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Chemomodulatory Effect of *Trachyspermum ammi* on Murine Skin and Forestomach Papillomagenesis

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***Trachyspermum ammi* seed consumed worldwide as a spice ingredient is much valued for its medicinal properties. However, it has not been investigated for its cancer chemopreventive efficacy. Herein, the chemopreventive effect of different doses (2%, 4%, and 6%) of test diets of *Trachyspermum ammi* seeds were examined on DMBA-induced skin and B(a)P-induced forestomach papillomagenesis, inducibility of drug metabolizing phase I and phase II enzymes, antioxidant enzymes(catalase, superoxide dismutase, glutathione peroxidase, glyoxalase I), reduced glutathione content, and peroxidative damage. Results exhibited a significant reduction in the skin as well as the forestomach tumor multiplicity with respect to all doses of test diet as compared to the control group. Biochemical assays revealed a significant increase in the activities of phase I enzymes especially with 6% test diet. A concomitant increase in the activities of the phase II enzymes and antioxidant enzymes were observed in *Trachyspermum ammi* treated groups. The content of reduced glutathione was significantly elevated, whereas the peroxidative damage along with lactate dehydrogenase activity exhibited a significant reduction with all the three doses of test diet. These findings were indicative of chemopreventive potential of *Trachyspermum ammi* seeds against carcinogenesis.**

INTRODUCTION

There is a growing interest to reduce the incidence of cancer in the human population. Since diet is closely linked to cancer (1), there is a need to test the various dietary agents for their potential to prevent cancer incidence. Spices are consumed worldwide and form a part of our regular dietary regime. *Trachyspermum ammi* Sprague (Ajowan) of Family Apiaceae is much valued for its antispasmodic, stimulant, tonic, and carminative properties. "Ajowan" usually refers to the dried seeds of *Trachyspermum ammi* and is extensively used as a spice for Indian culinary purposes. It is administered for flatulence, atonic dyspepsia, and diarrhea and is often recommended for cholera (2). The alcoholic and aqueous extracts of *Trachyspermum ammi* seeds have revealed anti-inflammatory effects (3), its essential oil exhibits fungitoxic properties (4), whereas an aqueous extract of the seeds were found to contain an aflatoxin inactivation factor (5). An ether extract of *Trachyspermum ammi* exhibits antiaggregatory effects and alters arachidonic acid metabolism in human platelets (6). Because of anti-inflammatory properties and potential for inactivation of aflatoxin, *Trachyspermum ammi* was expected to possess cancer chemopreventive ability.

Trachyspermum ammi seeds are known to be effective in case of various stomach ailments related to digestive disorders (2), and inflammation is considered to be one of the major mechanisms for skin tumor promotion (7). Therefore, in the present investigations, its chemopreventive efficacy has been evaluated against 7,12 dimethylbenz(a)anthracene (DMBA)-induced skin papillomagenesis and against benzo(a)pyrene [B(a)P]-induced forestomach papillomagenesis, at the peri-initiational level (exposure of the murine model system to the modulator around the event of initiation).

Carcinogens are metabolized and detoxified mainly by phase I and phase II enzymes (8). Further, the oxidative stress generated due to carcinogens is lowered by the enzymes involved in antioxidant function. Therefore, *Trachyspermum ammi* seeds were also assessed for their capacity to modulate the levels/activities of phase I and phase II detoxification enzymes, antioxidant enzymes, and toxicity in terms of peroxidative damage and activity of lactate dehydrogenase (LDH). The skin and forestomach papillomas were also examined histologically.

To the best of our knowledge, this finding is the first report showing the chemopreventive potential of *Trachyspermum ammi* against carcinogenesis.

MATERIALS AND METHODS

Chemicals

B(a)P, DMBA, 1 chloro 2,4-dinitrobenzene, 5,5′dithiobis-2-nitrobenzoic acid, 2,6-dichlorophenolindophenol, ethylenediamine tetraacetic acid, reduced glutathione (GSH), potassium ferricyanide, pyrogallol, bovine serum albumin, methylglyoxal, Triton X-100, thiobarbituric acid, reduced nicotinamide adenine dinucleotide (NADH), and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Sigma Chemical Co. (St. Louis, MO). The rest of the chemicals used

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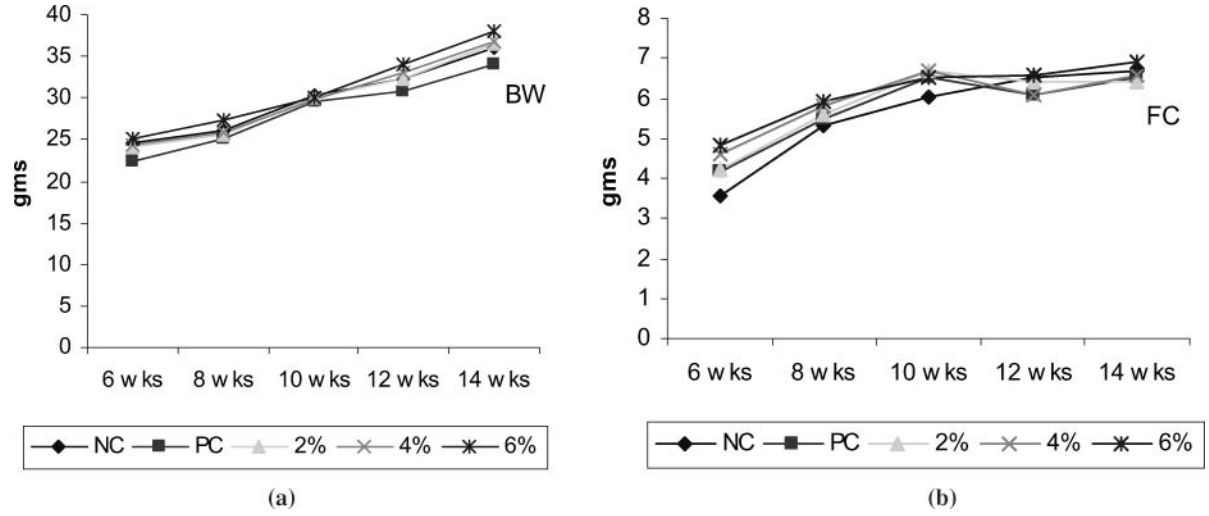

