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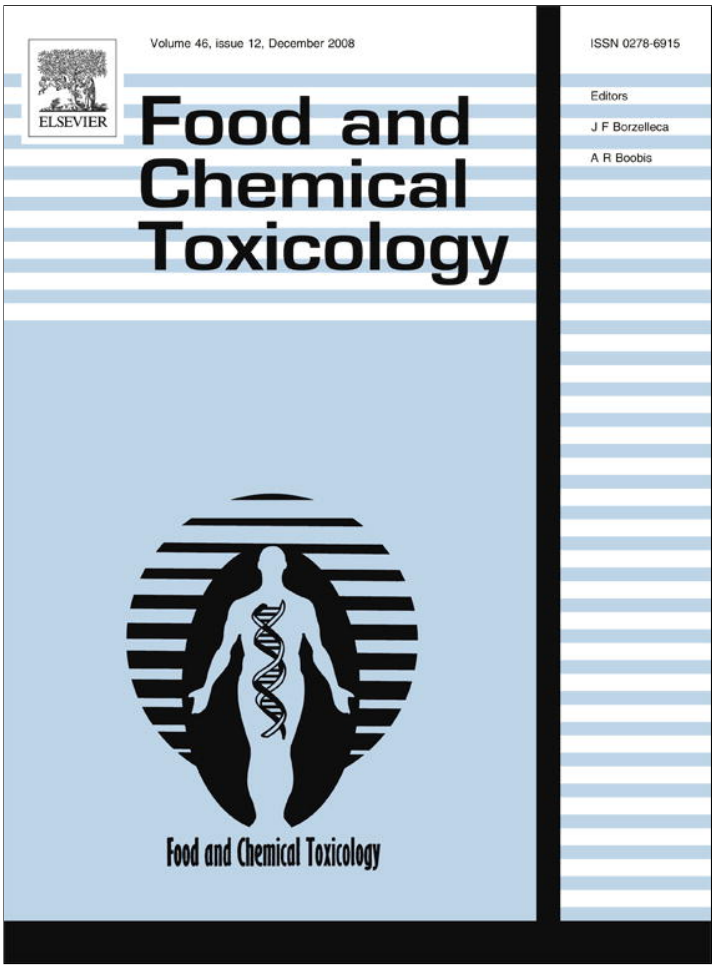

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Chemomodulatory action of *Foeniculum vulgare* (Fennel) on skin and forestomach papillomagenesis, enzymes associated with xenobiotic metabolism and antioxidant status in murine model system

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ABSTRACT

The chemopreventive effect of different doses of test diet of *Foeniculum vulgare* Mill (Fennel) seeds was examined on DMBA-induced skin and B(a)P-induced forestomach papillomagenesis in Swiss albino mice. To the best of our knowledge, this is the first report of Fennel seeds exhibiting a significant reduction in the skin and the forestomach tumor incidence and tumor multiplicity as compared to the control group. Further, biochemical assays showed a significant increase in the content/activities of phase I enzymes especially in the case of 6% test diet. A concomitant increase in the activities of the phase II enzymes were observed with all the doses of test diet under study. A significant enhancement in the activities of antioxidant enzymes were observed especially at 4% and 6% test diets of Fennel. Glyoxalase I activity and the content of reduced glutathione were significantly elevated. Expectedly, the levels of peroxidative damage along with lactate dehydrogenase activity, exhibited a significant reduction at all three doses of test diets. These findings were indicative of chemopreventive potential of Fennel against carcinogenesis.

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1. Introduction

Foeniculum vulgare Mill (Fennel) belonging to the Family Apiaceae (Umbelliferae) is a perennial herb native to the Mediterranean region. It is widely cultivated throughout the temperate and sub tropical regions of the world for its aromatic fruits extensively used as a culinary spice. It is a stout glabrous herb, 5–6 ft in height. The plant is pleasantly aromatic and is used as a pot herb. The leaves are used in fish sauce as garnishing and leaf stalks in salad. Thickened leaf stalks of Fennel are blanched and used as a vegetable. The leaves are reported to have diuretic properties. The roots are regarded as purgatives. Dried fruits of Fennel possess a fragrant odour and a pleasant aromatic taste. They are used for flavouring soups, meat dishes, sauces and confectionary items. The fruits are aromatic, stimulant, carminative and are considered to be useful in diseases of the chest, spleen and kidney (The Wealth of India, 1956).

Volatile oil of Fennel seeds has demonstrated carminative and stimulant activities as well as spasmolytic actions on the smooth muscles of experimental animals (Leung and Foster, 1996). The antiinflammatory, analgesic and antioxidant activities of the fruit of Fennel has been reported by Choi and Hwang (2004). Oral administration of fruit methanolic extract of Fennel exhibited inhibitory effect against acute and subacute inflammatory diseases and type IV allergic reactions and showed a central analgesic effect. It significantly increased the specific activities of superoxide dismutase (SOD) and catalase as well as significantly decreased the high density lipoprotein–cholesterol level along with a decrease in the peroxidative damage (Choi and Hwang, 2004). The essential oil of Fennel exhibited antibacterial activity (Ruberto et al., 2000) and antiviral activity (Shukla et al., 1988). The aqueous and ethanol extracts of Fennel seeds have exhibited potential antioxidant properties in *in vitro* studies (Oktay et al., 2003). Methanolic extracts of the whole plant of Fennel administered for four successive days ameliorated the amnesic effect of scopolamine (0.4 mg/kg) and aging induced memory deficits in mice. This property of the plant can be useful for the treatment of cognitive disorders such as dementia and Alzhemier's disease (Joshi, 2006).

Anethole is the principal active component of fennel seeds which has exhibited anticancer activity (Anand et al., 2008). The antitumor activity of anethole against Ehrlich ascites carcinoma have been reported (Al Harbi et al., 1995). Therefore, in the present investigation, the chemopreventive efficacy of Fennel seeds has

Abbreviations: B(a)P, benzo(a)pyrene; DMBA, dimethylbenz(a)pyrene; DTD, DT-diaphorase; Gly I, glyoxalase I; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GST, glutathione-S-transferase; LDH, lactate dehydrogenase; NADH, reduced nicotinamide adenine dinucleotide; NADPH, reduced nicotinamide adenine dinucleotide phosphate; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

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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 Fennel seeds around the event of initiation). Apart from above, Fennel seeds were also assessed for their capacity to modulate the levels/activities of phases I and II detoxification enzymes, antioxidant enzymes, content of reduced glutathione and toxicity in terms of peroxidative damage and activity of lactate dehydrogenase. Histological studies from the skin and forestomach papillomagenesis experiments were also performed.

To the best of our knowledge, our finding is the first report showing chemopreventive potential of seeds of Fennel against carcinogenesis. Since a large population worldwide consume fennel seeds, the present observation may have some significance.

2. Materials and methods

2.1. Chemicals

Benzo(a)pyrene [B(a)P], 7,12-dimethylbenz(a)anthracene (DMBA), 1-chloro 2,4-dinitrobenzene (CDNB), 5,5′dithiobis-2-nitrobenzoic acid (DTNB), 2,6-dichlorophenolindophenol (DCPIP), ethylenediamine tetraacetic acid (EDTA), reduced glutathione (GSH), potassium ferricyanide, pyrogallol, bovine serum albumin (BSA), methylglyoxal, Triton X-100, thiobarbituric acid (TBA), reduced nicotinamide adenine dinucleotide (NADH) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The rest of the chemicals used were obtained from local firms (India) and were of highest purity grade.

