Chapter-II

Antitumourogenic activity of Indole-3-Carbinol
in mouse skin model of carcinogenesis
Introduction

There has been growing interest in recent years in the potential of brassica vegetables as vectors for the introduction of anticarcinogenic compounds in the diet (McDanell et al., 1989). The diversity and widespread occurrence of these compounds in food make it virtually impossible to consume a diet that does not contain inhibitors of carcinogenesis.

Glucosinolates, are a group of secondary products commonly found in plants of the family Cruciferae. They upon enzymic hydrolysis give rise to a range of volatile pungent and physiologically active compounds the indole glucosinolates (glucobrassicins). This compound on chemically or enzymatically hydrolysed, gives rise to a range of involatile indole compounds which shows the anticarcinogenic and mixed function oxidase (MFO) stimulatory activities of brassica vegetables (McDanell et al., 1988).

Cabbage has been shown to cause a variety of physiological effects (Whitty and Bjeldanes, 1987). Cabbage and its juice has been reported in past to stimulate gastric secretion and peristalsis (Bykoff, 1922), lowered blood sugar levels (Dubin and Corbitt, 1928) and had antibacterial action (Sherman and Hodge, 1936). In bacterial mutagenic assays cabbage juice was found to
be remarkably effective in reducing the mutagenicity of several compounds (Morita, 1978). High ascorbic acid content of cruciferous vegetables, inhibited the nitrosation reaction otherwise, indole derivatives react with nitrite \textit{in vitro} to form compounds that were mutagenic in the Ames test (Wakabayashi \textit{et al.}, 1985).

I3C, a major indole metabolite in cruciferous vegetables has been found to inhibit the development of tumours in forestomach (Wattenberg and Loub, 1978; Wattenberg, 1990) mammary gland (Bresnick \textit{et al.}, 1990; Bradlow \textit{et al.}, 1991; Wattenberg, 1990), uterus (Kojima \textit{et al.}, 1994) and liver (Kim \textit{et al.}, 1994; Tanaka \textit{et al.}, 1990) of rodents when administered prior to or during carcinogenic exposure by gavage or in the diet. Brussels sprouts has putative antimutagenic and anticarcinogenic properties by decreasing the rate of oxidative damage to DNA (Verhagen \textit{et al.}, 1995).

Guo \textit{et al.} (1995) observed that I3C shows a protective role against PhIP-induced colon carcinogenesis through mechanism that alters the uptake or metabolism of the carcinogens and by suppression in the post-initiation phase. I3C was evaluated for its efficacy in the prevention of chemically-induced mammary tumours (Grubbs \textit{et al.}, 1995). Administration of I3C during the initiation phase (7 days prior to until 7 days post DMBA) was also highly effective as a chemopreventive agent (Grubbs \textit{et al.}, 1995). I3C also shows an inhibitory effects in studies of human breast and ovarian cancers (Stoewsand, 1995).

Shertzer (1984) observed that \textit{in vivo} administration of I3C shows protection for hepatic macromolecules against covalent binding of the metabolites of two indirect acting mutagens BaP and DMBA. Testosterone 6β-hydroxylase, the major testosterone hydroxylase activity in untreated mice was significantly inhibited (IC50 approximately 12 micrograms/ml) by the
Acid condensation products of I3C (Baldwin and Leblanc, 1992). Chung et al (1984) observed that indole L-tryptophan and I3C were strong inducers of N-nitrosodimethylamine (NDMA) and 4-(methyl nitrosamine)-1-(3-pyridyl)-1-butanone (NNK) demethylation.

I3C has an inhibitory effect against DMBA-induced mammary tumour formation in the rats and the occurrence of BaP induced-neoplasia of the forestomach in ICR/Ha mice (Wattenberg and Loub, 1978).