FIG. 1. Feed consumption and body weight of animals in different groups of treatments. gms = grams; BW, body weight; FC, feed consumption; NC, normal control group; PC, positive control group; 2%, 2% test diet treated group; 4%, 4% test diet treated group; 6%, 6% test diet treated group.

were obtained from local firms (India) and were of highest purity grade.

Animals

Random bred female Swiss albino mice (6–8 wk old) were used for this study. The animals were maintained in the air-conditioned animal facility (Jawaharlal Nehru University, New Delhi, India) with a 12-h light and 12-h dark cycle and provided (unless otherwise stated) with standard food pellets and drinking water ad libitum. Throughout the duration of experimentation, the animals were under strict observation with respect to food and water consumption and for manifestation of any toxicity syndrome. The experimental studies were conducted according to the ethical guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, on the use of animals for scientific research.

Modulator

Seeds of *Trachyspermum ammi* Sprague were obtained from the local market and were authenticated by a competent botanist. The collected seeds were powdered with the help of a mixer grinder and were mixed with the standard feed powder, according to the desired concentrations (2%, 4%, and 6%) wt/wt, and pellets were prepared. The pellets with different concentrations of the plant modulator were stored in clean bags in the feed store room of the animal house of the Jawaharlal Nehru University, under strict hygienic conditions, until the end of the experiment.

Preparation of Chemicals and Test Diets

DMBA was dissolved in acetone at a concentration of 50 μg per 50 μl acetone and was applied topically to the animals; 2% croton oil in acetone was prepared, which was used as a promoter in skin papillomagenesis study. B(a)P was dissolved in peanut oil, and the concentration was adjusted to 1 mg per 0.1 ml of peanut oil and was administered to the animals through an oral gavage. Test diet of *Trachyspermum ammi* (2%, 4%, and 6%) was prepared and orally administered to the animals. The food intake was monitored and found to be almost the same in each group (Fig. 1).

Experimental Design

In this study, 5 separate experiments were performed to delineate specific objectives as mentioned earlier. Experiments 1 and 2 were aimed to evaluate the probable efficacy of *Trachyspermum ammi* in chemoprevention of skin and forestomach papillomagenesis in murine model system, respectively. Experiment 3 was performed to study the chemomodulatory effect of the same on the hepatic phase I and phase II enzymes, antioxidant profile, and toxicity in terms of peroxidative damage and activity of LDH. Experiment 4 involved histological studies, and Experiment 5 was aimed at Western blot analysis for glutathione–S-transferase enzyme induction in the forestomach of tumor-bearing animals.

EXPERIMENT 1

Modulatory Effect of *Trachyspermum Ammi* on DMBA-Induced Skin Papillomagenesis

The experiment was performed as described by Yasukawa et al. (9) with some modifications. The hairs on the dorsal scapular region (2 cm diameter) of the mice were clipped off 3 days before the application of the carcinogen, and animals in the resting phase of hair growth cycle were selected for the experiment. The animals were randomly assorted into different groups as

TABLE 1
Experimental groups and their treatment[a]

Group	No. of Animals	Treatment
I (normal)	16	Animals were fed with normal diet.
II (positive control)	18	Animals were fed with normal diet. On Day 14, a single dose of DMBA (50 μg/50 μl acetone) was applied on the shaven area. Two wk after the carcinogen application, 0.1 ml of 2% croton oil in acetone was applied twice a week until termination of the experiment.
III	18	Animals were fed with 2% test diet of *Trachyspermum ammi* for 21 days. On Day 14, a single dose of DMBA was topically applied on the shaven area followed by croton oil treatment as given in Group II mice.
IV	18	Animals were fed with 4% test diet of *Trachyspermum ammi* for 21 days. On Day 14, a single dose of DMBA was topically applied on the shaven area followed by croton oil treatment as given in Group II mice.
V	18	Animals were fed with 6% test diet of *Trachyspermum ammi* for 21 days. On Day 14, a single dose of DMBA was topically applied on the shaven area followed by croton oil treatment as given in Group II mice.

[a]Abbreviation is as follows: DMBA, 7,12 dimethylbenz(a)anthracene.

shown in Table 1. The body weight of the animals were monitored at weekly intervals. The number of papillomas appearing in the shaven area of the skin were noted down at weekly intervals. The papillomas of the size above 1 mm in diameter were included in data analysis. The animals were sacrificed 120 days after commencement of the treatments. In each group, the number of papillomas per mouse (tumor multiplicity) was counted at the termination of the experiment.

