CHAPTER 8

- VITAMINS A, C AND E AS PROTECTIVE AGENTS AGAINST CANCER
INTRODUCTION

After years of "destructive" therapy with non-specific cytotoxic agents, each of which can be mutagenic in itself, emphasis seems to be shifting towards primary prevention for cancer control. Physiological regulation of cellular proliferation is also being attempted. Both approaches now tend to concentrate on nutritional manipulation and regulation. The importance of micronutrients, particularly vitamins as modulators of biological functions and the possible use of vitamins and their derivatives as possible chemopreventive or anticancer agents is being explored.

Consumption of alcohol, opium, nitrosamines and deficiencies of vitamins A, C, E and riboflavin have been associated with an increased risk of oesophageal cancer (1). The epidemiology of gastric cancer has been investigated in several countries. In gastric cancer, two distinct pathological entities are known (2) of which the intestinal type is often related to environmental factors. Deficiencies of vitamins A, C, and E were found to have a strong correlation with gastric cancer (3). Vitamin A seems to be of particular interest as many in vivo and in vitro studies on human and animal systems have suggested a role for this vitamin in cellular differentiation (4). Consideration of a vitamin A - cancer association is not new. As early as 1926, Burrows (5) hypothesized that cancer resulted from factors removing vitamin A and causing a local
vitamin A imbalance. The relationship between vitamin A and cancer was first demonstrated experimentally by Fujimaki (6), also in 1926, when he showed that rats fed on vitamin A deficient diet were found to develop carcinomas of the stomach. Further studies followed which produced much controversy as to the role of vitamin A in carcinogenesis and tumour progression. In recent times renewed interest has been evinced on the chemopreventive capabilities of vitamin A. Many reports are available on the effects of vitamin A and retinoids on tumour promotion, but information on the effects and role of vitamin A in cancer initiation are very scanty. Vitamin A has been reported to protect animals from many experimental tumours (7), but recently not much work has been done on its effects on stomach cancers.

Vitamin E is a hydroquinone derivative and is present in plant materials especially in wheat germ and corn. It is a major free radical trapping agent in biological membranes.(7).

It can protect cells against the toxic actions of free radical forming agents (7). The role of vitamin E in cancer is controversial. Vitamin E is inhibitory to the transplantation of sarcoma cells in mice (8) and to mammary gland tumour formation by dimethylbenzanthracene in rats (9). On the other hand a vitamin E rich diet promotes dimethylhydrazine induced colon cancer in mice (7). In addition, it fails to inhibit urinary bladder carcinogenesis
in rats by N-butyl-N-(4-hydroxy butyl) nitrosamine (10). The role of vitamin E in gastrointestinal tract cancers has therefore been investigated.

According to Graham (11), low intakes of vitamin C are associated with cancer at five sites viz., stomach, oesophagus, mouth, larynx and cervix. Of this, gastric cancer has been thoroughly studied. Nitrosamides formed in the stomach from amides and nitrite are potent carcinogens and they induce glandular stomach cancer in rats (2). Mirvish (2) has postulated that intragastric formation of nitrosamides is an important etiological factor in gastric cancer. Vitamin C is an efficient inhibitor of the nitrosation reaction and its role in prevention of gastric cancer has been attributed to this property (12). The efficacy of vitamin C as an antimutagen and its role in induction of detoxifying enzymes have been studied to investigate whether it can act as an inhibitor by other mechanisms.

Moreover the leafy vegetables and spices possessing chemopreventive capabilities also contain significant levels of vitamins A, C and E and therefore the chemopreventive potential of these three vitamins have been assessed and their roles in carcinogenesis elucidated and compared.

MATERIALS AND METHODS

Chemicals:

d, L-γ-Tocopherol, retinol, 2,4-dinitro phenyl hydrazine,
thiourea, \( \alpha, \alpha' \)-dipyridyl, Eosin Y, Vitamin K, riboflavin, pyrodoxine, pantothenic acid, niacin, vitamin B, biotin, folic acid, vitamin D and p-amino benzoic acid were purchased from Sigma Chemical Company, U.S.A. Ascorbic acid was obtained from Aldrich Chemical Company, U.S.A. All other chemicals, locally obtained were of ANALAR grade.

A. Determination of Vitamin A, C and E levels in serum of cancer patients:

Samples of blood (5 ml) were collected from cancer patients and serum separated. Controls consisted of normal subjects with similar background and age. Vitamins A, C and E levels were then estimated.

(i) Estimation of Vitamin C: (13)

Reagents

1. 2,4 Dinitrophenyl hydrazine (DNPH). - Dissolved 2 g of DNPH in 100 ml of 9N \( H_2 SO_4 \) and filtered.

2. TCA - 6% solution

3. Acid washed norit: 200 g of norit was suspended in 1 l of 10% HCl, heated to boiling and filtered under suction. The cake was removed and stirred with 1 l water and filtered. This procedure was repeated until the washings gave a negative test for ferric ions. The norit was dried overnight at 110 - 120 C.

4. \( H_2 SO_4 \) - 85%

5. Thiourea - 10 g dissolved in 100 ml of 50% v/v alcohol
Method:

To 2 ml of serum was added 6 ml of TCA, the mixture stirred, allowed to stand for 5 min and centrifuged at 100 g for 5 min. The supernatant was shaken vigourously with 300 mg norit and filtered. To incubate containing 1.6 ml filtrate, one drop of thiourea and 0.4 ml of 2,4-dinitrophenyl hydrazine reagent were added. The tube was incubated at 37 C for 3 h. This was then placed in an icewater bath along with a blank tube containing 1.6 ml norit filtrate and one drop of thiourea. Colour was developed by adding dropwise 2 ml of 85% H$_2$SO$_4$ while stirring. Finally 0.4 ml of DNPH reagent was added to the blank. The tubes were taken out of the water bath, allowed to stand for 30 min, and absorbance read at 540 nm. Aliquots from standard solution of ascorbic acid were treated in the same way and the absorbance determined.

Determination of Vitamin A in serum (14)

The reaction of vitamin with trichloroacetic acid (TCA) was used to determine vitamin A content of serum.