2.2. Modulator

Seeds of Fennel 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%) w/w and pellets were prepared for oral administration. Mice were provided with standard food pellets (Hindustan Lever Limited, India). Standard rodent diet was composed of (w/w) wheat (60.3%), roasted bengal gram (28.2%), refined peanut oil (5%), skimmed milk powder (1%), casein (1%), vitamin mixture (0.5%) and mineral mixture (4%), as recommended by the National Institute of Nutrition, Hyderabad, India (Padmavathi et al., 2005). The pellets with different concentrations of the Fennel seeds were stored in clean bags in the feed store room of animal house of the Jawaharlal Nehru University, under strict hygienic conditions, till the end of the experiment.

2.3. Animals

Random bred female Swiss albino mice (6–8 week old) were used for the present study. The animals were maintained in the air-conditioned animal facility (Jawaharlal Nehru University, New Delhi, India) with a 12 h light/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 and Jawaharlal Nehru University Institutional Animal Ethics Committee, on the use of animals for scientific research.

In the present study, four separate experiments were performed to delineate specific objectives as mentioned earlier. Experiments I and II were aimed to evaluate the probable efficacy of Fennel, in chemopreventing skin and forestomach papillomagenesis in murine model system, respectively. Experiment III was performed to study the chemomodulatory effect of the same on the hepatic phases I and II enzymes, antioxidant enzyme profile and toxicity in terms of peroxidative damage and activity of lactate dehydrogenase. Experiment IV included histological studies.

2.4. Experiment I: Modulatory effect of Fennel on DMBA-induced skin papillomagenesis

2.4.1. Preparation of chemicals and modulators

DMBA was dissolved in acetone at a concentration of 50 μg/50 μl acetone and was applied topically to the animals. Croton oil (2%) 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/0.1 ml of peanut oil and was administered to the animals through an oral gavage. Test diet of Fennel (2%, 4% and 6%) was prepared and orally administered to the animals.

2.4.2. Experimental design

The experiment was performed as described by Yasukawa et al. (1995) with some modifications. The hairs on the dorsal scapular region (2 cm diameter) of the mice were clipped off three 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 the following groups:

Group I ($n = 16$): Animals were fed with normal diet. This group served as the normal control.
Group II ($n = 18$): 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 weeks after the carcinogen application, 0.1 ml of 2% croton oil in acetone was applied twice a week until termination of the experiment. This group served as the positive control.
Group III ($n = 18$): Animals were fed with 2% test diet of Fennel 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.
Group IV ($n = 18$): Animals were fed with 4% test diet of Fennel 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.
Group V ($n = 18$): Animals were fed with 6% test diet of Fennel 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.

The body weight of the animals was 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 tumor incidence and tumor multiplicity (number of papillomas per mouse) were determined.

2.5. Experiment II: Modulatory effect of Fennel on B(a)P-induced forestomach papillomagenesis

2.5.1. Experimental design

The experiment was performed as described by Azuine and Bhide (1992). This is a modified method originally described by Wattenberg (1981). The animals were assorted into the following groups:

Group I ($n = 18$): Animals were kept on a normal diet and did not receive B(a)P treatment. This group of animals served as the normal control.
Group II ($n = 20$): Animals were kept on a normal diet for 2 weeks afterwhich each animal was administered with eight doses of 1 mg of B(a)P per 0.1 ml of peanut oil (twice weekly for four weeks) through an oral gavage. This group of mice served as the positive control group.
Group III ($n = 20$): Animals were kept on 2% test diet of Fennel starting 2 weeks before, during and 2 weeks after the carcinogen treatment (eight doses of 1 mg B(a)P per 0.1 ml of peanut oil) as given to group II animals.
Group IV ($n = 20$): Animals were kept on 4% test diet of Fennel starting 2 weeks before, during and 2 weeks after the carcinogen treatment (eight doses of 1 mg B(a)P per 0.1 ml of peanut oil) as given to group II animals.
Group V ($n = 20$): Animals were kept on 6% test diet of Fennel starting 2 weeks before, during and 2 weeks after the carcinogen treatment (eight doses of 1 mg B(a)P per 0.1 ml of peanut oil) as given to group II animals.

The 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 tumor incidence and tumor multiplicity were determined.

2.6. Experiment III: Modulatory effect of Fennel on the hepatic phases I and II enzymes, antioxidant enzymes, lactate dehydrogenase and peroxidative damage

2.6.1. Experimental design

The animals were randomly assorted into the following groups:

Group 1 ($n = 6$): Animals were kept on a normal diet for 15 days. This group of animals served as the control.
Group 2 ($n = 6$): Animals were kept on 2% test diet of Fennel for 15 days.
Group 3 ($n = 6$): Animals were kept on 4% test diet of Fennel for 15 days.
Group 4 ($n = 6$): Animals were kept on 6% test diet of Fennel for 15 days.

2.6.2. 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 (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% (w/v) of homogenate. An aliquot of this homogenate (0.5 ml)

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was used for estimation of reduced glutathione (GSH) content while the remainder was centrifuged at 10,500*g* for 45 min at 4 °C using RC5C Sorvall centrifuge (SM 24 rotor). The resultant supernatant was centrifuged at 1,05,000*g* for 60 min at 4 °C in a Beckman ultracentrifuge (Model L 780 M). The supernatant (cytosol fraction), after discarding any floating lipid layer and appropriate dilution, was used for the assay of GST, DTD, LDH, and antioxidant enzymes (catalase, SOD, GPx, GR, GLY I). 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), cytochrome P450 reductase (cyt P450 R), cytochrome b5 reductase (cyt b5 R) and lipid peroxidation.

2.6.3. Assay methods: cytochrome P450 and cytochrome b5

The estimation of cytochrome P450 content was based on the carbon monoxide difference spectra. The content of both cytochrome P450 and cytochrome b5 were determined in the microsomal suspension according to the method of Omura and Sato (1964), using an absorption coefficients of 91 and 185 cm^2/mmole, respectively.

2.6.4. NADPH–cytochrome P450 reductase and NADH–cytochrome b5 reductase

The specific activity of NADPH–cytochrome P450 reductase was determined by the method of Omura and Takesue (1970) with some modifications, measuring the rate of oxidation of NADPH at 340 nm. The reaction mixture contained 0.3 M potassium phosphate buffer (pH 7.5), 0.1 mM NADPH, 0.2 mM potassium ferricyanide and microsomal preparation in a final volume of 1 ml. The reaction was started at 25 °C by addition of NADPH and the rate of oxidation of NADPH was measured at 340 nm. The enzyme activity was calculated using extinction coefficient 6.22 mM^{-1} cm^{-1}. One unit of enzyme activity is defined as that causing the oxidation of 1 mol of NADPH per minute.

The specific activity of NADH–cytochrome b5 reductase was determined by the method of Mihara and Sato (1972) with some modifications, measuring the rate of reduction of potassium ferricyanide at 420 nm by NADH. The reaction mixture contained 0.1 M potassium phosphate buffer (pH 7.5), 0.1 mM NADH, 1 mM potassium ferricyanide and microsomal preparation in a final volume of 1 ml. The reaction was started at 25 °C by the addition of NADH and the rate of reduction of ferricyanide was measured at 420 nm. The enzyme activity was calculated using the extinction coefficient of 1.02 mM^{-1} cm^{-1}. One unit of enzyme activity is defined as that causing the reduction of 1 mol of ferricyanide per minute.

2.6.5. Glutathione-S-transferase

The specific activity of cytosolic GST was determined spectrophotometrically at 37 °C according to the method of Habig et al. (1974). The reaction mixture (1 ml) contained final concentration of 0.1 M phosphate buffer (pH 6.5), 1 mM CDNB in 95% ethanol and 1 mM GSH and was incubated at 37 °C for 5 min. The reaction was initiated by the addition of dilute cytosol sample and the enzyme activity was followed for 5 min at 340 nm. The specific activity of GST was calculated using the extinction coefficient 9.6 mM^{-1} cm^{-1} at 340 nm and expressed in terms of micromoles of GSH–CDNB conjugate formed/min/mg protein.