EXPERIMENT 2

Modulatory Effect of *Trachyspermum Ammi* on B(a)P-Induced Forestomach Papillomagenesis

The experiment was performed as described by Azuine and Bhide (10). This is a modified method originally described by Wattenberg (11). The animals were assorted into different groups as shown in Table 2. Body weight of animals were recorded at regular intervals. The animals were sacrificed after 180 days. The forestomach was cut open longitudinally, and the papillomas were counted under a dissecting microscope. In each group, the number of forestomach papillomas per mouse (tumor burden) was counted at the termination of the experiment.

EXPERIMENT 3

Modulatory Effect of *Trachyspermum Ammi* on the Hepatic Phase I and Phase II Enzymes, Antioxidant Enzymes, LDH, and Peroxidative Damage

The animals were assorted into different groups as shown in Table 3.

TABLE 2
Experimental groups and their treatment[a]

Group	No. of Animals	Treatment
I (normal)	18	Animals were kept on a normal diet and did not receive B(a)P treatment.
II (positive control)	20	Animals were kept on a normal diet for 2 wk after which each animal was administered with 8 doses of 1 mg of B(a)P per 0.1 ml of peanut oil (twice weekly for 4 wk) by an oral gavage treatment.
III	20	Animals were kept on 2% test diet of *Trachyspermum ammi* starting 2 wk before, during, and 2 wk after the carcinogen treatment (8 doses of 1 mg B(a)P per 0.1 ml of peanut oil) as given to Group II animals.
IV	20	Animals were kept on 4% test diet of *Trachyspermum ammi* starting 2 wk before, during, and 2 wk after the carcinogen treatment (8 doses of 1 mg B(a)P per 0.1 ml of peanut oil) as given to Group II animals.
V	20	Animals were kept on 6% test diet of *Trachyspermum ammi* starting 2 wk before, during, and 2 wk after the carcinogen treatment (8 doses of 1 mg B(a)P per 0.1 ml of peanut oil) as given to Group II animals.

[a]Abbreviation is as follows: B(a)P, benzo(a)pyrene.

TABLE 3
Experimental design for enzymatic assay

Group	No. of Animals	Treatment
I (control)	6	Animals were kept on a normal diet for 15 days.
II	6	Animals were kept on 2% test diet of *Trachyspermum ammi* for 15 days.
III	6	Animals were kept on 4% test diet of *Trachyspermum ammi* for 15 days.
IV	6	Animals were kept on 6% test diet of *Trachyspermum ammi* for 15 days.

Preparation of homogenate, cytosol and microsome fractions. Animals were sacrificed, and the entire liver was perfused immediately with ice cold NaCl (0.9%) and thereafter carefully removed, trimmed free of extraneous tissue, and rinsed in chilled 0.15 M of Tris KCl buffer (0.15 M KCl + 10 mM Tris HCl, pH 7.4). The liver was then blotted dry, weighed quickly, and homogenized in ice cold 0.15 M Tris KCl buffer (pH = 7.4) to yield 10% (wt/vol) of homogenate. An aliquot of this homogenate (0.5 ml) was used for estimation of reduced GSH content, whereas the remainder was centrifuged at 10,500 *g* for 45 min at 4°C using RC5C Sorvall centrifuge (SM 24 rotor; Thermo Fisher Scientific, Bengluru, India). The resultant supernatant was centrifuged at 105,000 *g* for 60 min at 4°C in a Beckman ultracentrifuge (Model L 780M; Beckman Coulter, Mumbai, India). The supernatant (cytosol fraction), after discarding any floating lipid layer and appropriate dilution, was used for the assay of glutathione-S-transferase (GST), DT-diaphorase (DTD), catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), glyoxalase I (GLY I), reduced GSH, and lactate dehydrogenase (LDH). The pellet representing the microsomal fraction was resuspended in the homogenizing buffer and was used for the assay of cytochrome P450 (cyt P450), cytochrome b5 (cyt b5), NADPH-cytochrome P450 reductase (cyt P450 R), NADH-cytochrome b5 reductase (cyt b5 R), and lipid peroxidation.

The methods for determination of levels/activities of phase I and phase II enzymes, antioxidant enzymes, reduced GSH, LDH, and peroxidative damage are summarized in Table 4.

EXPERIMENT 4

Histological Studies

At the termination of the experiments, the skin and the forestomach tumors were removed and fixed in 10% formalin. Following standard techniques, tissues were embedded in paraffin wax and sections, 4–5 microns thick, were stained with hematoxylin and eosin for histological study.

EXPERIMENT 5

Western Blot Analysis

A total of 50 μg of total cytosolic protein extracts was separated on a 12% SDS-polyacrylamide gel and transferred to a PVDF membrane. After blocking the membrane in 5% nonfat dry milk powder in PBS, incubation with primary antibody of monoclonal mouse GST (1:1000; Santa Cruz, Germany) was accomplished for 1 h at room temperature. Protein detection employed specific horseradish peroxidase-conjugated secondary antibody antimouse (1:1000; Santa Cruz, Germany) in enhanced chemiluminescence detection system.

Statistical Analysis

The mean and SD were calculated for the data sets from Experiments 1 and 2. The data was analyzed using 1-way analysis of variance followed by Dunnetts multiple comparison test to find any statistical significant differences between the control group and the *Trachyspermum ammi* treated groups in each experiment. A P value less than 0.05 was considered to be significant.

