Summary and Conclusions
5.1 SUMMARY

Cancer of the cervix is the seventh most common cancer in the world and the eighth most common cause of cancer deaths worldwide. It is the second most common cancer in women worldwide. In 1996, an estimated 5,24,000 new cases were diagnosed worldwide accounting for 10.5% of all new cancer cases in women. Developing countries account for 80% of the cases. In India, it is the most common cancer in women. There will be an estimated number of 1,00,000 cases of cancer of the uterine cervix in the country by 2001 AD, accounting for 1/4th of the cancer cases in women. The high incidence rates for the disease warrants attention towards its cure as well as prevention. In developed countries the incidence rates are declining due to extensive screening programmes for the disease.

Chemoprevention is an important strategy for cancer control. It involves administration of subtoxic doses of pharmacologic or natural agents that prevent the formation of carcinogen from precursor compounds, block DNA damage and arrest/reverse the progression of premalignant cells. The NCI (USA) has identified several agents for preclinical toxicology as well as phase II/III clinical efficacy trials for various cancers. For cancer of the uterine cervix 9-cis-retinoic acid has been identified for phase I trials while all-trans-retinoic acid, 4-HPR, β carotene, DFMO and folic acid are being tested for phase II/III clinical efficacy trials.

Animal studies have identified several compounds that show chemopreventive potential in chemically induced cervical carcinogenesis. The present study evaluates the modulation of 3-MCA induced cervical carcinogenesis by orally delivered Artemisia annua L. leaf extract, curcumin, aqueous suspension of Hippophae rhamnoides L. fruit, Piper betle L. leaf extract, aril of Punica granatum L. and Trigonella foenum-graecum L. seeds in Swiss albino mice.

Metabolic activation is the first step in the ultimate detoxification of most carcinogens, therefore any modulation in the activities of phase I and phase II hepatic xenobiotic enzymes will affect the ultimate fate of reactive electrophilic form of a carcinogen. Free radical damage plays an important role in tumor development. The
targets of free radical attack include proteins, lipids, carbohydrates and DNA. The antioxidant defense available in an organism provides the first line of protection against free radical damage. The effect of some modulators on mouse hepatic phase I and II xenobiotic detoxifying enzymes, and antioxidant defense system was also studied.

Swiss albino mice were maintained in the Animal facility, JNU in an air conditioned environment with a regulated 12 hr light/12 hr dark cycle. For cervical carcinogenesis study, the 8-9 week old animals were initially treated with the modulator for two weeks. Cervical carcinogenesis was then induced by inserting 600 μg MCA-impregnated thread in the uterine cervix of the animals. Treatment with the modulator was further continued for 90 days. After three months of post carcinogen exposure the animals were sacrificed and their cervical tissues fixed and studied histopathologically. For biochemical studies, the animals were orally treated with the modulator for 10 days (20 days for *Trigonella foenum-graecum* L. seeds). The biochemical parameters studied were cyt P< sub>450</sub>, cyt b< sub>5</sub>, GST, GPx and GR activities, and GSH content. The procedure of Omura and Sato (1964) was followed for cyt b< sub>5</sub> and cyt P<sub>450</sub> estimation. GST was estimated by the method of Habig et al. (1974), GPx and GR activities were studied by the procedure described by Paglia and Valentine (1967), and Carlberg and Mannervik (1975) respectively. The method described by Moron et al. (1979) was followed for sulphydryl group estimation.