2.6.6. DT-diaphorase

The specific activity of DTD was determined by the method described by Ernster et al. (1962) with NADH as the electron donor and DCPIP as the electron acceptor at 600 nm. The reaction mixture contained 50 mM Tris–HCl buffer (pH 7.5), 0.5 mM NADH, 40 μM DCPIP and 0.08% Triton X-100 as an activator in a final volume of 1 ml. The reaction was started at 25 °C by the addition of cytosolic fraction containing the enzyme and the rate of reduction of DCPIP was measured at 600 nm. The activity was calculated using extinction coefficient 21 mM^{-1} cm^{-1}. One unit of enzyme activity is defined as amount of enzyme required to reduce one micromole of DCPIP per minute.

2.6.7. Catalase

The specific activity of catalase was determined by monitoring the disappearance of H_2O_2 as described by Aebi (1984). The cytosolic fraction was treated with Triton X-100 (1%) and ethanol (10 μl/ml) and was incubated in ice for 30 min. The treated supernatant was added to the assay mixture which contained 0.05 M sodium phosphate buffer (pH 7.0) and 10 mM H_2O_2. The decrease in absorbance was measured at 240 nm. The activity was calculated using the extinction coefficient as 40 μmol^{-1} cm^{-1}. The specific activity of catalase has been expressed as moles of H_2O_2 reduced/min/mg protein.

2.6.8. Superoxide dismutase

The specific activity of SOD was determined by the method of Marklund and Marklund (1974), which involves inhibition of pyrogallol autooxidation at pH 8.0. A single unit of enzyme was defined as the quantity of superoxide dismutase required to produce 50% inhibition of autooxidation. The cytosolic supernatant treated with Triton X-100 (1%) was kept at 4 °C for 30 min and was added to the assay mixture (1 ml) which contained 0.05 M sodium phosphate buffer (pH 8.0), 0.1 mM EDTA, 0.27 mM pyrogallol. The absorbance was measured for 5 min at 420 nm. Solution of pyrogallol was made in 10 mM HCl.

2.6.9. Glutathione peroxidase

The specific activity of GPx was measured by the procedure described by Paglia and Valentine (1967). Briefly, 1 ml of the reaction mixture volume contained 50 mM sodium phosphate buffer (pH 7.0) containing EDTA (0.1 M buffer containing 1 mM EDTA), 0.24 U/ml yeast glutathione reductase, 0.3 mM glutathione (reduced), 0.2 mM NADPH, 1.5 mM H_2O_2 and cytosolic sample. Reaction was initiated by addition of NADPH and the decrease in the absorbance was monitored at 340 nm for 5 min. One unit of enzyme activity has been defined as nmoles of NADPH consumed/min/mg protein based on an extinction coefficient of 6.22 mM^{-1} cm^{-1}.

2.6.10. Glutathione reductase

The specific activity of GR was determined by the method of Carlberg and Mannervik (1985). The final concentration of reactants in 1 ml of reaction mixture was 0.2 M sodium phosphate buffer (pH 7.0) containing 2 mM EDTA, 1 mM GSSG, 0.2 mM NADPH and cytosolic sample. The enzyme activity was measured indirectly by monitoring the oxidation of NADPH following decrease in OD/min for 5 min at 340 nm. One unit of enzyme activity has been defined as nmoles of NADPH consumed/min/mg protein, based on an extinction coefficient of 6.22 mM^{-1} cm^{-1}.

2.6.11. Glyoxalase I

The specific activity of GLY I was determined according to the method of Racker as described by Thornalley (1993). The activity is assayed by measuring the initial rate of formation of the S-lactoylglutathione. The assay mixture, in a volume of 1 ml, was 50 mM sodium phosphate buffer (pH 7.0), 3.5 mM methylglyoxal, 1.7 mM reduced glutathione. It was incubated for 10 min followed by the addition of the cytosolic sample and increase in absorbance was measured for 5 min at 240 nm. The activity was calculated using the extinction coefficient value 2.86 mM^{-1} cm^{-1}. One unit of the enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 μmol of S-lactoylglutathione/min.

2.6.12. Reduced glutathione

Reduced glutathione content was estimated as the total non-protein sulphydryl group by the method as described by Moron et al. (1979). Liver homogenate was precipitated with 0.1 ml of 25% trichloroacetic acid and the precipitate was removed after centrifugation. Free –SH groups were assayed in a total 3 ml volume by adding 2 ml of 0.6 mM DTNB prepared in 0.2 M sodium phosphate buffer (pH 8.0) and 0.1 ml of the supernatant. The absorbance was read at 412 nm using a spectrophotometer (Shimadzu UV-160). Reduced glutathione (GSH) was used as a standard to calculate nmole of –SH content/g tissue.

2.6.13. Lactate dehydrogenase

The specific activity of LDH was determined by measuring the rate of oxidation of NADH at 340 nm according to the method of Bergmeyer and Bernt (1974). In brief, the reaction mixture contained 50 mM potassium phosphate buffer (pH 7.5), 0.5 mM sodium pyruvate, 0.1 mM NADH and the required amount of cytosolic fraction to make the final volume of 1 ml. The reaction was started at 25 °C by addition of NADH and the rate of oxidation of NADH was measured at 340 nm in Shimadzu UV-160, spectrophotometer. The enzyme activity was calculated using extinction coefficient 6.22 mM^{-1} cm^{-1}. One unit of enzyme activity has been defined as that causing the oxidation of 1 μmol of NADH per minute.

2.6.14. Peroxidative damage

Peroxidative damage in microsomes was estimated spectrophotometrically by thiobarbituric acid reactive substances (TBARS) method as described by Varshney and Kale (1990) and is expressed in terms of malondoialdehyde (MDA) formed per mg protein. In brief 0.5 ml of microsomal sample was mixed with 1.5 ml of 0.15 M Tris–KCl buffer (pH 7.4) to which 0.5 ml of 30% TCA was added. Then 0.5 ml of 52 mM TBA (thiobarbituric acid) was added. The tubes were placed in a water bath for 45 min at 80 °C, cooled in ice and centrifuged at room temperature for 10 min at 3000 rpm in REMI–T8 table top centrifuge. The absorbance of the clear supernatant was measured against reference blank of distilled water at 531.8 nm in spectrophotometer (Shimadzu UV-160).

2.6.15. Protein determination

The protein content of the samples were determined according to the method of Lowry et al. (1951) using bovine serum albumin (BSA) as standard.

2.7. Statistical analysis

After calculating the mean and standard deviation, data was analysed using one way ANOVA followed by Dunnetts multiple comparison test to find a statistical significant difference between the control group and the *F. vulgare* treated groups. A value of $p < 0.05$ was considered to indicate a significant difference between the experimental and the control groups.

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2.8. Experiment IV: 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 μm thick, were stained with Haematoxylin and Eosin for histological study.

3. Results

3.1. Modulatory effect of Fennel on DMBA-induced skin papillomagenesis

3.1.1. Tumor incidence

The skin tumor incidence in case of the DMBA and croton oil treated control group of animals was 100% whereas it was 71% and 67% in case of groups treated with 4% and 6% test diets of Fennel, respectively (Table 1).

3.1.2. Tumor multiplicity

The tumor multiplicity was 8 ± 2.9 in case of the DMBA and croton oil treated control group of animals. Relative to this control group, the tumor multiplicity was significantly reduced to 5 ± 2.5 ($p < 0.01$) and 5 ± 2.1 ($p < 0.01$) in case of the experimental groups fed with 4% and 6% test diets of Fennel, respectively. The percentage inhibition of tumor multiplicity were 29.4 and 33.3 in case of the groups treated with 4% and 6% test diets of Fennel, respectively, as compared to the control group. The results are depicted in Table 1.