Reagents

1. TCA - 50 g in 25 ml of chloroform
2. Ethanol
3. Light petroleum

To 5 ml of serum, 5 ml of ethanol and 10 ml of light petroleum were added. This was shaken in a mechanical shaker for 2 min and allowed to stand. An aliquot of the
petroleum layer was taken and solvent evaporated under vacuum. The residue was redissolved in chloroform and to this 2 ml of TCA was added rapidly, mixed and the absorbance immediately read at 620 nm in a spectrophotometer. Aliquots of a standard solution of retinol were prepared and treated in similar way and their absorbance determined.

**Determination of serum tocopherol:**

The method used was the Emmerie-Engel reaction which is based on the reduction by tocopherols of ferric ions to ferrous ions which form a red complex with $\alpha, \alpha'$ dipyridyl. Tocopherols and carotenoids are first extracted into xylene and absorbance read at 460 nm to measure the carotenoids. Then ferric chloride is added and absorbance read at 520 nm (15).

**Reagents**

1. $\alpha, \alpha'$dipyridyl - 1.2 g/l in n-propanol
2. FeCl$_3$ solution - 1.2 g FeCl$_3$ · 6H$_2$O/l in ethanol
3. Absolute ethanol
4. Xylene

**Method**

Serum (1.5 ml) and water (1.5 ml-blank) were taken in 2 stoppered centrifuge tubes. Xylene (1.5 ml) was added to the tubes and stoppered. The contents were mixed well and centrifuged. The xylene layer (1 ml) was transferred to other stoppered tubes and 1 ml of $\alpha, \alpha'$dipyridyl reagent added to each tube and mixed. These solutions (1.5 ml) were
taken in quartz cuvette and the absorbance read at 460 nm. Ferric chloride reagent (0.33 ml) was then added to the tubes and after 1.5 min the absorbance read at 520 nm. Aliquots of a standard solution of \(
\alpha\)-tocopherol were also treated in the same way, and absorbance (A) determined.

\[
\text{Serum tocopherol} = \frac{\text{A of test at 520 nm - A of test at 460 nm}}{0.29} \times \text{factor (Absorbance of standard)}
\]

B. Role of vitamins A and E deficiencies in tumourigenesis:

(i) Induction of deficiencies of Vitamin A and E respectively:

Male Swiss mice, divided into groups of 25 mice each, were used for the experiment. Vitamin A deficient diet was prepared as shown in Table 8.1 (16) and vitamin E deficient diet as shown in Table 8.2 (17).

Initially, the mothers of the suckling newborns were maintained on this diet. The young animals were weaned from the mothers after 28 days and they continued to receive the vitamin A and E deficient diet respectively for 2 months.

After 2 months, 5 animals from each group were sacrificed. Body weights, liver weights, liver vitamin A and E levels, GST activity and GSH levels were determined.

(ii) Determination of vitamin A levels in liver:

Vitamin A from the liver was extracted with cyclohexane, evaporated and the residue dissolved in
Table 8.1: Composition of vitamin A deficient diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A free caesin</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Starch (maize flour)</td>
<td>70.3 g</td>
</tr>
<tr>
<td>Refined oil</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td>10.0 U</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.4 mg</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>0.1 mg</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>1.0 mg</td>
</tr>
<tr>
<td>Niacin</td>
<td>3.0 mg</td>
</tr>
<tr>
<td>Vitamin B_{12}</td>
<td>2.0 µg</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.02 mg</td>
</tr>
<tr>
<td>Folic acid</td>
<td>0.2 mg</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>100.0 U</td>
</tr>
<tr>
<td>PABA</td>
<td>10.0 mg</td>
</tr>
<tr>
<td>Salt mixture</td>
<td></td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>600.0 mg</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>2.7 mg</td>
</tr>
<tr>
<td>Potassium monophosphate</td>
<td>500.0 mg</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>5.0 mg</td>
</tr>
<tr>
<td>Potassium iodide</td>
<td>2.0 µg</td>
</tr>
</tbody>
</table>
Table 8.2: Composition of vitamin E deficient diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A free casein</td>
<td>20.0 g</td>
</tr>
<tr>
<td>Starch (maize flour)</td>
<td>70.3 g</td>
</tr>
<tr>
<td>Refined oil</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>500.0 IU</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.4 mg</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>0.1 mg</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>1.0 mg</td>
</tr>
<tr>
<td>Niacin</td>
<td>3.0 mg</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>2.0 ug</td>
</tr>
<tr>
<td>Biotin</td>
<td>0.02 mg</td>
</tr>
<tr>
<td>Folic acid</td>
<td>0.2 mg</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>100.0 U</td>
</tr>
<tr>
<td>PABA</td>
<td>10.0 mg</td>
</tr>
<tr>
<td>Salt mixture</td>
<td></td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>600.0 mg</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>2.7 mg</td>
</tr>
<tr>
<td>Potassium monophosphate</td>
<td>500.0 mg</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>5.0 mg</td>
</tr>
<tr>
<td>Potassium iodide</td>
<td>2.0 ug</td>
</tr>
</tbody>
</table>
chloroform. The vitamin A content was then estimated as given earlier (14).

(III). Determination of Vitamin E in liver:

Liver tissue was weighed and cut into small pieces. It was then homogenised and extracted with hot ethanol. To this solution an equal volume of hexane and then an equal volume of water were added. The mixture was shaken well and the layers allowed to separate. The hexane layer was taken and evaporated to dryness under nitrogen. The residue was dissolved in xylene and vitamin E estimated as mentioned earlier (15).

IV. Chromosome aberrations were determined in the bone marrow of vitamin A and E deficient mice, which had been injected with BP (60 mg/kg body wt, i.p.) according to the procedure given under materials and methods, chapter - 2.

V. Sperm head abnormalities in Vitamin A and E deficient mice:

The sperm morphology assay is a relatively simple test that examines the effect in vivo, of compounds on germ cells of male mice. The end point in the sperm morphology assay is the percentage increase of morphologically aberrant sperms in the test series as compared to controls (18).

The cauda epididymis from vitamin A and E deficient mice and normal mice were removed. They were placed in 2 ml physiological saline, minced finely with scissors and then left for 30 min for the spermatozoa to diffuse into the saline. Three slides were prepared for each animal by
placing one drop of the suspension on a cleaned microscopic slide, and smearing with a clean coverslip. The slides were air dried and then fixed in absolute methanol for 1 min before staining in Eosin Y (1% aqueous solution) for 15 min. The slides were coded and 2000 sperms from each animal were examined at x 400 magnification.