Pence et al (1986) investigated that I3C was effective to enhance the tumourigenicity of dimethyl hydrazine (DMH). The incidence of adenomas of the small and large intestine was significantly increased in indole fed rats in comparison to control rats fed basal diet only. Birt et al (1987) observed that exposure of I3C during the post initiation stage was found to strongly enhance the N-nitrosobis (2-oxo-propyl) amine (BOP) induced pancreas tumourigenesis in hamsters. Kim et al (1997) found that I3C enhances liver and thyroid gland neoplastic development when given during the promotion phase in rat medium term multiorgan carcinogenesis model. Polycyclic aromatic hydrocarbons are ubiquitous environmental pollutants with high carcinogenic potencies (Melendez-colon et al, 1999). The effect of these chemical carcinogens can be easily checked in mouse skin model as it provides valuable information about the mechanism of tumour induction. This is the reason we selected here mouse skin as a model to investigate the antitumourigenic effect of I3C.

The present set of investigation shows the antitumour activity of I3C on polycyclic aromatic hydrocarbon i.e. DMBA and BaP. Here we found that the number of tumours was less in I3C treated group in comparison to DMBA and BaP treated groups.
Materials and Methods

Chemicals

DMBA, BaP and I3C were purchased from M/s Sigma Chemicals Co. USA. All other chemicals used in this study were of high purity analytical grades procured locally.

Animals and Treatment

Random bred male and female Swiss albino mice (Figure 5) of (12-15 gm) of weight were obtained from ITRC animal colony. They were kept in groups of 20 animals per polypropylene cages belonging to the same group under controlled humidity (65-75%) and temperature (22-25°C) with 12/12 hours (light and dark period). Animals were fed a standard diet and water ad libitum. Treatment was given on the shaved dorsal skin, in an area of 2 cm² in all the groups initially using electric clipper which was not lubricated with any oil or grease. Mice were shaved after every 15 days (Figure 6). The treatment was given according to the Table 10.

Animals from all the groups were checked weekly for gross changes on the skin, including loss of fur, development of tumours and ulcer during the entire period of experiment. At the end of experiment, all the surviving animals were sacrificed in order to check any tumour growth in their internal organs. Tissue from the treatment site was taken out for the histopathological evaluation. Skin tumours were cleaned, washed with saline and weighted. Blocks prepared according to the given process.

Washing

Skin tumours were cleaned, washed with normal saline and used balance for its weight.
Figure 5: Swiss albino mouse

Figure 6: Mouse with shaved dorsal skin
Table 10
Experimental schedule

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Control)</td>
<td>-</td>
</tr>
<tr>
<td>II (DMBA)</td>
<td>5 mg of DMBA dissolved in 50 ml acetone and its 10 μg/100 μl was applied topically 2X/week for 28 weeks</td>
</tr>
<tr>
<td>III (BaP)</td>
<td>10 mg of BaP dissolved in 40 ml acetone and its 25 μg/100 μl was applied topically 2X/week for 28 weeks</td>
</tr>
<tr>
<td>IV (DMBA+I3C)</td>
<td>DMBA same as in group II, I3C of 5 mg dissolved in 50 ml acetone and its 10 μg/100 μl was applied topically 2X/week for 28 weeks. DMBA applied 1 hour prior to I3C</td>
</tr>
<tr>
<td>V (I3C+DMBA)</td>
<td>Same as from group IV. I3C applied 1 hour prior to DMBA 2X/week for 28 weeks</td>
</tr>
<tr>
<td>VI (BaP+I3C)</td>
<td>BaP and I3C same as in group II and group IV. BaP applied 1 hour prior to I3C 2X/week for 28 weeks</td>
</tr>
<tr>
<td>VII (I3C+BaP)</td>
<td>I3C + BaP same as in group VI. I3C applied topically 1 hour prior to BaP 2X/week for 28 weeks</td>
</tr>
</tbody>
</table>
Fixation

After washing, fixed the tumours in 10% aqueous buffer formaline (conc. formalin 10 ml + Tap or Distilled water 90 ml) then fixed the tissues for 72 hours.