3.2. Modulatory effect of Fennel on B(a)P-induced forestomach papillomagenesis

3.2.1. Tumor incidence

The tumor incidences in forestomach were 100% in case of the control group of animals receiving only B(a)P, 80% in case of the animals treated with 2% test diet; 79% in case of 4% test diet and 76% in case of 6% test diet of Fennel (Table 2).

3.2.2. Tumor multiplicity

The tumor multiplicity was 6 ± 1.3 in case of the control group of animals, treated with B(a)P only. Relative to this control group, there was a significant dose dependent reduction in the tumor multiplicity exhibiting values of 5 ± 2.3 ($p < 0.01$); 4 ± 2 ($p < 0.01$) and 4 ± 1.4 ($p < 0.01$) in case of the groups of animals treated with 2%, 4% and 6% test diets of Fennel, respectively. The percentage inhibition of tumor multiplicity were 23.0, 35.3 and 40.5 in case of 2%, 4% and 6% test diets of Fennel, respectively. These results are depicted in Table 2.

3.3. Modulatory effect of Fennel on the phases I and II drug metabolizing enzymes

3.3.1. Phase I enzymes

There was a significant increase in the cytochrome P450 content by 1.12 fold ($p < 0.01$) in case of the group of animals treated with 6% test diet of Fennel relative to the control group of animals. Cytochrome b5 content exhibited a significant increase by 1.14 fold ($p < 0.01$), 1.16 fold ($p < 0.01$) and 1.21 fold ($p < 0.01$) with 2%, 4% and 6% test diets of Fennel, respectively, as compared to the control group. The specific activity of cytochrome P450 reductase exhibited a significant enhancement only with 6% test diet by 1.17 fold ($p < 0.01$). Cytochrome b5 reductase activity exhibited a significant enhancement with 4% and 6% test diets of Fennel by 1.21 fold ($p < 0.01$) and 1.30 fold ($p < 0.01$), respectively, as compared to the control group. These results are shown in Table 3.

3.3.2. Phase II enzymes

There was a significant enhancement in the specific activity of glutathione-S-transferase by 1.45 fold ($p < 0.01$) and 1.34 fold ($p < 0.01$) in case of the groups of animals treated with 4% and 6% test diets of Fennel, respectively, relative to the control group of animals. The specific activity of DT-diaphorase exhibited a significant enhancement by 1.15 fold ($p < 0.01$) and 1.18 fold ($p < 0.01$) with 4% and 6% test diets of Fennel, respectively, as compared to the control group. These results are shown in Table 3.

3.4. Modulatory effect of Fennel on the antioxidant enzymes

Relative to the control group of animals, the specific activity of catalase was significantly enhanced by 1.50 fold ($p < 0.01$) and 1.59 fold ($p < 0.01$) in case of the groups treated with 4% and 6% test

Table 1
Modulatory effect of Fennel on DMBA-induced skin papillomagenesis.

Groups	Body weight		Tumor incidence (%)	Tumor multiplicity (tumor/mouse)	Inhibition of tumor burden (%)
	Initial	Final			
Normal	22.88 ± 1.4 (100)	31.55 ± 1.4 (137.89)	Nil	Nil	Nil
DMBA + 2% croton oil	22.67 ± 2.5 (100)	30.77 ± 2.4 (135.73)	100	8 ± 2.9	–
DMBA + 2% croton oil + 2% test diet of Fennel	20.83 ± 1.9 (100)	28.66 ± 2.8 (137.59)	100	7 ± 2.6	7.5
DMBA + 2% croton oil + 4% test diet of Fennel	22.44 ± 1.3 (100)	30.83 ± 1.8 (137.38)	71	5 ± 2.5**	29.4
DMBA + 2% croton oil + 6% test diet of Fennel	22.44 ± 2.6 (100)	30.75 ± 2.5 (137.03)	67	5 ± 2.1**	33.3

Values are expressed as mean ± SD of 16–18 animals.
** Significant changes against the positive control group of animals ($p < 0.01$).

Table 2
Modulatory effect of Fennel on B(a)P-induced forestomach papillomagenesis.

Groups	Body weight		Tumor incidence (%)	Tumor multiplicity (tumor/mouse)	Inhibition of tumor burden (%)
	Initial	Final			
Normal	21.60 ± 1.5 (100)	33.19 ± 2.9 (153.65)	Nil	Nil	Nil
B(a)P only	23.11 ± 1.2 (100)	36.40 ± 2.8 (157.50)	100	6 ± 1.3	–
B(a)P + 2% test diet of Fennel	20.55 ± 0.9 (100)	35.733 ± 3.4 (173.88)	80	5 ± 2.3	23
B(a)P + 4% test diet of Fennel	24.88 ± 1.9 (100)	38.28 ± 3.2 (153.85)	79	4 ± 2.0**	35.3
B(a)P + 6% test diet of Fennel	21.60 ± 1.7 (100)	35.19 ± 1.7 (162.91)	76	4 ± 1.4**	40.5

Values are expressed as mean ± SD of 18–20 animals.
** Significant changes against the positive control group of animals ($p < 0.01$).

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Table 3
Modulatory effect of Fennel on the hepatic phases I and II drug metabolizing enzymes.

Groups	Cyt P450 (1)	Cyt b5 (1)	Cyt P450 R (2)	Cyt b5 R (3)	GST (4)	DTD (5)
Control	0.143 ± 0.001 (100)	0.141 ± 0.003 (100)	0.194 ± 0.004 (100)	1.607 ± 0.15 (100)	1.621 ± 0.35 (100)	0.058 ± 0.004 (100)
2% test diet of Fennel	0.145 ± 0.003 (101.39)	0.162 ± 0.008** (114.89)	0.202 ± 0.01 (104.12)	1.756 ± 0.15 (109.27)	1.757 ± 0.02 (108.30)	0.061 ± 0.001 (105.17)
4% test diet of Fennel	0.152 ± 0.004 (106.29)	0.164 ± 0.001** (116.31)	0.213 ± 0.009 (109.79)	1.959 ± 0.20** (121.90)	2.355 ± 0.03** (145.28)	0.067 ± 0.001** (115.51)
6% test diet of Fennel	0.161 ± 0.005** (112.58)	0.171 ± 0.005** (121.27)	0.227 ± 0.004** (117.01)	2.103 ± 0.16** (130.86)	2.179 ± 0.36** (134.42)	0.069 ± 0.005** (118.96)

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 livers of control mice).
(1) nmole/mg protein, (2) μmole of NADPH oxidized/min/mg protein, (3) μmole of NADH oxidized/min/mg protein, (4) μmole of CDNB–GSH conjugate formed/min/mg protein and (5) μmole of DCPIP reduced/min/mg protein.
Abbreviations: Cyt P450, cytochrome P450; Cyt b5, cytochrome b5; Cyt P450 R, cytochrome P450 reductase; Cyt b5 R, cytochrome b5 reductase; GST, glutathione-S-transferase; DTD, DT-diaphorase.
** Significant changes against the control group of animals ($p < 0.01$).

Table 4
Modulatory effect of Fennel on the hepatic antioxidant enzymes.