RESULTS

EXPERIMENT 1

Modulatory Effect of *Trachyspermum ammi* on DMBA-Induced Skin Papillomagenesis

Table 5 represents the results of skin papillomagenesis obtained from treatment of *Trachyspermum ammi* during the peri-initiational period. All the animals comprising the respective control and the experimental groups maintained a healthy body weight. There was no manifestation of any kind of toxicity syndrome among the animals fed with *Trachyspermum ammi* test diet. The animals of the normal group did not develop any spontaneous tumors. The tumor incidence was 100% in the case of the control and the experimental groups. The tumor multiplicity exhibited a significant reduction in case of 2%, 4%, and 6% test diets of *Trachyspermum ammi*. It was interesting that *Trachyspermum ammi* could not reduce the tumor incidence but was able to inhibit the tumor multiplicity. The percentages of inhibition of tumor multiplicity were 25, 37.5, and 50 with 2%, 4%, and 6% test diets of *Trachyspermum ammi,* respectively, as can be seen in Table 5.

EXPERIMENT 2

Modulatory Effect of *Trachyspermum Ammi* on B(a)P-Induced Forestomach Papillomagenesis

Unlike skin papillomagenesis, in the case of forestomach papillomagenesis, *Trachyspermum ammi* exhibited a significant dose-dependent reduction in the tumor incidence. Test diet containing 2%, 4%, and 6% of *Trachyspermum ammi* lowered the incidence of tumors to 85.7%, 80%, and 68.75%, respectively.

TABLE 4
The methods for estimations of levels/activities of phase I and phase II enzymes, antioxidant enzymes, reduced glutathione, lactate dehydrogenase and peroxidative damage

Experiment	Method	Examined Fraction	Molar extinction Coefficient	Units
Cyt P450	Omura and Sato (12)	Microsome	91 mM^{-1} cm^{-1}	nmole/mg protein
Cyt b5	Omura and Sato (12)	Microsome	185 mM^{-1} cm^{-1}	nmole/mg protein
Cyt P450R	Omura and Takesue (13)	Microsome	6.22 mM^{-1} cm^{-1}	μmole of NADPH oxidized/min/mg protein
Cyt b5R	Mihara and Sato (14)	Microsome	1.02 mM^{-1} cm^{-1}	μmole of NADH oxidized/min/mg protein
GST	Habig *et al* (15)	Cytosol	9.6 mM^{-1} cm^{-1}	μmole of CDNB-GSH conjugate formed/min/mg protein
DTD	Ernster *et al* (16)	Cytosol	21 mM^{-1} cm^{-1}	μmole of DCPIP reduced/min/mg protein
Catalase	Aebi (17)	Cytosol	40 mM^{-1} cm^{-1}	μmole of H_20_2 consumed/min/mg protein
SOD	Marklund and Marklund (18)	Cytosol	Not applicable	μmole/mg protein
GPx	Paglia and Valentine (19)	Cytosol	6.22 mM^{-1} cm^{-1}	nmole of NADPH consumed/mg protein
GR	Carlberg and Mannervik (20)	Cytosol	6.22 mM^{-1} cm^{-1}	nmole of NADPH consumed/mg protein
GLY I	Thornalley (21)	Cytosol	2.86 mM^{-1} cm^{-1}	μmole of s-lactoylglutathione formed/min/mg protein
GSH	Moron *et al* (22)	Liver homogenate	Not applicable	nmole GSH/gm tissue
LDH	Bergmeyer and Bernt (23)	Cytosol	6.22 mM^{-1} cm^{-1}	μmole/mg protein
Peroxidative damage	Varshney and Kale (24)	Microsome	Not applicable	nmole malondialdehyde formed/mg protein
Protein	Lowry *et al* (25)	Cytosol and Microsome	Not applicable	mg/ml

Abbreviations: Cyt P450: cytochrome P450, Cyt b5: cytochrome b5, Cyt P450 R: NADPH-cytochrome P450 reductase, Cyt b5R: NADH-cytochrome b5 reductase, GST: glutathione-S-transferase, DTD: DT- diaphorase, SOD: superoxide dismutase, GPx: glutathione peroxidase, GR: glutathione reductase, GLYI: glyoxalase I, GSH: reduced glutathione content, LDH: lactate dehydrogenase.

TABLE 5
Modulatory effect of test diets of *Trachyspermum ammi* Sprague on DMBA-induced skin papillomagenesis[a]

Measurement Indexes	Normal	DMBA +2% Croton Oil	DMBA +2% Croton Oil +2% Test Diet	DMBA +2% Croton Oil +4% Test Diet	DMBA +2% Croton Oil +6% Test Diet
Tumor incidence	Nil	100	100	100	100
Tumor multiplicity	Nil	8.0 ± 3.1	6 ± 1.0^b	5 ± 1.1^b	4 ± 1.4^b
Inhibition of tumor multiplicity (%)	Nil	—	25.0	37.5	50.0

[a]Abbreviation is as follows: DMBA, 7,12 dimethylbenz(a)anthracene. In skin papillomagenesis experiment, values are expressed as mean ± SD of 16–18 animals.
[b]$P < 0.01$; represents significant changes against the control group of animals.

The percentages of inhibition of tumor multiplicity, with the same compositions of test diet of *Trachyspermum ammi*, were 33.33, 50.0, and 66.66, respectively. The results are depicted in Table 6.

EXPERIMENT 3

Modulatory Effect of *Trachyspermum ammi* on the Hepatic Phase I and Phase II Drug Metabolizing Enzymes, Antioxidant Enzymes, Peroxidative Damage, and Activity of LDH

Phase I enzymes. The specific activity of cytchrome P450 reductase exhibited a significant enhancement by 1.35 fold ($p < 0.01$), 1.21 fold ($p < 0.01$), and 1.34 fold ($p < 0.01$) in case of 2%, 4% and 6% test diets of *Trachyspermum ammi* respectively, as compared to the control group (Table 7). cyt P450 and cyt b5 contents and cyt b5 R activity exhibited a significant enhancement (less than 20–30% of the control group) especially with 6% test diet as compared to the control group (data not shown); 2% and 4% test diets were found to have no significant effects on the cyt P450 and cyt b5 content in the liver of mice.