Washing

Again, after 72 hr of fixation, washed the tissue overnight. After overnight washing, put the tissues overnight in 70% alcohol (70 ml alcohol + 30 ml distilled water) and also changed the alcohol two times. After 70% alcohol, changed the tissues into 90% alcohol (90 ml alcohol + 10 ml distilled water) for 2 hr then transferred the tissues into 100% (100 ml) alcohol for 2 hrs for two times.

Block preparation

After 100% alcohol, transferred the tissues into xylene for overnight. For block preparation, used the melting wax at the 60-62 °C temperature. We put the tissues with the wax in an oven for 3 hrs. After this we prepared the paraffin blocks with the help of L shaped molds for section cutting, put the blocks in freezer for 2 hr before cutting. 5 µm thick sections were prepared with the help of microtome. Transferred the sections on Mayer's albumin (50 ml albumin + 50 ml glycerol) coated slides. As glycerol slows the oxidation of naturally oxidizing haematoxylin and improves the properties of material.

Staining

There are two processes in staining:

1. Rehydration

First of all removed the wax from the slides by dipping it into xylene for 5 minutes. After this put the slides in absolute alcohol for 5 minutes then transferred the slides into 90% and 70% alcohol for 5 minutes. Again put the
slides under running water for 5 minutes and then used Ehrlich's haemotoxylin
(haematoxylin 16 gms + ethylalcohol 480 ml + potassium or ammonium alum.
48 gms + Distilled water 240 ml + glycerol 240 ml + glacial acetic acid 24 ml)
for 5 minutes as we known that haematoxylin staining means staining of nuclei
by oxidized haematoxin (haematin) through mordant (Chelate), bonds of metals
such as alluminium.

After haematoxylin, put the slides under running water for 10 minutes.
Then used eosin (1 gm eosin + 100 ml distilled water) for 2 minutes for
counter staining as eosin colours in varying shades to different tissue fibers
and cytoplasm.

2. Dehydration

The process of dehydration starts by putting the slides into 70% alcohol
for 5 minutes. Again used 90% and 100% alcohol for 5 minutes for the
dehydration of slides.

Mounting

Before mounting first dipped the slides in xylene for 5 minutes. Then
mounted the slides with DPX.

For the analysis of skin tumour appearance, the Kaplan Meier method of
tumour free survival estimation was applied considering time for the first
appearance of skin tumour as the basic end-point. Fischer's (1950) Student 't'
test was applied for the statistical analysis

Results

Our findings suggests that the body weight of control group increased at
the end of experiment i.e. 28 weeks, when we compared it with other treated
groups. As the average body weight of control groups was 30 gms in
comparison to group II and III i.e. DMBA and BaP treated groups where it was 29 and 26 gms respectively (Figure 7). Body weight of other groups treated with I3C in combination to DMBA and BaP was also high. As in group IV and V i.e. DMBA + I3C and I3C+DMBA where it was 31 and 35 gms and in group VI and VII i.e. BaP+I3C and I3C+BaP where it was 30 and 36 gms (Figure 7). Similarly body weight of females was also high, when we compared it with control group as females of control group have 33 gms body weight at the end of experiment while in group II and III i.e. DMBA and BaP, where it was 32 and 30 gms (Figure 8). Body weight of I3C treated groups IV and V i.e. DMBA + I3C and I3C+DMBA have 31 and 32 gms while it was 34 and 35 gms in group VI and VII i.e. BaP+I3C and I3C+BaP at the end of experiment i.e. 28 weeks (Figure 8). From these results, we can conclude that I3C was more effective when we given it after the carcinogen.