VI. Mutagenesis assays :

Preparation of S9 from vitamin A and E deficient mice:

Vitamin A and E deficient mice, and normal mice (5 animals per group) were killed by cervical dislocation. The livers were removed aseptically, minced and homogenised in 0.15 M KCl. Homogenates were centrifuged at 9000 x g for 15 min and S9 fractions obtained were used in mutagenesis assays. S9 mix was prepared as given in Chapter 4 - materials and methods.

The mutagenicity of BP mediated by hepatic S9 from vitamin A and vitamin E deficient mice and the effects of retinol, tocopherol and glutathione on BP mutagenicity were evaluated in the Ames Test.

The method of Maron and Ames (19), (Chapter IV, materials and methods) was used. To 2.0 ml of molten top agar were added in the following sequence - 0.1 ml BP (in 10, 100 μg/plate DMSO) 0.1 ml of suspension of TA 1537 and 0.5 ml of S9 mix which contained 20 % S9. This was then poured on to petri plates containing minimal glucose agar medium. The plates were incubated at 37 C for 48 h. Duplicate plates were done for each experiment, and the
experiments were repeated thrice. The number of histidine revertants were counted after 48 h.

VII. Induction of stomach tumours in vitamin A and E deficient mice:

Four groups of 15 male Swiss mice each, comprising of

(i) Vitamin A deficient mice
(ii) Vitamin E deficient mice
(iii) Normal mice for negative controls
(iv) Normal mice receiving BP serving as positive controls.

The groups I, II and IV were fed BP in the diet, at a dose of 0.3 mg/g diet (dissolved in ground nut oil) thrice a week for 8 weeks. After that, till the end of the experiment, the mice were fed normal pellet diet. The animals were weighed weekly till the end of the carcinogen treatment and thereafter weighed once a fortnight till the end of the experiment. The animals were sacrificed 8 months later, and their stomachs processed (Chapter V, materials and methods) and examined for tumours histologically.

C. Assessment of chemopreventive potentials of vitamins A, C and E:

(i) Effects of vitamins A, C and E on GST activity and GSH levels in Swiss mice:

Male Swiss mice 8 weeks of age and weighing 25±3.3g were used. They were divided into 4 groups of 5 animals
each. The 1st group served as the control and was fed only pellet diet. Group II was administered retinol at a dose of 10 mg/g diet and Group III D, α-tocopherol at a dose of 10 mg/g diet. Group IV was administered 50 mg/g diet ascorbic acid. The animals were fed the different vitamins for 14 days.

After 14 days the animals were killed by cervical dislocation. The liver and stomach were dissected out and processed as given in chapter I — materials and methods. GST and GSH levels in the stomach and liver were estimated according to methods given in Chapter 1.

(ii) Effects of vitamins A, C and E on BP induced CA in Swiss mice:

Male Swiss mice, 8 weeks of age and weighing 27 ± 4.1 g were used for the experiment. They were divided into 5 groups of 5 mice each. One group served as the negative control and was fed pellet diet. Group II served as positive control and was fed standard pellet diet for 14 days after which the mice were injected BP (in 0.5 ml groundnut oil, i.p.) at a dose of 60 mg/kg body weight. Groups III, IV and V were fed retinol, d, α-tocopherol (both 10 mg/g diet) and ascorbic acid (50 mg/g diet) respectively, for 14 days, after which they were injected BP. Eighteen hours after the injection of BP, the mice were injected colchicine and sacrificed after 90 min. Chromosome specimens were prepared from bone marrow cells as given
under materials and methods of chapter II. Chromosome aberrations were then determined.

(iii) Antimutagenic effects of Vitamins A, C and E:

Reagents:

1. Vitamins A, C and E solution in DMSO: 50 \( \mu \)g, 100 \( \mu \)g and 200 \( \mu \)g of Vitamins A, E and C were dissolved in 100 \( \mu \)l of DMSO.

2. NMU - 5 \( \mu \)g of NMU in 100 \( \mu \)l of DMSO.

The DMSO solution (containing 50 \( \mu \)g, 100 \( \mu \)g and 200 \( \mu \)g) of the vitamins were mixed with 100 \( \mu \)l of 5 \( \mu \)g of NMU. The mixtures were incubated at 37°C for 30 min. The mutagenicity of these mixtures were determined by combining them with 100 \( \mu \)l of TA 1535 cells and 500 \( \mu \)l of phosphate buffer (pH 7.4) in soft agar tubes and pouring them onto minimal glucose agar plates. The plates were incubated at 37°C for 48 h and revertant colonies counted. Negative controls containing bacteria and solvent alone, were also done to determine spontaneous revertants. Positive control with 100 \( \mu \)l NMU, 100 \( \mu \)l DMSO, combined with 100 \( \mu \)l of bacteria and 500 \( \mu \)l of phosphate buffer pH 7.4 was also done. Duplicates were performed for each plate, and each experiment repeated thrice.

V. Anticarcinogenicity of vitamins A, C and E against BP-induced neoplasia:

Swiss mice, 8 weeks of age and weighing 26±3.4 g were used. They were divided into 5 groups of 15 animals each.
One group served as negative control and was fed only standard pellet diet throughout the experiment. The second group served as positive control and was fed BP alone. The 3rd, 4th and 5th groups of animals were fed vitamin A (10 mg/g diet), vitamin E (10 mg/g diet) and vitamin C (50 mg/g diet) respectively, for 14 days after which BP was administered to these animals. BP was administered at a dose of 0.3 mg/g diet thrice a week for 8 weeks. Throughout this period, the groups III, IV and V were fed the different vitamins. Thereafter, till the end of the experiment the mice of all groups were fed only pellet diet. The animals were weighed weekly during carcinogen treatment and thereafter once a fortnight till the end of the experiment. After 8 months the mice were sacrificed, the stomachs processed and examined for tumours.

VI. Effects of the three vitamins on the nitrosation of methylurea:

The three vitamins at doses of 7.5 mg and 5 mg in nitrosation mixtures were tested for their effects on the nitrosation of methyl urea as measured by Ames test, in strain TA 1535 of *Salmonella typhimurium* (For procedure see materials and methods of Chapter VI). An emulsion of the vitamins A and E in citrate-phosphate buffer pH 3.6 was prepared and used.