Groups	CAT (1)	SOD (2)	GPx (3)	GR (3)	GLY I (4)	GSH (5)
Control	15.74 ± 1.40 (100)	8.58 ± 1.30 (100)	13.06 ± 1.66 (100)	77.29 ± 1.78 (100)	2.46 ± 0.28 (100)	2.60 ± 0.42 (100)
2% test diet of Fennel	16.85 ± 1.27 (107.10)	9.24 ± 1.21 (107.69)	13.54 ± 1.15 (103.61)	77.60 ± 1.97 (100.39)	2.69 ± 0.24 (109.63)	3.84 ± 0.38** (147.33)
4% test diet of Fennel	23.64 ± 2.57** (150.18)	9.46 ± 1.0 (110.25)	13.68 ± 1.82 (104.68)	83.0 ± 3.96 (107.38)	2.99 ± 0.49** (121.65)	5.22 ± 0.78** (200.15)
6% test diet of Fennel	25.17 ± 1.75** (159.91)	10.60 ± 1.11** (123.54)	16.06 ± 1.02** (122.94)	85.38 ± 2.53** (110.46)	3.11 ± 0.19** (126.57)	5.27 ± 1.02** (202.06)

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 livers of control mice).
(1) μmol H_2O_2 consumed/min/mg protein, (2) specific activity expressed as μmole/mg protein, (3) nmole of NADPH consumed/min/mg protein, (4) μmole of s-lactoylglutathione formed /min, (5) nmole GSH/gm tissue.
Abbreviations: CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; GR, glutathione reductase; GLY I, glyoxalase I; GSH, reduced glutathione content.
** Significant changes against the control group of animals ($p < 0.01$).

diets of Fennel, respectively. The specific activity of superoxide dismutase was significantly enhanced by 1.23 fold ($p < 0.01$) in case of the group treated with 6% test diet of Fennel relative to the control group. A significant increase in the specific activity of glutathione peroxidase by 1.22 fold ($p < 0.01$) was observed with 6% test diet of Fennel as compared to the control group. The specific activity of glutathione reductase exhibited a significant enhancement by 1.10 fold ($p < 0.01$) with 6% test diet of Fennel as compared to the control group. A significant increase in the specific activity of glyoxalase I by 1.21 fold ($p < 0.01$) and 1.26 fold ($p < 0.01$) was observed in case of the groups treated with 4% and 6% test diets of Fennel, respectively, relative to the control group of animals. There was a significant enhancement in the reduced glutathione content as compared to the control group of animals, by 1.47 fold ($p < 0.01$), 2.0 fold ($p < 0.01$) and 2.02 fold ($p < 0.01$), in case of groups treated with 2%, 4% and 6% test diets of Fennel, respectively. These results are depicted in Table 4.

3.5. Toxicity related parameters

3.5.1. Lactate dehydrogenase

The specific activity of lactate dehydrogenase exhibited a significant reduction by 0.77 fold ($p < 0.01$), and 0.69 fold ($p < 0.01$) in case of groups treated with 4% and 6% test diets of Fennel, respectively, as compared to the control group. These results are shown in Table 5.

3.5.2. Peroxidative damage

The peroxidative damage exhibited a significant decrease by 0.83 fold ($p < 0.05$) with 6% test diet of Fennel as compared to the control group of animals. These results are depicted in Table 5.

Table 5
Modulatory effect of Fennel on toxicity related parameters.

Groups	LDH (1)	Lipid peroxidation (2)
Control	1.81 ± 0.41 (100)	1.57 ± 0.03 (100)
2% test diet of Fennel	1.60 ± 0.005 (88.47)	1.43 ± 0.01 (90.90)
4% test diet of Fennel	1.40 ± 0.003** (77.66)	1.39 ± 0.01 (88.42)
6% test diet of Fennel	1.26 ± 0.003** (69.66)	1.30 ± 0.34* (83.08)

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 liver of mice receiving test substance to activity in liver of control mice).
(1) μmole/mg protein and (2) nmol malondialdehyde formed/mg protein.
Abbreviation: LDH, lactate dehydrogenase.
* Significant changes against the control group of animals ($p < 0.05$).
** Significant changes against the control group of animals ($p < 0.01$).

3.6. Histological studies of skin 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. 1A). Sections from the skin of animals treated with DMBA and croton oil exhibited development of benign squamous papillomas arising from the epidermis (Fig. 1B). The histological sections of the papillomas from the experimental groups treated with DMBA and croton oil along with Fennel at 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 keratinisation as compared to the DMBA and croton oil treated control group. Keratinisation is a sign of differentiation. Overall, the histological features are suggestive of a protective effect of Fennel. The

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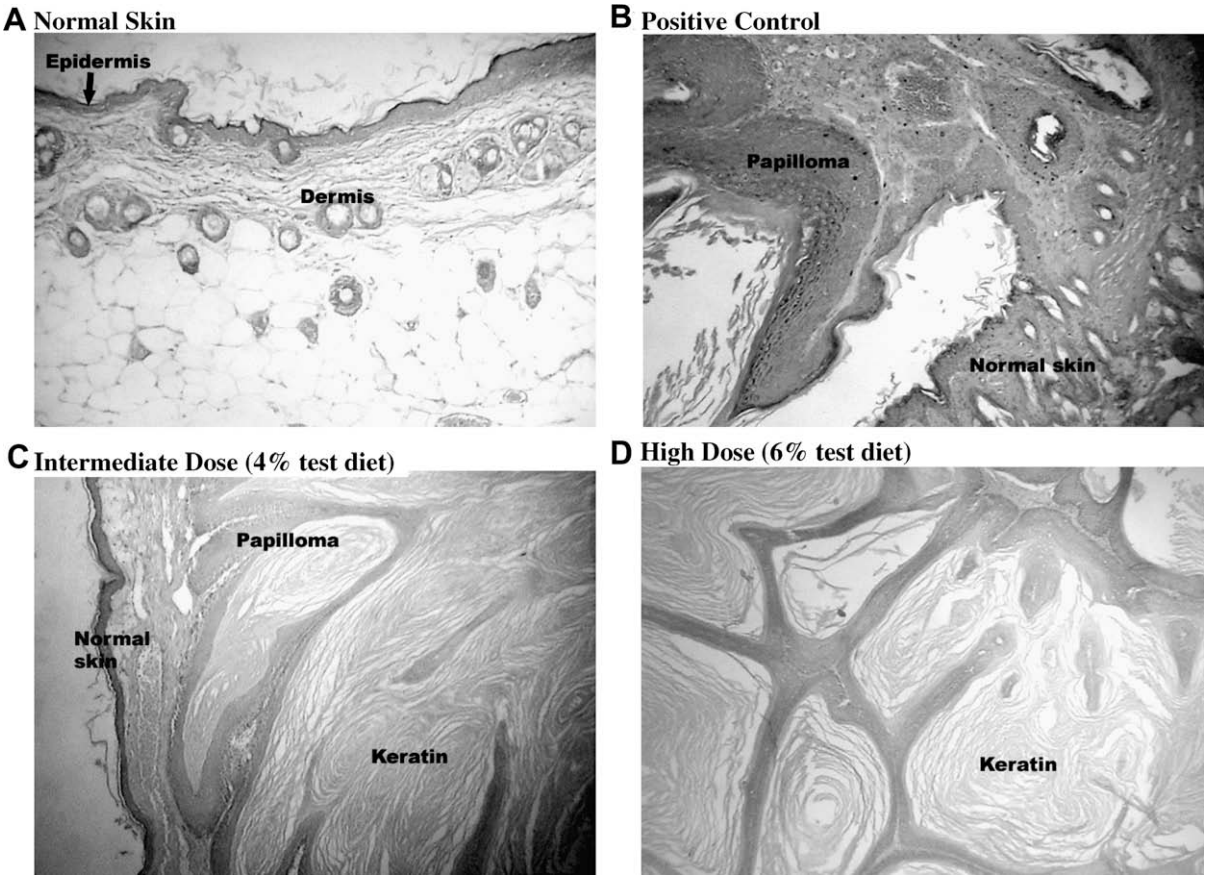

Fig. 1. Histological study of skin papillomagenesis. A: Photomicrograph of skin from normal control group showing normal histological appearance of epidermal and dermal layers (HE × 40). B: Photomicrograph of skin from group treated with DMBA + croton oil (positive control) (HE × 100). C: Photomicrograph of skin from group treated with Fennel (4%) + DMBA + croton oil showing prominent keratin formation (HE × 40). D: Photomicrograph of skin from group treated with Fennel (6%) + DMBA + croton oil showing prominent keratin formation (HE × 40).

results of histological studies with 4% and 6% test diets are shown in Fig. 1C and D, respectively.