Phase II enzymes. There was a significant dose dependent increase in the GST activity. As compared to the control group, the specific activity of GST was significantly enhanced by 1.18-fold ($P < 0.05$), 1.45-fold ($P < 0.01$), and 1.48-fold ($P < 0.01$) in case of 2%, 4%, and 6% test diets of *Trachyspermum ammi,* respectively. The specific activity of DTD was significantly enhanced by 1.73-fold ($P < 0.05$), 2.0-fold ($P < 0.01$), and 2.47-fold ($P < 0.01$) relative to the control group in the case of the groups treated with 2%, 4%, and 6% test diets of *Trachyspermum ammi,* respectively. These results are shown in Table 7.

Antioxidant enzyme profile. Relative to the control group, the specific activity of catalase was significantly enhanced by 1.65-fold ($P < 0.01$), 1.84-fold ($P < 0.01$), and 1.98-fold ($P < 0.01$) in the case of the groups treated with 2%, 4%, and 6% test diets of *Trachyspermum ammi,* respectively. The specific activity of SOD was enhanced with 4% and 6% test diets, whereas that of GPx was significantly enhanced with all 3 doses of *Trachyspermum ammi*. Relative to the control group, the SOD activity was significantly enhanced by 1.35-fold ($P < 0.01$) and 1.66-fold ($P < 0.01$) in the case of the groups treated with 2%, 4%, and 6% test diets of *Trachyspermum ammi,* respectively. In the case of GPx, the specific activity was significantly enhanced by 1.66-fold ($P < 0.01$), 1.90-fold ($P < 0.01$), and 2.46-fold ($P < 0.01$) in the case of the groups of animals treated with 2%, 4%, and 6% test diets of *Trachyspermum ammi,* respectively. However, relative to the control group, significant enhancement in the specific activity of GR by 1.24-fold ($P < 0.01$) was observed only in the case of the group of animals treated with 6% test diet of *Trachyspermum ammi.* The specific activity of GLY I showed 1.38-fold ($P < 0.01$), 1.65-fold ($P < 0.01$), and 1.72-fold ($P < 0.01$) enhancement in the specific activity with 2%, 4%, and 6% test diets of *Trachyspermum ammi,* respectively, as compared to the control group. The content of reduced GSH was significantly enhanced by 1.62-fold ($P < 0.05$), 2.15-fold

TABLE 6
Modulatory effect of test diets of *Trachyspermum ammi* Sprague on B(a)P-induced forestomach papillomagenesis[a]

Measurement Indexes	Normal	B(a)P Only	B(a)P + 2 % Test Diet	B(a)P + 4% Test Diet	B(a)P + 6% Test Diet
Tumor incidence	Nil	100	85.7	80	68.75
Tumor multiplicity	Nil	6 ± 1.4	4 ± 1.3^b	3 ± 1.1^b	2 ± 0.93^b
Inhibition of tumor multiplicity (%)	Nil	—	33.33	50.0	66.66

[a]Abbreviation is as follows: B(a)P, benzo(a)pyrene. In the forestomach papillomagenesis experiment, values are expressed as mean ± SD of 18–20 animals.
[b]$P < 0.01$; represents significant changes against the control group of animals.

TABLE 7
Modulatory effect of test diets of *Trachyspermum ammi* on the phase I and phase II drug metabolizing enzymes, antioxidant enzymes, peroxidative damage, and lactate dehydrogenase activity in the liver of mice[a]

Measurement Indexes	Control	2% Test Diet	4% Test Diet	6% Test Diet
Cyt P450 R (1)	0.170 ± 0.004 (100)	0.231 ± 0.01[b] (135.88)	0.206 ± 0.01 (121.17)	0.228 ± 0.02[b] (134.11)
GST (2)	1.524 ± 0.15 (100)	1.807 ± 0.09[c] (118.56)	2.217 ± 0.17[b] (145.47)	2.268 ± 0.35[b] (148.81)
DTD (3)	0.019 ± 0.007 (100)	0.033 ± 0.009[c] (173.68)	0.038 ± 0.01[b] (200.0)	0.047 ± 0.01[b] (247.36)
CAT (4)	23.839 ± 2.53 (100)	39.439 ± 3.58[b] (165.43)	44.0 ± 1.25[b] (184.59)	47.214 ± 2.14[b] (198.04)
SOD (5)	12.054 ± 1.37 (100)	13.930 ± 1.02 (115.56)	16.346 ± 2.90[b] (135.60)	20.082 ± 1.29[b] (166.60)
GPx (6)	5.890 ± 1.90 (100)	9.806 ± 1.22[b] (166.48)	11.224 ± 2.18[b] (190.56)	14.508 ± 0.91[b] (246.31)
GR (6)	37.083 ± 1.21 (100)	37.154 ± 3.93 (101.19)	37.638 ± 2.11 (101.49)	46.343 ± 3.45[b] (124.97)
GLY I (7)	2.065 ± 0.80 (100)	2.865 ± 0.36[b] (138.74)	3.425 ± 0.43[b] (165.85)	3.567 ± 0.23[b] (172.73)
GSH (8)	1.342 ± 0.14 (100)	2.185 ± 0.28[c] (162.81)	2.895 ± 0.83[b] (215.72)	3.141 ± 1.02[b] (234.05)
LDH (9)	0.863 ± 0.001 (100)	0.428 ± 0.005[b] (49.59)	0.408 ± 0.003[b] (47.27)	0.195 ± 0.003[b] (22.59)
Peroxidative damage (10)	1.964 ± 0.015 (100)	1.615 ± 0.077[b] (82.23)	1.593 ± 0.346[b] (81.10)	1.412 ± 0.036[b] (71.89)