Students "t" test was used to evaluate the significance of the results (20).
RESULTS

A. The levels of vitamins A, C and E in cancer patients are shown in Table 8.3. The serum levels of all the three vitamins were significantly decreased in stomach, lung and oesophageal cancer patients as compared to controls. Vitamin C levels were not significantly decreased in cervical cancer patients and other patients with miscellaneous cancers. Vitamin A and E levels were significantly low in cervical cancer patients also.

B. Studies on Vitamin A and E deficient mice:

Table 8.4 shows that the body weights, liver weights, liver GSH levels and vitamin A levels of the vitamin A deficient mice were significantly low when compared to control mice. GST activity in vitamin A deficient mice was not significantly different from those of control mice. The percentage of sperm head abnormalities in vitamin A deficient mice was not significantly different from that of controls but a significant increase (p<0.001) in the percentage of chromosomal aberrations was observed in vitamin deficient mice when BP was injected, compared to normal mice (Table -8.6).

Figure 8.1 shows the different types of sperm head abnormalities seen in vitamin E deficient mice. They include amorphous shaped sperms, spear shaped sperms, pear shaped sperms etc.

In vitamin E deficient animals, body and liver weights,
Table 8.3: Levels of vitamins A, C and E in the serum of cancer patients

<table>
<thead>
<tr>
<th>Type of cancer</th>
<th>No. of persons analysed</th>
<th>Vitamin A (ug/dl)</th>
<th>Vitamin C (mg/dl)</th>
<th>Vitamin E (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(Volunteers) 20</td>
<td>52.6 ± 15.1</td>
<td>1.2 ± 0.1</td>
<td>7.3 ± 1.1</td>
</tr>
<tr>
<td>(Normal persons)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oesophageal cancer patients</td>
<td>40</td>
<td>23.1 ± 11.1</td>
<td>0.2 ± 0.03</td>
<td>2.3 ± 0.9</td>
</tr>
<tr>
<td>Stomach cancer patients</td>
<td>20</td>
<td>24.0 ± 5.7</td>
<td>0.2 ± 0.06</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td>Cervix cancer patients</td>
<td>10</td>
<td>22.7 ± 6.4</td>
<td>1.7 ± 0.4</td>
<td>2.2 ± 0.9</td>
</tr>
<tr>
<td>Lung cancer patients</td>
<td>7</td>
<td>31.2 ± 9.6</td>
<td>0.8 ± 0.1</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td>Miscellaneous cancer patients</td>
<td>7</td>
<td>50.1 ± 14.3</td>
<td>1.1 ± 0.31</td>
<td>5.3 ± 0.2</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SD

a : p < 0.001  b : p < 0.01  c : p < 0.05
Table 8.4: Effects of vitamin A deficiency on different parameters in Swiss mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vitamin A deficient mice</th>
<th>Control mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>$25.6 \pm 2.5^b$</td>
<td>$35.7 \pm 4.7$</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>$1.0 \pm 0.09^a$</td>
<td>$1.7 \pm 0.07$</td>
</tr>
<tr>
<td>Liver GST (nm/min/mg protein)</td>
<td>$1680.0 \pm 110$</td>
<td>$1786.0 \pm 106$</td>
</tr>
<tr>
<td>Stomach GST (nm/min/mg protein)</td>
<td>$703.0 \pm 29$</td>
<td>$759.0 \pm 59$</td>
</tr>
<tr>
<td>Liver GSH (um/g tissue)</td>
<td>$5.2 \pm 0.4^c$</td>
<td>$6.7 \pm 0.6$</td>
</tr>
<tr>
<td>Liver vitamin A (ug/g)</td>
<td>$3.5 \pm 0.9^a$</td>
<td>$8.0 \pm 1.3$</td>
</tr>
<tr>
<td>Sperm head abnormality (%)</td>
<td>$1.5 \pm 0.3$</td>
<td>$1.3 \pm 0.2$</td>
</tr>
</tbody>
</table>

Values are Mean ± SD, n = 5
a: p < 0.001        b: p < 0.01        c: p < 0.05
Figure 8.1

Shows some sperm head abnormalities seen in vitamin E deficient mice

A: Shows a normal sperm

B: Shows an abnormal sperm, whose head is amorphous shaped
Figure 8.1

C: Reveals a banana shaped head

D: Depicts an abnormal sperm with spear shaped head
vitamin E levels, GST activities of stomach and liver and GSH levels were significantly lower than those of control mice (Table 8.5). In vitamin E deficient mice, sperm head abnormalities and chromosome aberrations induced by BP were present to a significant extent as compared to normal mice (Table 8.5 and 8.6).

The mutagenicity of BP was significantly higher when mediated with S9 from vitamin A deficient mice than when incubated with S9 from normal mice (Table 8.7). When retinol or glutathione was added to the reaction mixture, a dose dependent inhibition of the mutagenicity of BP was seen.

S9 from vitamin E deficient mice was also found to significantly increase the mutagenicity of BP as compared to S9 from normal mice. (Table 8.8). α-tocopherol and GSH were also extremely efficient in suppressing the mutagenicity of BP at both doses studied significantly, \( p<0.001 \), Table - 8.8.

The effects of vitamin A and E deficiency on BP induced neoplasia are shown in Table 8.9. Vitamin A and E deficiency did not decrease the latent period of tumour formation, but vitamin A and E deficiency were responsible for increasing the tumour incidence by 19%, and 12% respectively, but these were not statistically significant.

C. Chemopreventive potential of Vitamins A, C and E:

The effects of feeding the three vitamins to Swiss
Table 8.5: Effect of deficiency of vitamin E on different parameters in Swiss mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vitamin E deficient mice</th>
<th>Normal mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>27.0 ± 1.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35.7 ± 4.7</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>1.03± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7 ± 0.07</td>
</tr>
<tr>
<td>Liver GST activity&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1040.0 ± 89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1786 ± 106</td>
</tr>
<tr>
<td>Stomach GST activity&lt;sup&gt;*&lt;/sup&gt;</td>
<td>623.0 ± 36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>759.0 ± 59</td>
</tr>
<tr>
<td>Liver GSH (um/g tissue)</td>
<td>5.4 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.7 ± 0.6</td>
</tr>
<tr>
<td>Liver vitamin E levels (ug/g)</td>
<td>20.1 ± 5.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.3 ± 10.8</td>
</tr>
<tr>
<td>Sperm head abnormality</td>
<td>2.5 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3 ± 0.2</td>
</tr>
</tbody>
</table>