When we compared the significant values of treated groups with control group using student 't' test at different time intervals, we observed that at starting week, there was no significance in the values of body weight with control groups as p<0.05 while at 4 weeks, only two groups i.e. group IV (DMBA+I3C) and group VI (BaP+I3C) showed significant values. At 8 weeks, when we compared the control group with treated groups, we found that group II, IV, V, VI and VII i.e. DMBA, DMBA+I3C, I3C+DMBA, BaP+I3C and I3C+BaP were significant, when we compared the values at 12 weeks, we found that group II, III, IV, V and VII i.e. DMBA, BaP, DMBA+I3C, I3C+DMBA and I3C+BaP have different significant values. Groups II, III, IV, V, VI and VII i.e. DMBA, BaP, DMBA+I3C, I3C+DMBA, BaP+I3C and I3C+BaP have different significant values when compared with control group at 16 weeks of time interval. At 20 weeks, group II, IV, V and VII i.e. DMBA, DMBA+I3C, I3C+DMBA and I3C+BaP have different significant values in comparison to control groups. Group II, III, IV, VI and VII i.e. DMBA, BaP,
Figure 7: Showing average body weight of male Swiss albino mice at different time intervals.
Figure 8: Showing average body weight of female Swiss albino mice at different time intervals.
DMBA+I3C, BaP+I3C and I3C+BaP have significant values compared to control group at the end of 24 weeks while group II, III, IV, V and VII i.e. DMBA, BaP, DMBA+I3C, I3C+DMBA and I3C+BaP have different significant values in comparison to control groups at the end of experiment i.e. 28 weeks.

The next parameter of this experiment was to observe the total number of surviving animals when we compared the control group with group II and III i.e. DMBA and BaP, we observed that the number of animals was 14 and 15 out of 25 at the end of experiment i.e. 28 weeks while when we compared the surviving animals of group IV and V i.e. DMBA+I3C and I3C+DMBA with control group, the numbers of surviving animals was 17 and 18 out of 25, while it was 19 and 20 in group VI and VII i.e. BaP+I3C and I3C+BaP treated animals (Figures 9,10). The number of surviving animals was high in I3C treated groups in comparison to group II and III i.e. DMBA and BaP treated groups (Tables 11,12).

First tumour induction time was also observed primary in group II i.e. DMBA treated group as it was observed in the 8th week of experiment while in all the other groups, first tumour induction was seen in the 12th week of the experiment (Tables 11,12). The next parameter we used was the percentage of animals with tumours. We can see that 100% tumourigenesis was observed in group II i.e. DMBA treated group while it was 73.3 and 94.1% in BaP treated group i.e. group III (Figures 11,12; Tables 11,12). Group IV and V i.e. DMBA+I3C and I3C+DMBA treated groups have 94.1 and 88.2% of tumours while group VI and VII have 89.4, 94.6 and 70, 80% tumour (Figures 11,12). From the above results we can say that 100% tumorigenesis never achieved in I3C treated group, and I3C is more effective in terms of percentage of animals with tumour when given after the carcinogenic dose (Figures 11,12).
Figure 9: Showing number of surviving animals in male Swiss albino mice.
Figure 10: Showing number of surviving animals in female Swiss albino mice.
Table 11
Appearance of tumour dynamics in male Swiss albino mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Number of surviving animals at end point</th>
<th>1st Induction of tumour (in days)</th>
<th>Average tumour/mouse</th>
<th>Cumulative number of tumours</th>
<th>Percentage of animals with tumour</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>23</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>DMBA</td>
<td>14</td>
<td>56</td>
<td>6.4</td>
<td>90</td>
<td>100</td>
<td>6.07±0.233</td>
</tr>
<tr>
<td>III</td>
<td>BaP</td>
<td>15</td>
<td>84</td>
<td>4.7</td>
<td>52</td>
<td>73.3</td>
<td>4.9±0.251</td>
</tr>
<tr>
<td>IV</td>
<td>DMBA+I3C</td>
<td>17</td>
<td>84</td>
<td>3.8</td>
<td>62</td>
<td>94.1</td>
<td>3.8±0.180</td>
</tr>
<tr>
<td>V</td>
<td>I3C+DMBA</td>
<td>18</td>
<td>84</td>
<td>3.3</td>
<td>40</td>
<td>66.6</td>
<td>3.4±0.217</td>
</tr>
<tr>
<td>VI</td>
<td>BaP+I3C</td>
<td>19</td>
<td>84</td>
<td>3.7</td>
<td>63</td>
<td>89.4</td>
<td>3.7±0.237</td>
</tr>
<tr>
<td>VII</td>
<td>I3C+BaP</td>
<td>20</td>
<td>84</td>
<td>3.9</td>
<td>55</td>
<td>70</td>
<td>3.4±0.232</td>
</tr>
</tbody>
</table>
### Table 12