[a] Abbreviations are as follows: Cyt P450 R, cytochrome P450 reductase; GST, glutathione-S-transferase; DTD, DT-diaphorase; CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; GR, glutathione reductase; GLY I, glyoxalase I; GSH, reduced glutathione content; LDH, lactate dehydrogenase; (1), μmole of NADPH oxidized/min/mg protein; (2), μmole of CDNB-GSH conjugate formed/min/mg protein; (3), μmole of DCPIP reduced/min/mg protein; (4), μmole H_2O_2 consumed/min/mg protein; (5), specific activity expressed as μmole/mg protein; (6), nmole of NADPH consumed/min/mg protein; (7), μmole of s-lactoylglutathione formed/min; (8), nmole GSH/gm/tissue; (9), μ mole/mg protein; (10), nmole malondialdehyde formed/mg protein. Values are expressed as mean ± SD of 6–8 animals. Values in parentheses represent relative change in parameters assessed (i.e., levels of activity in livers of mice receiving test substance to activity in liver of control mice).
[b] $P < 0.01$; represents significant changes against the control group of animals.
[c] $P < 0.05$; represents significant changes against the control group of animals.

($P < 0.01$), and 2.34-fold ($P < 0.01$) in the case of the groups of animals treated with 2%, 4%, and 6% test diets of *Trachyspermum ammi,* respectively. These results are shown in Table 7.

LDH and peroxidative damage. The specific activity of LDH exhibited a significant decrease, as compared to the control group, by 0.49-fold ($P < 0.01$), 0.47-fold ($P < 0.01$), and 0.22-fold ($P < 0.01$) in the case of the groups treated with 2%, 4%, and 6% test diets of *Trachyspermum ammi,* respectively. Lipid peroxidation was also significantly reduced by 0.82-fold ($P < 0.01$), 0.81-fold ($P < 0.01$), and 0.71-fold ($P < 0.01$), relative to the control group, in the case of animals treated with 2%, 4%, and 6% test diets of *Trachyspermum ammi,* respectively. These results can be seen in Table 7.

EXPERIMENT 4

Histological Studies of Skin Papillomagenesis and Forestomach Papillomagenesis

Histological sections of skin from the normal group of animals exhibited a normal histological appearance with the epidermal and the dermal layers (Fig. 2A). Sections from the skin of animals treated with DMBA and croton oil shows the development of benign squamous papillomas arising from the epidermis (Fig. 2B). The histological sections of the papillomas from the experimental groups treated with DMBA and croton oil along with the respective plant modulators at 2% (low dose), 4% (intermediate dose), and 6% (high dose) test diets exhibited the development of both exophytic and endophytic forms of papillomas, reduction in the size of papillomas, and increase in keratinization as compared with the control group. These results are shown in Figs. 2C through 2E.

Histological sections from the forestomach of the normal group of animals exhibited histological details of normal forestomach (Fig. 2F). Sections from the forestomach tissue of group of animals treated orally with only B(a)P exhibited development of benign papillomas arising from the squamous epithelium of the forestomach wall. The papillomas grow in an exophytic manner and demonstrate simple branching (Fig. 2G). Sections from the experimental groups receiving B(a)P along with the test diets of *Trachyspermum ammi* with respect to all 3 doses (2%, 4%, and 6%) exhibited a decrease in papilloma size. Maximum reduction in the papilloma size was observed with 6% test diet treated groups. The findings with 2%, 4%, and 6% test diets, representing protective effect of *Trachyspermum ammi,* are shown in Fig. 2H, Fig. 2I, and Fig. 2J.

EXPERIMENT 5

Western Blot Analysis

Western blot analysis of GST protein, from the cytosolic fractions of the forestomach tissue bearing B(a)P induced papillomas, exhibited gradual induction of GST, which was markedly evident in the case of the group treated with 6% test diet of *Trachyspermum ammi* (data not shown).