Values are Mean ± SD  

n = 5  

<sup>a</sup>: p < 0.001  
<sup>b</sup>: p < 0.01  
<sup>c</sup>: p < 0.05

<sup>*</sup> Specific activity of GST expressed as nanomoles S-(2,4-dinitrobenzyl) glutathione formed/min/mg protein
**Table 8.6**: Effects of deficiencies of vitamins A and E on BP induced chromosome aberrations in Swiss mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Per centage of cells with</th>
<th>Per cent incidence of aberrant cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gaps</td>
<td>Breaks</td>
</tr>
<tr>
<td>BP injected mice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(controls)</td>
<td>20.2±1.9</td>
<td>43.8±6.1</td>
</tr>
<tr>
<td>BP injected vitamin A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>deficient mice</td>
<td>32.0±3.2a</td>
<td>48.4±6.4</td>
</tr>
<tr>
<td>BP injected vitamin E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>deficient mice</td>
<td>35.0±5.6a</td>
<td>50.0±6.7</td>
</tr>
</tbody>
</table>

Values are Mean ± SD; n = 5

a: p < 0.001  

b: p < 0.05
Table 8.7: Effects of vitamin A and glutathione on the mutagenic activity of BP in *S. typhimurium* TA 1537 in presence of S9 from vitamin deficient mice

<table>
<thead>
<tr>
<th>S9</th>
<th>Compound added (ug/plate)</th>
<th>Histidine revertants per plate</th>
<th>BP added (ug/plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Normal</td>
<td>-</td>
<td>167 ± 23</td>
<td>368 ± 39</td>
</tr>
<tr>
<td>Vitamin A deficient</td>
<td>-</td>
<td>181 ± 35</td>
<td>489 ± 66^c</td>
</tr>
<tr>
<td>Vitamin A deficient</td>
<td>Retinol (200)</td>
<td>169 ± 26</td>
<td>302 ± 39^a</td>
</tr>
<tr>
<td></td>
<td>(400)</td>
<td>153 ± 19</td>
<td>241 ± 36^a</td>
</tr>
<tr>
<td></td>
<td>Glutathione (200)</td>
<td>173 ± 22</td>
<td>295 ± 33^a</td>
</tr>
<tr>
<td></td>
<td>(400)</td>
<td>159 ± 29</td>
<td>249 ± 27^a</td>
</tr>
</tbody>
</table>

Values are Mean ± SD  

Spontaneous revertants (140 ± 25) are not subtracted

a: p < 0.001 (as compared to number of histidine revertants/plate produced when BP incubated with S9 from vitamin A deficient mice)

B: p < 0.001; c: p < 0.01 (when compared to the number of histidine revertants/plate produced when BP incubated with S9 from normal mice)
Table 8.8: Effects of vitamin E and glutathione on the mutagenic activity of BP in
*S. typhimurium* TA 1537 in presence of S9 from vitamin E deficient mice

<table>
<thead>
<tr>
<th>Compound added (ug/plate)</th>
<th>Histidine revertants per plate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BP added (ug/plate)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin E deficient</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin E deficient</td>
<td>∞-Tocopherol (200) 162±24</td>
</tr>
<tr>
<td></td>
<td>(400) 159±19</td>
</tr>
<tr>
<td>Glutathione (200) 171±18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(400) 166±21</td>
</tr>
</tbody>
</table>

Values are Mean ± SD of 6 plates

Spontaneous revertants (152±19) are not subtracted

\(a: p < 0.001, \ b: p < 0.01\) (as compared to number of his\(^+\) revertants/plate produced when BP incubated with S9 from vitamin E deficient mice)

\(*: p < 0.001\) (when compared to the number of his revertants/plate produced when BP incubated with S9 from normal mice)
Table 8.9: Effects of deficiencies of vitamins A and E on BP induced neoplasia in Swiss mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals at risk</th>
<th>No. of animals with tumours</th>
<th>Per cent incidence of tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BP alone</td>
<td>15</td>
<td>11</td>
<td>73</td>
</tr>
<tr>
<td>Vitamin A deficient mice + BP</td>
<td>15</td>
<td>13</td>
<td>87</td>
</tr>
<tr>
<td>Vitamin E deficient mice + BP</td>
<td>11</td>
<td>9</td>
<td>82</td>
</tr>
</tbody>
</table>

The results were not significant, using $x^2$ 2x2 contingency test with correction for continuity (Yates). (20).
mice on GST activities and GSH levels in stomach and liver are shown in Table 8.10. The three vitamins significantly increased GSH levels in the stomach and liver of Swiss mice. Vitamin C failed to increase GST activity significantly in the liver and stomach. Vitamin A increased GST activity by 52% in the liver and by 66% in the stomach. Vitamin E increased GST activity by 110% in the stomach and by 86% in the liver.

The effects of the vitamins A, C and E on BP induced CA in Swiss mice are shown in Table 8.11. Vitamin C did not significantly inhibit BP induced CA. Vitamins A and E however were found to significantly (p<0.001, and p<0.01 respectively) decrease the incidence of aberrant cells by 55% and 26% respectively.

The effects of vitamins A, C and E on the mutagenicity of NMU in TA 1535 strain of Salmonella typhimurium are shown in Figure 8.2. Vitamin A and E moderately inhibited the mutagenicity of NMU at 200 μg/plate by 20% and 25% respectively (p<0.001). At 100 μg/plate dose, percent inhibition of mutagenicity were 14 and 17 for vitamin A and E respectively (p<0.01). Both the vitamins failed to significantly inhibit the mutagenicity of NMU, when added at a dose of 50 μg/plate. Vitamin C failed to significantly inhibit the mutagenicity of NMU.