Appearance of tumour dynamics in female Swiss albino mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Number of surviving animals at end point</th>
<th>1st Induction of tumour (in days)</th>
<th>Average tumour/mouse</th>
<th>Cumulative number of tumours</th>
<th>Percentage of animals with tumour</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>24</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>DMBA</td>
<td>15</td>
<td>84</td>
<td>5.6</td>
<td>85</td>
<td>100</td>
<td>5.57±0.233</td>
</tr>
<tr>
<td>III</td>
<td>BaP</td>
<td>17</td>
<td>84</td>
<td>4.4</td>
<td>71</td>
<td>94.1</td>
<td>4.4±0.226</td>
</tr>
<tr>
<td>IV</td>
<td>DMBA+13C</td>
<td>17</td>
<td>84</td>
<td>4.8</td>
<td>73</td>
<td>88.2</td>
<td>4.8±0.230</td>
</tr>
<tr>
<td>V</td>
<td>13C+DMBA</td>
<td>17</td>
<td>84</td>
<td>4.3</td>
<td>56</td>
<td>76.4</td>
<td>4.3±0.241</td>
</tr>
<tr>
<td>VI</td>
<td>BaP+13C</td>
<td>18</td>
<td>84</td>
<td>4.6</td>
<td>79</td>
<td>94.6</td>
<td>4.6±0.172</td>
</tr>
<tr>
<td>VII</td>
<td>13C+BaP</td>
<td>20</td>
<td>84</td>
<td>3.7</td>
<td>60</td>
<td>80</td>
<td>3.7±0.202</td>
</tr>
</tbody>
</table>
Figure 11: Percentage of Swiss male albino mice developed tumours during the study period at different time intervals.
Figure 12: Percentage of Swiss female albino mice developed tumours during the study period at different time intervals.
The next parameter of this investigation was to observe the average tumour per mouse as we can say from (Figures 13,14) that in group II i.e. DMBA, the average tumour per mouse ratio was high in comparison to other groups as it was 6.4 and 5.6 in group II, while in group III i.e. BaP, it was 4.7 and 4.4 per mouse (Figures 13,14; Tables 11,12). Average number of tumours of group IV and V i.e. DMBA+I3C and I3C+DMBA was 3.8 and 3.3 in males (Figure 13) and 4.8 and 4.3 in female (Figure 14) while in group VI and VII i.e. BaP+I3C and I3C+BaP it was 3.7 and 3.9 in males (Figure 13) and 4.6 and 3.7 in females. So from the above we can conclude that I3C was effective as all the groups which was treated with I3C either pre or post have less numbers of average tumour in comparison to group II and III i.e. DMBA and BaP (Figures 13,14; Tables 11,12).

The last parameter of our investigation was to check the cumulative numbers of tumours (CNT). Group II and III i.e. DMBA and BaP have 90 and 52 CNT in males and 85 and 71 CNT in females (Figures 15,16). While group IV and V i.e. DMBA+I3C and I3C+DMBA have 62 and 40 CNTs in male group and 73, 56 CNTs in females groups (Figures 15,16; Tables 11,12). Group VI and VII i.e. BaP+I3C and I3C+BaP have 63 and 60 CNTs in male groups and 79 and 70 CNTs in female groups (Figures 15,16). So from the above we can say that I3C was also effective in terms of CNT numbers as the CNT was less in I3C treated groups in comparison to group II and III i.e. DMBA and BaP.