The sections from the forestomach of the normal group of animals exhibited normal histological features (Fig. 2A). 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 grew in an exophytic manner and demonstrated simple branching (Fig. 2B). Sections from the experimental groups receiving B(a)P along with the test diet of Fennel with respect to all the three doses (2%, 4% and 6%) exhibited a decrease in papilloma size, branching and cell layer thickness which was maximum in the high dose (6% test diet) treated group. Overall the histological data indicated a chemoprotective effect of the test diet of Fennel against the papillomagenetic effect of B(a)P on the forestomach epithelium. For clarity, findings with 2% and 6% test diets, representing protective effect of Fennel are shown in Fig. 2C and D, respectively.

4. Discussion

Fennel finds extensive use in culinary purposes thus forming a part of the regular dietary regime. It possesses a plethora of medicinal properties which have been well documented in the Indian medicinal Ayurvedic text. The anti-inflammatory, analgesic, antibacterial and antioxidant activities of the fruit of Fennel have also been reported (Choi and Hwang, 2004; Ruberto et al., 2000; Oktay et al., 2003). With this backdrop, the present study was an attempt to evaluate the chemopreventive efficacy of Fennel seeds by screening for its modulatory effect against DMBA-induced skin papillomagenesis and B(a)P-induced forestomach papillomagenesis, at the peri-initiational level (exposure of the murine model system to the Fennel seeds around the event of initiation). Fennel seeds were also assessed for their capacity to modulate the phases I and II detoxification enzymes, antioxidant enzymes, reduced glutathione content and toxicity related parameters such as peroxidative damage and lactate dehydrogenase activity.

The present investigation has clearly shown the effective reduction in the tumor incidence and tumor multiplicity in case of DMBA-induced skin papillomagenesis and B(a)P-induced forestomach papillomagenesis, especially at 4% and 6% test diets (Tables 1 and 2, respectively) as compared to the positive control group. Similar chemopreventive effect of Fennel seeds was also observed histologically. In case of skin papillomagenesis, the histological features exhibited a protective effect of Fennel seeds in terms of smaller and more differentiated papillomas. The group treated with the Fennel seeds, revealed keratinisation which is a sign of differentiation (Fig. 1C and D). In the forestomach, the group treated with Fennel seeds, showed a decrease in papilloma size. These effects were more clearly visible in the 6% (high dose) test diet as compared to the positive control group treated only with B(a)P (Fig. 2C and D). These findings were indicative of chemopreventive potential of Fennel against skin and forestomach papillomagenesis.

The cytochrome P450 enzyme system catalyses the oxidative metabolism of a wide variety of xenobiotics including carcinogens

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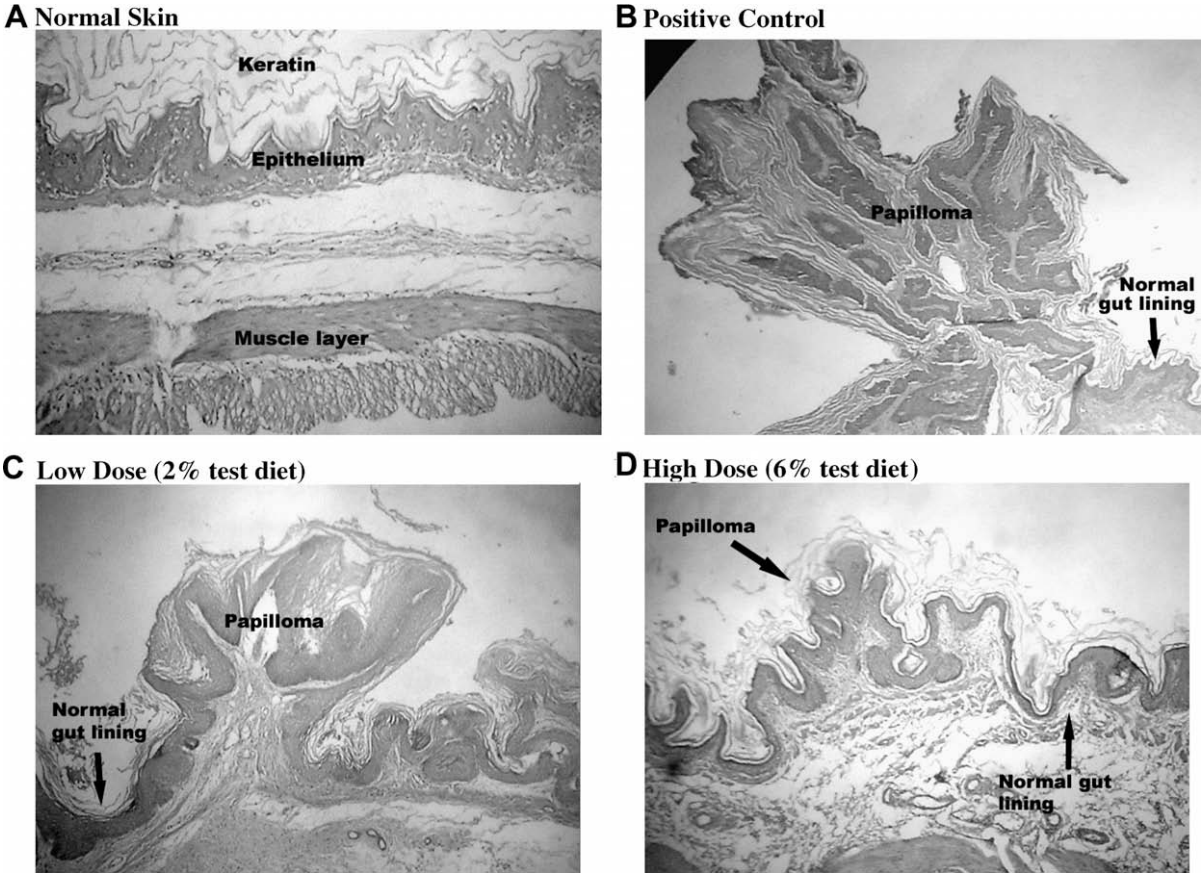

Fig. 2. Histological study of forestomach papillomagenesis. A: Photomicrograph of forestomach wall from normal control group (HE × 40). B: Photomicrograph of forestomach wall from group receiving B(a)P only (Positive control) (HE × 40). C: Photomicrograph of forestomach wall from group receiving Fennel (2%) + B(a)P (HE × 40). D: Photomicrograph of forestomach wall from group receiving Fennel (6%) + B(a)P. The papillomas exhibited a reduction in size (HE × 40).

(Shimada et al., 1994). The present experimental investigation exhibited a significant increase in the components of cytochrome P450 system (cytochrome P450, cytochrome b5, cytochrome P450 reductase and cytochrome b5 reductase) especially with 4% and 6% test diets of Fennel. These observations were suggestive of the fact that Fennel seeds in diet might have enhanced the metabolism of DMBA and B(a)P. It may be mentioned in this context that indole-3-carbinol is known to exhibit chemopreventive properties in various animal models through its ability to induce cytochrome P450 system (Vang et al., 1999). In the present study, Fennel has also shown an ability to modulate the phase I enzyme system when taken in diet (Table 3); which might contribute to the detoxification of DMBA and B(a)P.

The phase II enzymes GST and DTD, facilitate the detoxification of the reactive metabolites of phase I enzymatic reactions. The major function of GST is to catalyse the conjugation of electrophilic xenobiotics or carcinogens to the endogenous nucleophile GSH and in turn protect the cellular components against the toxic compounds (Awasthi et al., 1994). Since, the diet containing seeds of Fennel was found to enhance the specific activity of GST, possibly its chemopreventive action against DMBA and B(a)P-induced skin papillomagenesis and forestomach papillomagenesis, respectively, was closely linked to this enhanced activity of GST. It could be mentioned that epoxides and other metabolites constituting the reactive intermediates of B(a)P and DMBA metabolism reported to be responsible for carcinogenesis are detoxified by GST (Cooper et al., 1980; Christou et al.,1989). In view of this, it was significant that Fennel seeds exhibited an elevation in the GST activity with respect to 4% and 6% test diets as compared to the control group (Table 3).