5.1.1 Cervical tumor Incidence in Normal and Control Animals

The Swiss albino strain maintained at the Animal facility, JNU dose not show spontaneous tumor incidence in the cervix. Cervical carcinogenesis was induced in 8-9 week old females by insertion of 600 μg MCA-impregnated thread in the uterine cervix. The animals were sacrificed after 90 days of carcinogen exposure. The effective number of animals were taken as animals with the intracervical thread remaining intact and without any infection in the cervical area. The cervical lesions were assessed histopathologically. The tumor incidence values in different experiments varied from 71.43% (20/28 animals) to 75% (21/28 animals).
5.1.2 Modulatory Effect of *Artemisia annua* L. Leaf Extract

5.1.2.1 Tumor Modulation

Animals were treated with 12.5 mg and 15 mg/ kg b.wt. of aqueous leaf extract of *A. annua* L. The treatment was started 2 weeks prior to carcinogen exposure and was continued for 90 days post- carcinogen exposure. There was no effect on body wt. gain on treatment with the modulator. Histopathological analysis of cervical tissue revealed a 51.85% tumor incidence for the lower dose and 42.86% for the higher dose of the modulator. The values for the higher dose was significantly ($P < 0.02$) lower than the observed tumor incidence for the control (75%) group.

5.1.2.2 Biochemical Studies

Animals were treated with 5, 10, 15 mg/ kg b.wt. of the modulator for 10 days. The hepatic GSH content increased significantly ($P < 0.05$) at the two higher doses of the modulator. Cyt P$_{450}$ activity showed a significant increase at the 15 mg/ kg b.wt. dose. GST activity increased significantly ($P < 0.05$) at the 10 mg dose, and highly significantly ($P < 0.01$) at the 15 mg/ kg b.wt. dose. GPx activity showed a significant ($P < 0.05$) increase at the two higher doses while GR activity increased significantly ($P < 0.05$) at the highest dose of the modulator.

5.1.3 Modulatory Effect of Curcumin

5.1.3.1 Tumor Modulation

The animals were orally treated with 7 and 15 mg/ kg b.wt. curcumin for 2 weeks following which cervical carcinogenesis was induced by insertion of 3-MCA impregnated thread in the uterine cervix. Treatment was continued 90 days post- carcinogen exposure following which the animals were sacrificed and their cervical tissues examined histopathologically. The tumor incidence values obtained were 71.43%, 44.44% and 37.04% for the control (corn oil treated) group, lower dose and higher dose respectively. The decrease in tumor incidence was significant in both cases ($P < 0.05$ for 7 mg/ kg b.wt. dose and $P < 0.02$ for 15 mg/ kg b.wt. dose). Treatment with the modulator did not result in any loss in body weight gain of the animals. Also no mortality was observed in the modulator treated groups.
5.1.4 Modulatory Effect of *Hippophae rhamnoides* L. Fruit

5.1.4.1 Tumor Modulation

Animals were treated with 50 mg and 100 mg/ kg b.wt. of the modulator for 2 weeks following which cervical cancer was induced by insertion of MCA- impregnated thread in the uterine cervix of the animals. The modulator treatment was continued for 90 days post- carcinogen exposure. The animals were then sacrificed and their cervical tissues examined histopathologically. The tumor incidence values observed in the control group was 71.43%. The tumor incidence values observed for the lower dose and the higher dose of the modulator were 40.74% and 30.76% respectively. Both the values are significantly (P < 0.05 for 50 mg/ kg b.wt. and P < 0.02 for 100 mg/ kg b.wt.) lower than the tumor incidence value for the control group.

5.1.4.2 Biochemical Studies

Animals were treated with 25 mg, 50 mg and 100 mg/ kg b.wt. of the modulator for 10 days following which they were sacrificed and their hepatic tissues analysed for biochemical assays. The modulator induced the xenobiotic detoxifying enzymes as well as the antioxidant defense system. The cyt b\textsubscript{3} activity increased significantly (P < 0.05) at the highest dose while the cyt P\textsubscript{450} was induced at the two higher doses. GST was induced with all the three doses (P < 0.05 for the lowest dose and P < 0.001 for the two higher doses). GPx activity was induced at the two higher doses (P < 0.01). GR was also induced at the two higher doses of the modulator (P < 0.05 for 50 mg/ kg b.wt. and P < 0.01 for 100 mg/ kg b.wt. dose).