Skin lesions were observed in experimental groups. The lesions were confined to the dorsal painted area of skin and they were classified as tumours and non tumours lesions. The non-tumourus lesions included loss of fur followed by poor hair growth at the site of application, hyperkeratinization and scaly skin. The tumours encountered in the study were mostly benign in
Figure 13: Showing average tumour per mouse in different groups (in male).
Figure 14: Showing average tumour per mouse in different groups (in female).
Figure 15: Showing cumulative number of tumours in male Swiss albino mice.
Figure 16: Showing cumulative number of tumours in female Swiss albino mice.
nature and rest of the tumours were showing malignancy. Mouse skin is a complex dynamic organ, which produces a bewildering variety of tumours such as epithelial and mesenchymal tumours. These included keratoacanthomas, papillomas, squamous cell carcinomas, sebaceous adenomas, basal cell tumours dermofibromas and dermato fibrosarcomas. Characteristic features of some of these are as follows:

**Papilloma**

These tumours are composed of papillary or finger like processes. These tumours may occur in hairy or glabrous skin. Papillomas of hairy skin are produced by exfoliation of a type-1 keratoacanthoma. The whole mechanism of papilloma formation is that the cell proliferates by the neoplastic process and this produces an elongation and thickening of the epithelium, which is then thrown into a series of folds so as to accommodate itself in a given space. Increase foldings of this type ultimately produces a projecting branched sessile or pedunculated mass (Figure 17).

**Keratoacanthoma**

This is one of the common benign cutaneous tumour in man and experimental animals. It grows rapidly but at the same time, it regresses also.

Under electron microscope keratoacanthoma cells contain abundant intra cytoplasmic filaments, some rough endoplasmic reticulum and slightly enlarged nuclei with altered nucleoli. During the growth phase, nucleous become enlarged and comes to lie close to the nuclear envelope (Figure 18).

**Squamous cell carcinoma**

Squamous cell carcinoma shows well differentiated pleomorphism. Ulcerated squamous cell carcinomas invariably shows a brilliant red fluorescence while a benign epithelial tumour shows a weak red fluorescence.
Figure 17: Squamous cell papilloma in mouse skin.

Figure 18: Keratoacanthoma in mouse skin.
This red fluorescence is due to the presence of protoporphyrin which lies on the surface but not in the substance of the carcinoma (Figure 19).

**Basal cell tumour**

These resembles with the basal cells of the epidermis and rarely produced in the skin of rabbit and mouse.

The antitumour activity of I3C was evident in the present investigation as 100% tumourigenesis was not achieved in I3C supplemented animals during the entire course of experiment. Survival rate was high in I3C treated groups i.e. DMBA+I3C, I3C+DMBA, BaP+I3C and I3C+BaP. Topical application of I3C prior to or after carcinogen exposure leads to a significant reduction in the average number of tumours per mouse and tumour latency period during the course of study. Therefore, the results indicate that I3C has the potential to inhibit the development of neoplasia in experimental animals.

**Discussion**

The results indicate that I3C has the potential to inhibit the development of neoplasia in experimental animals. Brookes and Lawley (1964) studied the covalent binding of various PAHs in mouse skin with the relative carcinogenic potency of it. The metabolically formed intermediates involved in the binding of PAHs to epidermal DNA were identified as dihydrodiol epoxides (Sims et al, 1974). (+)-anti-B[a]P-7,8-dihydrodiol 9,10 epoxide [(+)-Anti-B[a]PDE] was identified as the specific stereoisomer responsible for the majority of the binding of B[a]P to DNA of stable adducts on the exocyclic amino group of deoxyguanosine (dG) [(+)-anti-B[a]PDE-dG adducts (Kooreda et al, 1978; Meehan and Calvin, 1979). Moschel et al (1977) and Bigger et al, (1983) observed that the DMBA-3,4-dihydrodiol 1,2-epoxides (DMBADEs) are the ultimate carcinogenic metabolites responsible for covalent binding of DMBA to DNA.
Figure 19: Squamous cell carcinoma in mouse skin (two different stages).
The dihydrodiol epoxides of DMBA bind to the exocyclic amino groups of both deoxyadenosine (dA) and deoxyguanine in DNA, a common property of dihydrodiol epoxides of non-planar PAHs (Dipple et al, 1983). Devanesan et al (1993) reported that 99% of all the PAH adducted purines in mouse skin exposed to DMBA are lost from the DNA by depurination. The activation of B(a)P in mouse skin has been reported to result in the formation of >70% depurinating (BaP-DNA) adducts (Rogan et al, 1993). Barrcelo et al (1997) observed that the broccoli constituent sulforapane being able to inhibit CYP2E1 isoform, which may offer chemoprevention against carcinogenic substrates of this enzymes. The antitumourigenic effect of dietary indoles indicates that I3C may inhibit the risk of spontaneous mammary and endometrial tumour formation and the incidence and/or the tumour multiplicity of chemically induced tumours of breast, forestomach, liver or tongue (Barrcelo et al, 1997).