DT-diaphorase (DTD) is another important member of phase II system and plays a key role in protecting the cells against the toxicities of a variety of xenobiotics (Benson et al., 1980). It catalyses the two electron reduction of quinones, quinone imines, azo dyes and other nitrogen oxides (Ernster, 1987; Riley and Workman, 1992). Fennel seeds led to a significant increase in DTD activity, especially at 4% and 6% test diets (Table 3) and was also likely to provide protection against carcinogenesis induced by DMBA and B(a)P (Fahey et al., 1997). As an activation of phases I and II systems are known to facilitate the xenobiotic metabolism leading to detoxification, the reduction in the skin and forestomach tumor incidence as well as tumor multiplicity in the present study may be attributed to the elevation of activities of enzymes of phases I and II system. This possibility has been supported by various studies using different plant modulators (Dasgupta et al., 2003, 2004). As Fennel has elevated both the phases I and II enzymes, it could possibly be considered as bifunctional inducer (Prochaska and Talalay, 1988). Phytochemicals from different plants such as isothiocyanates in Cruciferous plants (Zhu and Loft, 2003), epigallocatechin gallate in green tea (Chou et al., 2000) and indoles in *Brassica* (Fowke et al., 2006) are known to induce phases I and II enzymes Fennel seeds are also known to contain phytochemicals like anethole, β-myrcene, D-limonene with antioxidant potential. These phytochemicals of Fennel seeds might have also induced the phases I and II enzymes in the present system and contributed towards the detoxification of DMBA and B(a)P leading to the inhibition of carcinogenesis.

The reactive oxygen species which cause oxidative stress are implicated in the etiology and progression of many diseases

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including cancer (Dreher and Junod, 1996). The defense system comprising of various enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GP_X), glutathione reductase (GR) and non-enzymatic antioxidants present in the body scavenge the reactive oxygen species and confers protection against oxidative stress and in turn serve as inhibitors of neoplastic process (Bagchi et al., 2000). In the present study, significant elevation in the activities of antioxidant enzymes due to presence of Fennel seeds in test diet were expected to detoxify reactive oxygen species generated during the metabolism of DMBA and B(a)P and inhibit their carcinogenic effect.

Methyl glyoxalase system is considered vital for biological function. Apart from its involvement in the regulation of cell division and differentiation, the glyoxalase system is suggested to have antioxidant function as the electrophiles and cytotoxic-2-oxaldehydes are converted to less reactive chemical species (Thornalley, 1998; Agrawal et al., 2001). Glyoxalase I activity was elevated at all the three doses of the test diet of Fennel and was likely to contribute in lowering the oxidative stress related to initiation of carcinogenesis (Table 4). Reduced glutathione (GSH) is a non-protein sulphydryl compound, 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 (Ketterer, 1988; Meister, 1994). It has a redox potential of about 230 mV which perhaps makes it behave as an antioxidant and protect against the electrophiles, free radicals and in turn oxidative stress (Agrawal et al., 2001). Some of the GSH dependent antioxidant reactions are catalysed by the enzymes GST and methyl glyoxalases. A significant enhancement of GSH content with all the three doses of test diets of Fennel as compared to the control group (Table 4) was likely to play an important role in chemoprevention.

The enhanced antioxidant status in the animals, upon administration of Fennel was an important observation. In the event of increased antioxidant status, the lowered level of oxidative damage was expected in the group of animals treated with Fennel seeds in diet. To confirm this possibility, the peroxidative damage was estimated in terms of TBARS. As expected, a significant decrease in the level of peroxidative damage was observed particularly with 6% test diet of Fennel (Table 5). Significant reduction in the activity of lactate dehydrogenase, with 4% and 6% test diets of Fennel seeds given to animals (Table 5) also supported this possibility. The decreased level of peroxidative damage is correlated well in accordance with the induction of antioxidant enzymes above the basal level.

The plants which have chemopreventive potential are known to contain various antioxidants. These antioxidants interact with the reactive oxygen species and neutralize them. As mentioned earlier, reactive oxygen species are intimately linked with the process of carcinogenesis (Dreher and Junod, 1996).The antioxidant species such as anethole, β-myrcene and D-limonene present in the Fennel seeds, as mentioned earlier, might also have interacted with reactive oxygen species and neutralized them leading to chemopreventive effect. Therefore, apart from the induction of phases I and II enzymes and activation of antioxidant enzymes, Fennel might have also contributed to prevention of carcinogenesis induced by DMBA and B(a)P through scavenging of reactive oxygen species by its antioxidants.

Although Fennel is known to contain carcinogenic alkylbenzene components, still it showed a significant inhibitory effect against DMBA and B(a)P-induced papillomas. It suggested that the ability of Fennel seeds to induce phases I and II enzymes as well as enhanced antioxidant potential of animals might have suppressed effectively the carcinogenic effect of these alkylbenzene components through the same process of detoxification and scavenging of reactive oxygen species. Thus the alkylbenzene components might have been metabolized along with DMBA and B(a)P leading to the inhibition of their carcinogenic effect.

In conclusion, the result of the present study showed that Fennel seeds in diet were able to inhibit skin and forestomach papillomagenesis induced by DMBA and B(a)P, respectively, in Swiss albino mice. Fennel seeds seemed to possess the property to elevate both phases I and II enzymes, leading to removal of the carcinogenic metabolites and in turn prevention of papillomagenesis. Enhanced antioxidant status by Fennel was also likely to contribute in inhibiting the skin and forestomach papillomagenesis. Our findings might have some significance and suggest that inclusion of Fennel seeds in the diet is likely to reduce the risk of cancer in human population.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

Aebi, H., 1984. Catalase *in vitro*. In: Colowick, S.P., Kaplan, N.O. (Eds.), Methods in Enzymology, vol. 105. Academic Press, New York, pp. 21–126.

Agrawal, A., Choudhary, D., Upreti, M., Rath, P.C., Kale, R.K., 2001. Radiation induced oxidative stress. I: studies in Ehrlich solid tumor in mice. Mol. Cell. Biochem. 223, 71–80.

Al Harbi, M.M., Qureshi, S., Raza, M., Ahmed, M.M., Giangreco, A.B., Shah, A.H., 1995. Influence of anethole treatment on the tumor induced by Ehrlich ascites carcinoma cells in paw of Swiss albino mice. Eur J Cancer Prev. 4, 307–318.

Anand, P., Kunnumakara, A.B., Sundaram, C., Harikumar, K.V., Tharakan, S.T., Lai, O.S., Sung, B., Aggarwal, B.B., 2008. Cancer is a preventable disease that requires major lifestyle changes. Pharmaceut. Res. 25, 2097–2116.

Awasthi, Y.C., Sharma, R., Singhal, S.S., 1994. Human glutathione-S-transferases. Int. J. Biochem. 26, 295–308.

Azuine, M.A., Bhide, S.V., 1992. Chemopreventive effect of turmeric against stomach and skin tumors induced by chemical carcinogens in Swiss mice. Nutr. Cancer 17, 77–83.

Bagchi, D., Bagchi, M., Stohs, J.S., Das, K.D., Ray, D.S., Kuszynski, A.C., Joshi, S.S., Pruess, G.H., 2000. Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicology 148, 187–197.

Benson, A.M., Hunkeler, M.J., Talalay, P., 1980. Increase of NAD(P)H: quinone reductase by dietary antioxidants: possible role in the protection against carcinogenesis and toxicity. Proc. Natl. Acad. Sci. USA 77, 5216–5220.