5.1.5 Modulatory Effect of *Piper betle* L. Leaf Extract

5.1.5.1 Tumor Modulation

The animals were treated with 10 mg and 20 mg/ kg b.wt. doses of the modulator for 2 weeks, following which cervical carcinogenesis was induced by insertion of 3-MCA impregnated thread into the uterine cervix of the animals. The modulator treatment was continued 90 days post- carcinogen exposure. The animals were then sacrificed and their cervical tissues examined histopathologically. The control (vehicle) treated group was observed to have a tumor incidence value of 75% (21/28 animals). The tumor incidence values for the lower and higher doses of the modulator were 62.96% (17/27 animals) and
57.14% (16/28 animals) respectively. The decrease observed is not statistically significant.

5.1.5.2 Biochemical Studies

Animals were given 1 mg, 5 mg and 10 mg/ kg b.wt. of Piper betle L. leaf extract by oral gavage for 10 days. They were then sacrificed and their liver tissues examined for biochemical parameters. Treatment with the modulator does not result in loss of body weight. The modulator does not induce/inhibit the hepatic xenobiotic detoxifying enzymes. The GPx and GR activities were observed to increase significantly (P < 0.05) at the 10 mg/ kg b.wt. dose.

5.1.6 Modulatory Effect of Punica granatum L. Aril

5.1.6.1 Tumor Modulation

Animals were given 50 μl and 100 μl/animal/day of the crushed aril of Punica granatum L. fruit by oral gavage for 2 weeks. Cervical carcinogenesis was then induced by insertion of MCA- impregnated thread. Treatment with the modulator was continued 90 days post- carcinogen induction. The animals were then sacrificed and their cervical tissues examined histopathologically. The control (vehicle treated) group showed a 73.91% (17/23 animals) tumor incidence. The tumor incidence values for the groups treated with the lower dose of the modulator was 75% (18/24 animals), and for the higher dose was 69.56% (16/23 animals).

5.1.6.2 Biochemical Studies

Animals were given 50 μl and 100 μl of the crushed aril of Punica granatum L. for 10 days. The animals were then sacrificed and their liver tissues assessed for biochemical parameters. Treatment with the modulator does not induce/inhibit the hepatic phase I and II xenobiotic detoxifying enzymes as well as the antioxidant system.

5.1.7 Modulatory Effect of Trigonella foenum-graecum L. Seeds

5.1.7.1 Tumor Modulation

Animals were given the modulator in diet. The control group was given pulverised standard animal feed and a known quantity of the modulator was added in the experimental groups to give the desired concentration. The doses given were 5% and 10%
modulator in diet. After 2 weeks of treatment with the modulator cervical tumors were induced in the animals by insertion of MCA- impregnated thread in the uterine cervix. Treatment with the modulator was continued 90 days post-carcinogen exposure. The animals were then sacrificed and their cervical tissues processed histopathologically. There was no loss in body weight gain in the modulator treated groups. The tumor incidence values observed were 73.91%, 70.83% and 65.22% for the control, 5% diet dose and 10% diet dose respectively. The modulator does not have any inducing/inhibiting effect on the cervical carcinogenesis in Swiss albino mice.

5.1.7.2 Biochemical Studies

Animals were treated with the 2% and 5% concentration of the modulator in diet for 20 days. The animals were then sacrificed and their liver tissues examined for biochemical parameters. The xenobiotic phase I enzymes are not modulated by the T. foenum-graecum L. seeds. GST activity was induced (P < 0.05) at the 5% dose of the modulator. GSH content was also observed to increase (P < 0.05) on treatment with the 5% dose. GPx and GR activities however were not modulated

5.2 CONCLUSIONS

1. Treatment with the modulators did not result in any loss of body weight in the animals. The groups treated with the modulators and inserted with carcinogen impregnated thread did not develop cancerous lesions in the cervical tissues. The modulators therefore did not have any deleterious effects at the doses used for this study.