Paolini et al (1995) reported that I3C has anticarcinogenic activity, when given throughout the experimental period. Several evidences suggest that isothiocyanates derived from the hydrolysis of methylsulphinyl alkyl glucosinolates found in the cruciferous family could protect against chemically induced cancer. Chung et al (1984) observed that isothiocyanates are potential inhibitors of carcinogenesis by N-nitrosopyrrolidine and N'-nitrosonornicotine. Reddy et al (1983) investigated that butylated hydroxytoluene (BHT), ethoxyquin, disulfiram, I3C, indole-3-acetonitrile, sodium selenite and α-tocopherol were non-mutagenic.

Dashwood et al, (1989) showed the successive increase in I3C, in a dose related decrease in AFB1-DNA binding, which results in a series of curves of decreasing slope, that shows pure anti-initiating activity by a natural anticarcinogen.
Tawfiq et al (1995) studied that sulforaphane (4-methyl sulphinyl) butyl isothiocyanate derived from the glucosinolate found in broccoli, blocks the formation of mammary tumours in Sprague Dawley rats treated with DMBA.

I3C in women has produced a beneficial effect through a modification of oestrogen metabolism. It seems that I3C may be a very useful preventive agent against hormone related cancers (Michnovicz and Bradlow, 1994). I3C exhibits anticarcinogenic effects in many experimental protocols that suggested the anticarcinogenic mechanism of I3C in mammals including induction of phase I enzymes, induction of phase II enzymes, inhibition of enzyme activation, inhibition of steroid hormone binding, scavenging of electrophiles and protection against oxidative damage (Bradfield and Bjeldanes, 1984; Danger et al, 1992; Fong et al, 1990; Shertzer and Sainsbury, 1991; Shertzer and Tabor, 1988).

A significant (p<0.01) decrease in hepatic AFB1-DNA binding caused by Brussels sprouts accompanied by increased hepatic intestinal glutathione S-transferase (GST) activities (Salbe and Bjeldanes, 1989). I3C shows inhibitory potency even at low level. AFB1-DNA binding suppressed by almost 95% at the highest I3C dose (Dashwood et al, 1988). I3C was most effective as a radical scavenger in the microsomal carbon tetrachloride (CCl4) initiated system by inhibiting lipid peroxidation in a dose dependent manner with 50% inhibition at 35-40 mM I3C (Shertzer et al, 1988).

The covalent binding to DNA and hepatic macromolecules to metabolites of indirectly acting carcinogens, BaP and N-nitrosodimethylamine and some indirect acting mutagens was prevented when mice were pretreated by gavage with I3C (Shertzer, 1984). Shertzer et al (1986) suggested that I3C inhibits formation of thiobarbituric acid reactive material in a dose dependent manner.
However, the species specific anticarcinogenic mechanism for this compound remains to be clearly defined. Melendez-Colon et al (1999) observed that genomic damage induced by stable dihydrodiol epoxide is responsible for cancer initiation in mouse skin by PAHs. But there is a need to study the mechanism of antitumour activity of I3C in the model of mouse skin.