. The reaction was stopped by addition of
1 ml of trichloroacetic acid (10%) followed by
addition of 1.0 ml thiobarbituric acid (0.67%). All
tubes were placed in a boiling water bath for a
period of 20 minutes. At the end, tubes were
shifted to crushed ice bath and then centrifuged at
2500 rpm for 10 minutes. The amount of the
formed TBA reacting species which were
assessed by the measurement of the optical
density of the supernatant at 535 nm against a
reagent blank. The results were expressed as nmol
of malondialdehyde (MDA) formed/hr/mg of
tissue protein at 37°C by the use of the molar
extinction coefficient of 1.56x10^3 M^-1 cm^-1.

Biochemical studies were performed on four
groups of mice (6 mice in each group). Group I
and II received a single intramuscular injection of
saline (0.2 ml/mouse) whereas Group III and IV
received an intramuscular injection of imferon (5
mg iron/0.2 ml/mouse). Twenty four hours after
injection of saline or imferon, the group IV
animals received a topical application of TA (400
mg iron/0.2 ml acetone/mouse). One hour after the TA
treatment, group II, III and IV animals received a
single topical application of croton oil (0.5% in
200 µl acetone/mouse) and after 12 hours, all
animals were killed by cervical dislocation. Their
dorsal skin were excised and processed for tissue
preparation.

The effect of TA on inhibition of iron overload
augmented, DMBA-initiated and croton oil
promoted tumorigenesis in mice were studied on
four groups of animals (20 mice in each group).
Group I and III received an intramuscular
injection of saline and Group II and IV were
given an intramuscular injection of imferon daily
for a period of 15 days. Twenty-four hours after
the last injection of saline or imferon, group III
and IV animals were treated with TA (400 mg/200
µl acetone) and after one hour animals of all four
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groups were treated with a single topical application of DMBA (40 ìg/200 ìl acetone/mouse). One week after DMBA treatment, all groups received a twice weekly topical application of croton oil (0.5% in 200 ìl acetone) for a period of 20 weeks. These animals were observed weekly for recording tumors for a period of 25 weeks. The number of tumors and incidence of tumorigenesis were recorded and plotted against the time of the test (weeks).

Statistical analysis: The level of significance between different groups was based on t-test followed by analysis of variance test. The level of significance was chosen at p<0.05 or less.

RESULTS
The effect of the pretreatment with TA on croton oil induced lipid peroxidation in normal and iron overload mice is given in figure 1. While croton oil treatment in iron overloaded animals augmented lipid peroxidation in comparison to saline, prior topical application of TA diminished induction significantly (p < 0.05). The effect of pretreatment of TA on the tumor yield and tumor incidences are given in figures 2 and 3. Figure 2 shows that the tumor yield decreased significantly in normal and iron overloaded animals by pretreatment with TA and at the end of week 13, the tumor yield in normal and iron-overloaded animals were 1.75 and 2.0/mouse respectively whereas in TA pretreated group it was 0.3 in both groups. Similarly by the week of 19, the tumor yield in normal animals was 3.5/mouse and in iron overloaded animals it was 4.7/mouse which reduced in TA pretreated group to 0.3 and 1.1/mouse respectively. Similarly, the tumor incidences as shown in figure 3, by week of 13, was 35% in normal and 70% in iron overload group animals whereas in TA pretreated animals were 5% and 10% respectively. By the week of 19, incidences were more than 95% in iron overload animals versus 80% in normal animals whereas in TA pretreated normal and iron overloaded animals these percentages were 25% and 40% respectively. The tumor incidences reached to 100% by week of 25 in normal and iron overloaded animals and maximum incidences in TA pretreated were recorded as 55% and 60% respectively.

DISCUSSION
Tumor prevention is becoming more popular day by day because of the limited success in the treatment of cancer. Recently dietary manipulations which affect cancer development has been highlighted. A large number of studies have been conducted to ensure that diet plays an important role in etiology and development of cancer. Several epidemiological studies have also suggested that diet influences human cancer risk (15). A large number of chemical compounds which have anticarcinogenic potential have been identified and shown to be present in normal human diet (16). These compounds are predominantly herbal products which have a highly diversified chemical structures. Polyhydroxy plant phenols such as tannic acid is one of the classical example of such agents which is present in a variety of plants (17) and many of them are consumed in human diet (18). TA has also been shown to possess beneficial pharmaceutical effects (19,20). It has been shown that dietary supplementation of TA results in protection against the onset of forestomach, lung and skin neoplasia but effects are more pronounced in forestomach followed by lung and skin (21). Also, dermal application of TA show a significant protection against cutaneous carcinogenesis. The mechanism of protection in these studies seems to be inhibition of PAH metabolizing enzymes and their subsequent binding to DNA. The observed decrease in tumor yield and tumor incidence in animals receiving a pretreatment of TA before application of DMBA suggest that inhibition in skin tumorigenesis in iron overloaded animals may be due to the inhibition of tumor promotion responses. The ability of TA to inhibit tumor-promoting response is evident from the data of the present study where prior application of TA before application of croton oil in normal and iron overload animals decreased lipid peroxidation. Also TA proved to be a potent inhibitor of papilloma formation in mouse skin. In another study, it has been shown that application of oxidant on papilloma induced by chemicals as well as ultraviolet light enhances the malignant transformation of benign papilloma into carcinoma (22). Our results therefore show that TA inhibits the iron-dextran augmented skin tumorigenesis and it may be proposed that subsequent chelation with iron, causes diminish generation of the free radical. It may be suggest that the chelated iron with TA may be eliminated faster from the body as compared to unchelated iron, which remains for a longer period of time in tissues.
**Figure 1.** Effect of TA on the inhibition of croton oil-dependent enhancement of cutaneous lipid peroxidation in iron overloaded mice. Treatment protocol and experimental procedures are given in the text. Each value represents mean ± S.E. of six animals. *P< 0.05 when compared to croton oil treatment in iron overloaded animals groups, ** P<0.05 when compared to untreated groups.

**Figure 2.** Effect of TA treatment before the tumor initiation on the yield of tumors developed as a result of DMBA-initiation and croton oil promotion on mouse skin in normal and iron overload animals. The tumors were counted every week and represented as the number of tumors/mouse plotted as a function of weeks on test. Each value represents mean number of tumors/mouse calculated on the basis of 20 animals. The analysis of variance test indicated a significant difference in number of tumors / mouse among the groups (P<0.001).
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Figure 3. Effect of TA treatment before the tumor initiation on percent incidence of tumors in DMBA-initiated and croton oil-promoted normal and iron overload mice. The data were recorded every week and plotted as a function of weeks on test. Each value represents % incidence tumors calculated on the basis 20 animals. The analysis of variance test indicated a significant difference in % incidence of tumors among the groups (P<0.001).

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REFERENCES