2. Effect of Artemisia annua L. leaf extract:

a) Artemisia annua L. leaf extract (15 mg/kg b.wt.) has a chemopreventive effect on cervical carcinogenesis in Swiss albino mice.

b) The modulator induced the antioxidant defense system. It is also a bifunctional inducer of hepatic phase I and II enzymes.

c) Artemisinin and dihydroartemisinin present in A. annua L. leaf are cytotoxic to certain tumor cell lines. Also the drug detoxifying enzyme inducing property of camphor (a constituent of the essential oil) are well documented. In the present study the whole leaf was given was a modulator, therefore it is not possible to identify the active substance(s)
responsible for the chemopreventive effect. Further studies are required with regards to its active constituents and their role in chemoprevention.

3. Effect of curcumin:
   a) Curcumin (7 and 15 mg/kg b.wt.) has a chemopreventive effect on cervical carcinogenesis induced by 3-MCA in Swiss albino mice.
   b) Curcumin (diferuloyl methane) has antioxidant and anticarcinogenic properties. In this study, curcumin has proved to be an effective chemopreventor in cervical carcinogenesis elicited by chemical carcinogen. The chemopreventive efficacy of curcumin is related to its inhibition of arachidonic acid metabolism, ODC and PKC activity; its antioxidant property; and, modulation of xenobiotic detoxifying enzymes.

4. Effect of *Hippophae rhamnoides* L. fruit:
   a) *Hippophae rhamnoides* L. aqueous fruit suspension, at the doses 50 and 100 mg/kg b.wt. shows a preventive effect on the cervical carcinogenesis model.
   b) The modulator induces the phase I and II hepatic xenobiotic enzymes. It also induced the antioxidant enzymes and increased the GSH content.
   c) The berries are rich in carotene (30- 40 mg/ 100 gm), vitamin C (360- 2500 mg/ 100gm) and vitamin E (160 mg/ 100 gm). This may account for its tumor modulation property and, induction of antioxidant system and detoxifying enzymes. However, there may be other bioactive constituent(s) responsible for this action. The plant warrants further studies in this regard.

5. Effect of *Piper betle* L. leaf extract:
   a) *Piper betle* L. leaf extract at the doses 10 and 20 mg/kg b.wt. does not show any modulatory effect on cervical carcinogenesis.
   b) The modulator induces the hepatic GPx and GR activities. It also increases the GSH content in the liver tissue.
   c) Studies have reported the inhibition of chemically induced carcinogenesis in laboratory animals on oral feeding with *P. betle* L. leaf extract. The chief constituent of the essential oil of *Piper betle* L. leaf are eugenol, isoeugenol and hydroxychavicol. These compounds are known to be free radical scavengers and inducers of phase II enzymes. The constituents
of essential oil of *P. betle* L. leaf vary widely within the different cultivars in the country with eugenol varying from 10.9%-63.5%, isoeugenol from 5.2- 10.59%, and chavicol from 5.1- 16.7%. Seasonal variations are also present. Investigations are necessary to document the essential oil constituents of the betelvine types cultivated at different regions and seasons in India and respective analysis of their modulatory potential in carcinogenesis.

6. Effect of *Trigonella foenum-graecum* L. seeds and *Punica granatum* L. aril:

a) 2% and 5% of *Trigonella foenum-graecum* L. seeds in the diet does not modulate the cervical tumor incidence in Swiss albino mice. 50 µl and 100 µl/animal/day of *Punica* aril L. also does not have any modulatory effect on the cervical carcinogenesis model system.

b) *Trigonella foenum-graecum* L. (5% in diet) induced the GST activity and increases the GSH content. However the phase I and II enzymes are not modulated. *P. granatum* L. aril does not modulate the detoxifying enzyme system and the antioxidant defense system.

c) *Punica granatum* L. aril and *Trigonella foenum-graecum* L. seeds show estrogenic activity in laboratory bioassays. Phytoestrogens are diverse in their chemical structures and functions. Though they generally have a low potency in bioassays and a low affinity for estrogens receptors, they have recently emerged as an area of interest for chemoprevention studies due to the anticarcinogenic activity of some compounds. They are observed to have estrogen agonist/antagonist effects. The estrogenic activity of the modulators, their effect on estrogen responsive tissues and their action vis-à-vis the endogenous estrogen pool requires further examination.