The effects of vitamins A, C and E on the food intakes and body weights of mice fed BP are shown in Table 8-12.
Table 8.10: Effects of feeding vitamins A, C and E on GST activity and GSH levels in Swiss mice

<table>
<thead>
<tr>
<th>Vitamin administered to mice</th>
<th>GST specific activity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>GSH levels&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Stomach</td>
</tr>
<tr>
<td>None</td>
<td>1800 ± 101</td>
<td>715 ± 53</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>2760 ± 226&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1202 ± 99&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>3374 ± 302&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1527 ± 103&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>1793 ± 108</td>
<td>743 ± 59</td>
</tr>
</tbody>
</table>

Values are Mean ± SD   n = 5

<sup>a</sup>: Specific activity of GST expressed as nanomoles S-(2,4-dinitrobenzyl)glutathione formed/min/mg protein

<sup>b</sup>: GSH expressed as umoles/g tissue

<sup>c</sup>: p < 0.001
Table 8.11: Effects of vitamins A, C and E on BP induced chromosome aberrations in Swiss mice bone marrow cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Per centage of cells with</th>
<th>Per cent incidence of aberrant cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gaps</td>
<td>Breaks</td>
</tr>
<tr>
<td>BP alone</td>
<td>20.2 ± 1.9</td>
<td>43.8 ± 6.1</td>
</tr>
<tr>
<td>BP + Vitamin A</td>
<td>8.4 ± 2.7 a</td>
<td>19.0 ± 1.6 a</td>
</tr>
<tr>
<td>BP + Vitamin C</td>
<td>18.6 ± 2.4</td>
<td>38.8 ± 2.1</td>
</tr>
<tr>
<td>BP + Vitamin E</td>
<td>9.8 ± 2.5 a</td>
<td>32.8 ± 4.8 c</td>
</tr>
</tbody>
</table>

Values are Mean ± SD

n = 5

a : p < 0.001  
b : p < 0.01  
c : p < 0.05
Figure 8.2

Effects of vitamins A, C and E on the mutagenicity of NMU in TA 1535 of *Salmonella typhimurium*.

Mutagenicity experiments were carried out without the addition of S9.

Spontaneous revertants were 20±2 and were not subtracted. No. of rev/plate for NMU alone was 935±78.
Table 8.12: Effects of vitamins A, C and E on food intakes and body weights in mice fed BP

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Food intake g/mouse/day</th>
<th>Body weight (g) at the end of 16 weeks</th>
<th>Body weight (g) at the end of 32 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>None (Negative control)</td>
<td>3.5 ± 0.4</td>
<td>30.9 ± 2.6 (15)</td>
<td>40.1 ± 5.0 (15)</td>
</tr>
<tr>
<td>2.</td>
<td>BP alone (Positive control)</td>
<td>3.3 ± 0.2</td>
<td>28.4 ± 3.1 (15)</td>
<td>37.9 ± 4.2 (14)</td>
</tr>
<tr>
<td>3.</td>
<td>Vitamin A + BP</td>
<td>3.2 ± 0.3</td>
<td>28.6 ± 3.8 (14)</td>
<td>39.4 ± 3.2 (14)</td>
</tr>
<tr>
<td>4.</td>
<td>Vitamin C + BP</td>
<td>3.4 ± 0.4</td>
<td>29.2 ± 2.8 (14)</td>
<td>37.8 ± 4.3 (12)</td>
</tr>
<tr>
<td>5.</td>
<td>Vitamin E + BP</td>
<td>3.5 ± 0.2</td>
<td>28.9 ± 3.0 (15)</td>
<td>39.8 ± 4.5 (13)</td>
</tr>
</tbody>
</table>

Values are Mean ± SD

Numbers in parentheses indicate the number of mice alive at the time of measurement.

There were no significant differences (p > 0.05) in food intakes and body weights among the different groups of mice.
There were no significant differences in body weights and food intakes of the mice fed different vitamins + BP, or BP alone, when compared to those of mice fed pellet diet alone.

The effects of vitamins A, C and E on BP-induced stomach carcinoma are presented in Table 8.13. Vitamins A and E inhibited the incidence of squamous cell carcinoma of the stomach by 46% and 32% respectively, and Vitamin C had negligible effects on BP-induced neoplasia.

The effects of vitamins A, C and E on the nitrosation of methylurea are shown in Figure 8.3. Vitamins C and E significantly inhibited the nitrosation of methylurea at both doses studied. Vitamin A failed to inhibit the nitrosation of methylurea, significantly, at both concentrations.

DISCUSSION

The levels of vitamins A, C and E which are important micronutrients, were significantly decreased in patients with gastro-intestinal tract cancers. This suggests an etiological role of their deficiency in cancers of the stomach and oesophagus. Many workers have related low levels of vitamins A and C to increased incidence of GI tract cancers (21, 11) but not much information is available on the role of vitamin E in stomach and oesophageal cancers. Our results show that vitamin E levels were also decreased
Table 8.13: Effects of vitamins A, C and E on BP induced neoplasia in mice

<table>
<thead>
<tr>
<th>Vitamin administered to mice</th>
<th>No. of mice at risk</th>
<th>No. of mice developing tumours</th>
<th>Per cent incidence of tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BP alone</td>
<td>14</td>
<td>11</td>
<td>79</td>
</tr>
<tr>
<td>Vitamin A + BP</td>
<td>14</td>
<td>6</td>
<td>43</td>
</tr>
<tr>
<td>Vitamin C + BP</td>
<td>12</td>
<td>9</td>
<td>75</td>
</tr>
<tr>
<td>Vitamin E + BP</td>
<td>13</td>
<td>7</td>
<td>54</td>
</tr>
</tbody>
</table>

The results were not statistically significant. $X^2$ 2x2 test with correction for continuity (Yates) was used (25).
Figure 8.3

Inhibition of the nitrosation of MU by vitamins A, C and E (assessed by the mutagenicity of the NM in *Salmonella typhimurium* TA 1535).

NM - Nitrosation mixture alone

Mutagenicity was tested without the addition of S9.
in serum of stomach and oesophageal cancer patients.

In mice with induced deficiency of vitamins A and E there was an increased incidence of CA in bone marrow cells, induced by BP. These results suggest that deficiency of vitamins A and E may increase the susceptibility of the organism to clastogenic agents. It is also seen that there is an enhancement of BP induced mutagenicity in Ames assay by S9 from vitamin A and E deficient mice. This may be due to increased metabolism of BP to its ultimate carcinogen or due to a decrease of cellular scavengers in the liver. GST activity in vitamin A deficient mice is not significantly different from that of controls. Alzieu et al (22) have shown that the activities of BP metabolising enzymes (BP - monooxygenase and epoxide hydrolase) were decreased in vitamin A deficient rats. The amount of glutathione and retinol in the liver of vitamin A deficient mice are low. Therefore the increased mutagenicity of BP can be related to the lack of vitamin A, and to a decrease in the level of free radical scavengers like GSH in the cellular fractions of deficient mice. Thus GSH and retinol significantly decreased the mutagenicity of BP mediated by S9 from vitamin A deficient mice, when added to the incubation mixture. Qin and Huang (23) reported that retinol had no effect on the mutagenesis of BP in the Ames assay. However, they used a maximum dose of 40 μg/plate, while in our experiments we have added retinol at doses of 100 μg and 200 μg/plate in
the Ames assay.