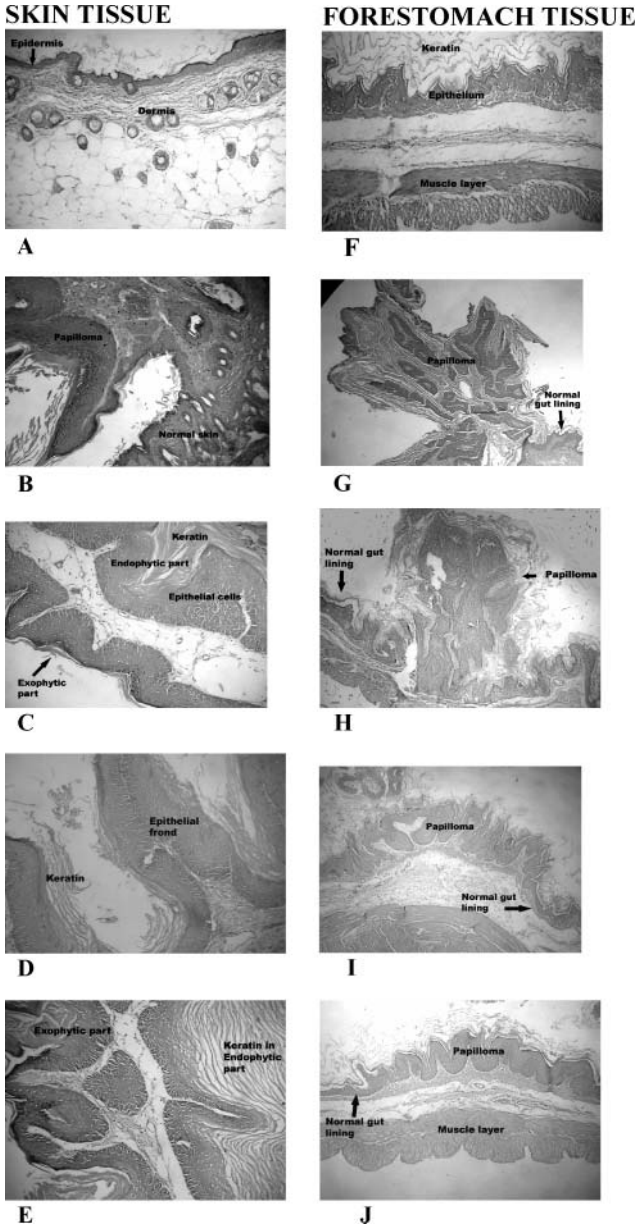

FIG. 2. Histopathological study of skin and forestomach papillomagenesis. 2A: Photomicrograph of skin from normal control group (HE × 40); 2B: Photomicrograph of skin from positive control group (HE × 100). 2C: Photomicrograph of skin from group treated with 2% modulator, DMBA + croton oil (HE × 100). 2D: Photomicrograph of skin from group treated with 4% modulator, DMBA + croton oil (HE × 100). 2E: Photomicrograph of skin from group treated with 6% modulator, DMBA + croton oil (HE × 40). 2F: Photomicrograph of forestomach wall from normal control group (HE × 40). 2G: Photomicrograph of forestomach wall from group receiving B(a)P (HE × 40). 2H: Photomicrograph of forestomach wall from group receiving 2% modulator and B(a)P (HE × 40). 2I: Photomicrograph of forestomach wall from group receiving 4% modulator and B(a)P (HE × 40). 2J: Photomicrograph of forestomach wall from group receiving 6% modulator and B(a)P (HE × 40). Modulator, *Trachyspermum ammi.*

DISCUSSION

Spices form an integral part of the diet worldwide. *Trachyspermum ammi* seeds are widely used as a spice ingredient. The importance of spices as potent cancer chemopreventive agents have been well established (26–28). In the present study, therefore, the chemopreventive potential of *Trachyspermum ammi* seeds was examined against skin and forestomach papillomagenesis.

For the DMBA-induced skin papillomagenesis model, dietary administration of 2%, 4%, and 6% test diets of *Trachyspermum ammi* significantly reduced the skin tumor multiplicity in a dose-dependent manner. However, it failed to inhibit the incidence of skin tumors (Table 5). In comparison, for the B(a)P-induced forestomach papillomagenesis, the dietary administration of 2%, 4%, and 6% test diets of *Trachyspermum ammi* seeds significantly inhibited both tumor multiplicity and tumor incidence at the peri-initiational level (Table 6).

The difference in the chemopreventive effects exerted by *Trachyspermum ammi* on the skin and forestomach papillomagenesis can possibly be associated with its bioavailability and the actual doses received by them (29). It is speculated that the skin and forestomach tumor development mechanisms may follow different pathways since DMBA-inducing skin papillomas and B(a)P-inducing forestomach papillomas have different pathways of metabolism (30,31). As a result, the action of the various carcinogenic metabolites of DMBA and B(a)P (32) with *Trachyspermum ammi* may have differential reactions. This may result in the differential manifestation of the tumor incidence and multiplicity profile in case of the skin and forestomach papillomagenesis with *Trachyspermum ammi* treated group.

The histological features of skin cancer indicated a protective effect of *Trachyspermum ammi* in terms of smaller and more differentiated papillomas. The papillomas from the group of animals treated with *Trachyspermum ammi* revealed keratinization, which is a sign of differentiation (Figs. 2C–2E). In the case of the forestomach papillomagenesis, the group treated with *Trachyspermum ammi* revealed a decrease in the papilloma size (Figs. 2H–2J). These findings were supportive of chemopreventive potential of *Trachyspermum ammi.*

The phase I and phase II enzymes are known to play an important role in the metabolism of xenobiotics including carcinogens (8). The present experimental investigation reported a significant elevation in the phase I enzyme system (Table 7). The content of cyt P450 and cyt b5 along with cyt P450 R activity showed a significant increase especially with 6% test diet of *Trachyspermum ammi,* which was likely to enhance the biotransformation of carcinogens (26). Since similar to indole-3-carbinol (33), *Trachyspermum ammi* exhibited a modulatory effect on cyt P450 system and at the same time has shown the chemopreventive activity in both tumor models, it could perhaps be considered as a blocking agent.