Bergmeyer, H.U., Bernt, E., 1974. Lactic dehydrogenase. Meth. Enzym. Anal. 2, 574–579.

Carlberg, I., Mannervik, B., 1985. Glutathione reductase. Methods in Enzymology, 113. Academic Press, New York. pp. 484–490.

Choi, E.M., Hwang, J.K., 2004. Antiinflammatory, analgesic and antioxidant activities of the fruit of *Foeniculum vulgare*. Fitoterapia 75 (6), 557–565.

Chou, F.P., Chu, Y.C., Hsu, J.D., Chiang, H.C., Wang, C.J., 2000. Specific induction of glutathione-S-transferase GST M2 subunit expression by epigallocatechin gallate in rat liver. Biochem. Pharmacol. 60, 643–650.

Christou, M., Jovanovich, M.C., Jefcoate, C.R., 1989. Epoxide hydratase: sex specific expression and rate limiting role in DMBA metabolism. Carcinogenesis 10, 1883–1890.

Cooper, C.S., Hewer, A., Ribeiro, O., Grover, P.L., Sims, P., 1980. The enzyme catalysed conversion of anti-benzo(a)pyrene 7,8-diol 9,10-oxide into a glutathione conjugate. Carcinogenesis 1, 1075–1080.

Dasgupta, T., Rao, A.R., Yadava, P.K., 2003. Modulatory effect of Henna leaf (*Lawsonia inermis*) on drug metabolizing phases I and II enzymes, antioxidant enzymes, lipid peroxidation and chemically induced skin and forestomach papillomagenesis in mice. Mol. Cell. Biochem. 245, 11–22.

Dasgupta, T., Rao, A.R., Yadava, P.K., 2004. Chemomodulatory efficacy of Basil leaf (*Ocimum basilicum*) on drug metabolizing and antioxidant enzymes, and on carcinogen-induced skin and forestomach papillomagenesis. Phytomedicine 11, 139–151.

Dreher, D., Junod, A.F., 1996. Role of oxygen free radicals in cancer development. Eur. J. Cancer 32A, 30–38.

Ernster, L., 1987. DT-diaphorase: a historical review. Chem. Scripta 27A, 1–13.

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Ernster, L., Danielson, L., Ljunggren, M., 1962. DT-diaphorase, I. Purification from soluble fraction of rat liver cytoplasm. Biochim. Biophys. Acta 58, 171–188.
Fahey, J.W., Zhang, Y., Talalay, P., 1997. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc. Natl. Acad. Sci. USA 94, 10367–10372.
Fowke, J.H., Jason, D.M., Motley, S., Bostik, R.M., Ness, R.M., 2006. *Brassica* vegetable consumption reduces urinary F 2-isoprostane levels independent of micronutrient intake. Carcinogenesis 27, 2096–2102.
Habig, W.H., Pabst, M.J., Jacoby, W.B., 1974. Glutathione-S-transferase: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7129–7130.
Joshi, H., 2006. Cholinergic basis of memory-strengthening effect of *Foeniculum vulgare* Linn. J. Food Med. 9 (3), 413–417.
Ketterer, B., 1988. Protective role of glutathione and glutathione-S-transferases in mutagenesis and carcinogenesis. Mutat. Res. 202, 343–361.
Leung, A.Y., Foster, S., 1996. Encyclopedia of Common Natural Ingredients Used in Food Drugs and Cosmetics, second ed. John Wiley and Sons, Inc., New York.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 193, 265–275.
Marklund, S., Marklund, G., 1974. Involvement of superoxide anion radical in autooxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47, 469–474.
Meister, A., 1994. Glutathione. In: Arias, I.M., Boyer, J.L., Jakoby, W.B., Fausto, D., Schacter, D., Shafritz, D.A. (Eds.), The Liver Biology and Pathobiology. Raven Press, New York, pp. 401–417.
Mihara, K., Sato, R., 1972. Partial purification of NADH cytochrome b5 reducatse from rabbit liver microsomes with detergents and its properties. J. Biochem. 71, 725–735.
Moron, M.A., Dipierre, I.W., Mannervick, B., 1979. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochim. Biophys. Acta 582, 67–78.
Oktay, M., Gulcin, I., Kufrevioglu, I., 2003. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Lebensm. Wiss. Technol. 36, 263–271.
Omura, T., Sato, R., 1964. The carbon monoxide binding pigment of liver microsomes. J. Biol. Chem. 239, 2370–2378.
Omura, T., Takesue, S., 1970. A new method for simultaneous purification of cytochrome b5 and NADPH–cytochrome c reductase from rat liver microsomes. J. Biochem. 67, 249–257.
Padmavathi, B., Rath, P.C., Rao, A.R., Singh, R.P., 2005. Roots of *Withania somnifera* inhibit forestomach and skin carcinogenesis in mice. eCAM 2, 99–105.
Paglia, D.E., Valentine, W.M., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70, 158–169.
Prochaska, H.J., Talalay, P., 1988. Regulatory mechanisms of monofunctional and bifunctional anticarcinogenic enzyme inducers in murine liver. Cancer Res. 48, 4776–4782.
Riley, R.J., Workman, P., 1992. DT-diaphorase and cancer chemotherapy. Biochem. Pharmacol. 43, 1657–1669.
Ruberto, G., Baratta, M.T., Deans, S.G., Dorman, H.J., 2000. Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. Planta Med. 66 (8), 689–693.
Shimada, T., Yamazaki, H., Mimura, M., Inui, Y., Guengerich, F.P., 1994. Interindividual variation in human liver cytochrome P450 enzymes involved in oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Causasians. J. Pharmacol. Exp. Ther. 270, 414–423.
Shukla, H.S., Dubey, P., Chatuvedi, R.V., 1988. Antiviral properties of essential oils of *Foeniculum vulgare* and *Pimpinella anisum* L. Agronomia 9, 277–279.
The Wealth of India, 1956. A Dictionary of Indian Raw Materials and Industrial Products, Raw Materials, vol. IV. Council of Scientific and Industrial Research, New Delhi, India, pp. 52–55.
Thornalley, P.J., 1993. Modification of the glyoxalase system in disease processes and prospects for therapeutic strategies. Biochem. Soc. Trans. 21, 531–534.
Thornalley, P.J., 1998. Glutathione dependent detoxification of (oxoaldehydes by the glyoxalase system: involvement in disease mechanisms and antiproliferative activity of glyoxalase I inhibitors. Chem. Biol. Interact. 137, 111–112.
Vang, O., Frandsen, H., Hansen, K.T., Nielsen, J.B., Anderson, O., 1999. Modulation of drug - metabolising enzyme expression by condensation products of indole-3yl carbinol, an inducer in cruciferous vegetables. Pharmacol. Toxicol. 84, 59–65.
Varshney, R., Kale, R.K., 1990. Effects of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. Int. J. Radiat. Biol. 58, 733–743.
Wattenberg, L.W., 1981. Inhibition of carcinogen-induced neoplasia by sodium cynate, tert-butylisocyanate and benzyl isothiocyanate administered subsequent to carcinogen exposure. Cancer Res. 41, 2991–2994.
Yasukawa, K., Yu, S., Yamanouchi, S., Takido, M., Akihisa, T., Tamura, T., 1995. Some lupene–type triterpenes inhibit tumor promotion by 12-o-tetradecanoylphorbol-13-acetate: in two stage carcinogenesis in mouse skin. Phytomedicine 4, 309–313.
Zhu, C.Y., Loft, S., 2003. Effect of chemopreventive compounds from *Brassica* vegetables on NAD(P)H: quinone reductase and induction of DNA strand breaks in murine hep a1C1C 7 cells. Food Chem. Toxicol. 41, 455–462.