In livers of vitamin E deficient mice, GST activity as well as GSH levels were found to be significantly low than those found in normal mice. Therefore the detoxification of ultimate carcinogens may be decreased. Thus, the increased mutagenicity of BP mediated by S9 from vitamin E deficient mice may be attributed to both, decreased detoxification of BP metabolites and decreased levels of GSH and vitamin E in the cellular fraction of deficient animals.

Thus, it is seen that vitamin A and E deficient mice are more susceptible to carcinogenesis. This is confirmed by our findings that the percent incidence of squamous cell carcinomas in the stomach of Swiss mice induced by BP were increased in vitamin A and E deficient mice, but not significantly.

The three vitamins act as moderate chemopreventive agents but do so by different mechanisms. Vitamins C and E are efficient inhibitors of the nitrosation reaction and thus prevent the formation of N-nitroso compounds endogenously. Vitamin E increases GST activity by more than 78% in the stomach and liver and also increases GSH levels significantly. However, it only moderately inhibited BP induced neoplasia. Vitamin E in the diet also enhances liver microsomal activity (24). This may result in an increase in the level of enzymes metabolising BP to ultimate carcinogens. Vitamin C failed to increase GST levels, and to decrease significantly BP induced CA and neoplasia. Vitamin
A failed to inhibit the nitrosation of methylurea, and though it caused only a modest increase of GST, it significantly inhibited BP-induced neoplasia. The inhibitory effects of retinol may be due to its ability to inhibit certain forms of cytochrome P-450 isoenzymes which are required for metabolic activation (25). It may also be that the observed anticarcinogenic effect of vitamin A results from an interference with cellular proliferation and differentiation of the epithelial mucosa in the direction of the keratinising squamous type (26). It is known that vitamin A is important in maintaining the normal histology of certain types of epithelium (26). These observations are of interest because of the metaplastic change which frequently precedes or accompanies the development of squamous cell neoplasms of uterine cervix, oral region and lung in humans (27).

Vitamin C is not an effective antimutagen against NMU in Ames assay. Vitamin C has been shown to enhance the mutagenicity of tryptophan pyrolysates (28) and also failed to decrease the mutagenicity of tobacco smoke or fried meat in *S. typhimurium* (29). Vitamin A and E exhibit moderate antimutagenicity against NMU which is a direct acting mutagen. Vitamin E was reported to decrease the mutagenicity of 2-aminofluorene and 2-aminoanthracene in Ames test (24). Vitamin A is found to be more effective
against carcinogens requiring metabolic activation in the Ames assay. This may be attributed to the fact that it has the capacity to inhibit the enzymes that act during the process of metabolic activation of mutagens (30).

Extensive work on the influence of vitamins A and E on carcinogenesis of colon, skin, lung and breast induced by chemical carcinogens have been performed (7). Data on the role of these vitamins in stomach cancer is however insufficient. Our results suggest that vitamins A and E do not have a significant protective effect against development of stomach neoplasia and can inhibit the development of stomach cancer when ingested. By inhibiting endogenous nitrosation in the stomach, vitamin C can also protect against gastric cancer.
SUMMARY

1. The roles of vitamins A, C and E in relation to cancer have been studied.

2. The levels of vitamins A, C and E in the serum of stomach and oesophageal cancer patients were found to be significantly lower than those of normal persons.

3. Deficiencies of vitamins A and E in Swiss mice were induced. Body weights, liver weights, GSH levels, and vitamin A levels were decreased in vitamin A deficient mice. Body weights, liver weights, GST activity, GSH levels and vitamin E levels were decreased in vitamin E deficient mice, while sperm head abnormalities were higher.

4. Chromosome aberrations induced in vitamin A and E deficient mice by BP were significantly greater than CA induced in normal mice by BP.

5. The mutagenicity of BP was significantly higher, when mediated with S9 from vitamin A deficient mice than when incubated with S9 from normal mice. Retinol and glutathione significantly decreased the mutagenicity of BP mediated by S9 from vitamin A deficient mice.

6. The mutagenicity of BP was significantly higher when mediated with S9 from vitamin E deficient mice than when incubated with S9 from normal mice. Tocopherol and glutathione significantly inhibited the mutagenicity of BP mediated by S9 from vitamin E deficient mice.

7. Deficiencies of vitamins A and E significantly increased the incidence of BP-induced neoplasia in mice.
8. Vitamins A and E significantly increased GST activity and GSH levels in the stomach and liver of Swiss mice on oral administration. Vitamin C failed to increase GST activity and GSH levels significantly.

9. Vitamins A and E significantly inhibited BP-induced CA, but not vitamin C.

10. Vitamins A and E were found to be moderate antimutagens against NMU in the Ames assay. Vitamin C failed to decrease the mutagenicity of NMU.

11. Vitamins C and E inhibited the nitrosation of methylurea considerably, while vitamin A failed to exert such an inhibitory effect.

12. These results suggest that vitamin E is a "general" protective agent against all carcinogens, while vitamin A is a good protective agent, particularly in those cancers due to BP and related carcinogens. On the other hand, vitamin C has only a very limited role as a protective agent, limited to those involving nitrosamines and nitrosamides.
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Complex effects of retinol on the metabolic activation
of 2- aminofluorene.
GENERAL SUMMARY
GENERAL SUMMARY

Prevention is better than cure; especially so with cancer, for some forms of which, the cure is either uncertain or practically non-existent. Hence, as part of the strategy for the prevention of cancer, a comprehensive survey for protective agents against cancer in the Indian diet (food components) has been carried out.

Twenty commonly consumed plant products (spices and leafy vegetables) have been screened for their abilities to increase the tissue levels of the carcinogen - detoxifying enzyme, Glutathione-S-transferase (GST), in laboratory animals (Swiss mice), on oral administration for a fortnight. Ten of them - cumin and poppy seeds, kandathipili, turmeric, asafoetida, neem flowers, drumstick, manathakkali, ponnakanni and basil leaves - have been found to increase the levels of this enzyme sufficiently high (more than 78% above normal) as to be considered protective agents.