Trachyspermum ammi seeds also exhibited a significant elevation in the activities of hepatic GST and DTD (Table 7). Both these enzymes belong to the phase II enzyme system, which acts upon the substrates generated by the action of phase I enzymes on the carcinogens to convert them into metabolites that are solubilized in water and subsequently excreted. Since the major function of GST is known to catalyze the conjugation of electrophilic xenobiotics or carcinogens to the endogenous nucleophile GSH and in turn protect the cellular components (34), the enhanced activity of GST due to *Trachyspermum ammi* might also contribute to the prevention of DMBA- and B(a)P-induced skin and forestomach papillomagenesis. It could be noted that B(a)P 4,5-epoxide and anti-BPDE which constitute the reactive intermediates of B(a)P metabolism, were reported to serve as substrates for GST (35). Benzo (a) pyrene-7,8-diol-9,10-epoxide, an ultimate carcinogenic metabolite of B(a)P, is also detoxified by GST (36). An elevation in DTD activity due to *Trachyspermum ammi* might also contribute to inhibition of carcinogenesis induced by DMBA and B(a)P, as it is known to play a key role in protecting the cells against the toxicities of a variety of quinones and their metabolic precursors including polycyclic aromatic hydrocarbons (37,38) as well as against oxidative stress (39,40). Thus a concomitant increase in the specific activities of phase I and phase II enzymes is expected to enhance the detoxification of DMBA and B(a)P and control their carcinogenic effect.

The induction of GST by numerous compounds including food phytochemicals results in protection against toxicity and chemical carcinogenesis, especially during the initiation phase (41). Inducers of GST have received much attention as a potential chemopreventive agent. Therefore, a preliminary and representative experiment was carried out to assess its induction in the forestomach tissues bearing B(a)P-induced papillomas. Western blot analysis of GST protein from the cytosolic fractions of the forestomach tissue, bearing B(a)P-induced papillomas, from the groups treated with *Trachyspermum ammi,* exhibited a higher fold induction in GST, particularly at 6% test diet as compared to the control group. It appears that *Trachyspermum ammi* had an ability to induce the enzymes involved in metabolism of carcinogenesis and in turn reduce its carcinogenic effect.

A plethora of experimental reports provide evidence of the role of oxidative stress in mutagenesis, which is intimately linked with carcinogenesis (42). The reactive oxygen species that cause oxidative stress are implicated in the etiology and progression of many diseases including cancer (43). The enzymatic and nonenzymatic antioxidants present in the body are suggested to scavenge the reactive oxygen species and confer protection against oxidative stress and in turn serve as inhibitors of carcinogenesis (44). Metabolism of carcinogens and the application of tumor promoters are associated with generation of active oxygen species such as O_2^- and H_2O_2. A significant elevation in the activities of catalase, SOD, and GPx in case of all 3 doses of *Trachyspermum ammi* (Table 7) were expected to detoxify reactive oxygen species and a wide variety of reactive metabolites leading to protection against oxidative stress. Further, a significant enhancement of GR activity, particularly at 6% test diet of *Trachyspermum ammi,* is likely to regulate GSH-GSSG cycle in cells (45) and increase the reduction of oxidized glutathione to GSH. GLY I suggested to have antioxidant function as the electrophiles and cytotoxic-2-oxaldehydes are converted to less reactive chemical species (46) also exhibited a significant enhancement in its activity with all 3 doses of *Trachyspermum ammi* (Table 7). Therefore, a possible reduction in oxidative stress due to the test diet might increase chemopreventive potential of animals.

Reduced glutathione (GSH) is the principle nonprotein sulphydryl, which has been endowed with an important function in maintaining the reduced milieu of the cells. Due to its conjugating ability, GSH is involved in detoxification of xenobiotics including carcinogens (36). It has a redox potential of about (-)230 mV, which makes it behave as an antioxidant and protects against the electrophiles, free radicals, and in turn oxidative stress (47). Some of the GSH dependent antioxidant reactions are catalyzed by the enzymes GST and methyl glyoxalase. A significant enhancement of GSH content in this study, with all 3 doses of the test diet (Table 7), would perhaps help in the elimination of free radicals generated during carcinogen metabolism resulting in inhibition of their papillomagenetic effect. A dose-dependent decrease in the level of peroxidative damage due to test diet of *Trachyspermum ammi* (Table 7) was suggestive of the enhanced antioxidant status of animals. A reduction in the activity of LDH with all the doses of test diet (Table 7) was also supportive of protective potential of *Trachyspermum ammi* (27,48).

In addition to the preceding mechanisms, the seeds of *Trachyspermum ammi* might have provided chemoprevention through their antioxidative, immunomodulatory, and anti-inflammatory actions. Seeds of *Trachyspermum ammi* are known to contain antioxidants such as thymol and monoterpenes (2), which are the focus of investigations in the arena of cancer prevention and therapy, being associated with the regression of tumors (49).

Thus, the findings of this investigation suggested that *Trachyspermum ammi* could be regarded as a chemopreventive agent with respect to its ability to reduce the skin and forestomach papilloma burden. The elevation of specific activities of both the phase I and phase II systems by *Trachyspermum ammi* might have metabolized and detoxified DMBA and B(a)P and in turn contributed to inhibition of skin and forestomach papillomagenesis. Enhanced activities of antioxidant enzymes and level of GSH content are likely to metabolize reactive oxygen species and contribute to chemopreventive efficacy. In addition, an antioxidant content of seeds of *Trachyspermum ammi* and their anti-inflammatory property might have also caused chemoprevention of papillomagenesis.

From these findings, it could be inferred that regular use of *Trachyspermum ammi* seeds in the diet may reduce the risk of cancer, particularly stomach cancer. However, to confirm

the chemopreventive efficacy and associated mechanisms, *Trachyspermum ammi* seeds have to be examined using different tumor model systems.

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