These ten plant products were then investigated further through a series of short-term and long-term tests to assess exactly their potentials as chemopreventive agents against cancer. Nine of these plant products i.e. excepting neem flowers, when fed simultaneously with the potent carcinogen, benzo(a)pyrene (BP), to Swiss mice, suppressed the induction of chromosomal aberrations by BP. Pretreatment with cumin seeds, poppy seeds, ponnakanni or manathakkali leaves was
found to be most effective in suppressing the genotoxic effects of benzo(a)pyrene, in a dose-dependent manner. Turmeric and kandathipili were moderately effective, while basil leaves and drumstick leaves were only slightly effective.

The abilities of these spices and leafy vegetables to counteract in vivo and in vitro the microsomal degranulations produced by the carcinogens, BP or 3'Methyl 4-dimethylaminoazobenzene (3MeDAB) have been determined through measurements of the microsomal RNA/protein and RNA/phospholipid ratios. Cumin seeds, poppy seeds turmeric and kandathipili significantly inhibited the microsomal degranulations due to both BP and 3MeDAB. So also basil, manathakkali and drumstick leaves. But ponnakanni leaves were only slightly effective, while asafoetida had no effect at all.

All these plant products were found to be non-mutagenic through the Ames Test, except asafoetida which is mutagenic. The antimutagenicities of all these plant products against the potent mutagens, sodium azide and nitrosomethylurea were then investigated, also through the Ames Test with the histidineless mutants of S. typhimurium TA 98, and TA 1535. Cumin seeds, poppy seeds and turmeric have been found to be potent antimutagens, while kandathipili had moderate inhibitory effects against these mutagens. On the other hand, asafoetida had no antimutagenic action. It actually
increased the mutagenicity of NMU. This anomalous behaviour of asafoetida may be due to the fact that it is actually a mixture of resins, which may have opposing effects. All the four leafy vegetables tested have pronounced antimutagenic effects, manathakkali leaves alone exhibiting antimutagenicity at higher doses only.

After ascertaining the antimutagenicities of these plant products, their anticarcinogenicities were determined. The effects of simultaneous feeding of these plant products on the induction of
1. Squamous cell carcinomas of the stomach in Swiss mice by BP; and
2. Hepatomas (adenocarcinomas) in Wistar rats by 3MeDAB, were investigated. Cumin seeds and basil leaves were most effective in preventing induction of tumours by these carcinogens, followed by poppy seeds, turmeric and ponnakanni leaves, drumstick leaves, manathakkali leaves and kandathipili showed moderate antitumour effects. Also asafoetida, on the other hand, had practically no inhibitory effect.

Besides polycyclic aromatic hydrocarbons as exemplified by BP, and azodye carcinogens like 3MeDAB, nitrosamines and nitrosamides form a very important group of carcinogens, to be reckoned with in the origins of human cancers. Hence, the protective effects of these plant products against these carcinogens have also been investigated. Nitrosamines and nitrosamides can arise endogenously also (particularly in
the stomach) through interactions between secondary or tertiary amines and nitrites (derived from nitrates), both from the diet, in the pH range 3-6. Whether these plant products can inhibit this formation, this nitrosation reaction, has been investigated, using the nitrosations of 1. triethanolamine to nitrosodiethanolamine; 2. Methylurea to nitrosomethylurea as model reactions. Cumin seeds, poppy seeds, turmeric, drumstick leaves, and ponnakanni leaves strongly inhibited the formation of these nitroso compounds. Manathakkali leaves were effective only at a higher dose of 7.5 mg. Basil leaves and kandathipili inhibited the nitrosation reaction only at a dose of 5 mg alone. Asafoetida failed to inhibit the nitrosation reaction.

As cumin seeds, poppy seeds and basil leaves exhibited pronounced antitumour effects, the volatile essential oils from them were extracted out and investigated for anticancer properties. Oral administration of these three oils significantly increased the GST activities and glutathione levels in the stomach, liver and oesophagus of Swiss mice; and significantly decreased BP-induced microsomal degranulation both in vivo and in vitro. All the three essential oils were non-mutagenic with all the strains of S. typhimurium tested; and exhibited potent antimutagenicity against both nitrosomethylurea and sodium azide. All of them profoundly inhibited the induction of squamous cell carcinomas of the stomach by BP, cumin oil being the most
effective. When administered to Swiss mice on different initially days after transplantation with Daltons lymphoma, all the three oils retarded the growth of this tumour and increased the life span of these animals significantly. Basil oil was the most effective. These results suggest that cumin seed, poppy seed and basil leaf oils have, undoubtedly, considerable protective effects against carcinogenesis; and moderate therapeutic effects against established cancers. The chemopreventive potential exhibited by cumin seeds, poppy seeds, and basil leaves may be due to their respective essential oils.

The roles of vitamins A, C and E in relation to cancer were also investigated, as they all occur in the plant products studied.

1. The levels of these vitamins in the serum of patients suffering from stomach and oesophageal cancers were found to be significantly low as compared to those of normal persons.

2. Swiss mice with deficiencies of vitamins A and E had greater susceptibilities to the effects of carcinogens; but the incidence of BP-induced neoplasia was not increased in their deficiencies.

3. The chemopreventive potentialities of vitamins A, C and E were explored. Vitamin E alone increased GST activity by more than 78% in the stomachs and livers of Swiss mice, while vitamin A produced only modest increases in GST activity. Vitamin C failed to significantly increase GST levels. Vitamins A and E significantly inhibited the
genotoxicity and carcinogenicity of BP. Vitamin C, however, had negligible effect. Vitamin A and E were also found to be modest antimutagens, while vitamin C failed to suppress the mutagenicity of nitrosomethylurea.

4. However, vitamins C and E efficiently inhibited the nitrosation of methylurea, while vitamin A failed to inhibit the formation of nitrosomethylurea. These results suggest that vitamin E can act as a general protective agent against all cancers, while vitamin A will be particularly useful in some cases involving benzo(a)pyrene and related carcinogens. Vitamin C, on the other hand, has a limited role only as a protective agent, limited to those due to nitrosamines and nitrosamides.