SUMMARY AND CONCLUSION
Cancer of the uterine cervix, on a global basis, is the second most common cancer that can occur in women. Epidemiological studies have placed India among the countries that record very high cervical cancer incidence. The etiological factors responsible for the development of cervical cancer have been the subject of considerable speculation and several risk factors have been attributed for its incidence, the most important of which are (a) age at first intercourse (b) HPV infection (c) Multiple sexual partner (d) use of steroidal contraceptives (e) socioeconomic status (f) poor hygiene.

Since the introduction of oral contraceptive pills scientists have started suspecting the possible role of OC pills use in the increased risk of getting cervical cancer. Although the epidemiological studies have yielded equivocal results regarding the possible association of OC pill use and cervical cancer, a number of recent prospective studies have shown trends of increasing cervical cancer with pill use for longer duration.

The possible cervical cancer risk among pill users elicits two main questions (1) do the oral contraceptive pills induce cervical cancer among the pill users? (2) do the oral pills have any modulatory influence on the precancerous and cancerous lesions elicited by known or
unknown carcinogens? While the first problem is being adequately investigated, the second one has yet to receive sufficient epidemiological as well as experimental attention.

Injectable contraceptive steroids, like oral contraceptives, have also generated a lot of controversies around the world regarding their possible association with neoplasia which led to their ban in USA. Some epidemiological studies have suggested a possible increased risk of getting cervical cancer with the use of injectable contraceptive steroid. The available information regarding the tumor induction in some of the animals by MPA, a widely used injectable contraceptive steroid, treatment do not provide any reason to associate MPA use with increased risk of neoplasia. Moreover we do not have any information regarding the modulatory action of injectable contraceptive steroids on the process of cervical carcinogenesis.

Besides this the phase I and phase II drug metabolizing enzymes and the pathways responsible for biotransformation of xenobiotic and endobiotic chemical compounds have been found to be regulated or modulated by a large number of environmental and hormonal factors. Thus any substance with the potential of altering the phase I and II drug
metabolizing enzymes and glutathione level can make the host more susceptible or resistant to different xenobiotic and endobiotic chemicals including carcinogens.

Interaction of contraceptive steroids with liver drug metabolizing enzymes has been reported in a number of publications. However, various animals species, different experimental approaches and various contraceptive steroidal combinations have shown conflicting results.

In the present study an attempt has been made to evaluate the modulatory influence of oral contraceptive and injectable contraceptive steroids on (1) MCA-induced cervical carcinogenesis in mice (2) Phase I and phase II drug metabolizing enzyme and acid soluble sulphydryl level in the liver of mouse which can be summarized as follows:

1. **INDUCTION OF CERVICAL CARCINOGENESIS IN MOUSE**

Cervical carcinogenesis was induced by inserting 300 ug MCA-impregnated thread into the uterine cervix of mouse. After 30, 60 and 90 days of carcinogen thread insertion the percentage of animals with cervical cancer was 0.0%, 8.0% and 27% respectively. Incidence of hyperplasia and dysplasia were also observed.
2. MODULATORY INFLUENCE OF COMBINED ORAL CONTRACEPTIVE PILLS "OVRAL" (CONTAINING ETHINYLESTRADIOL AND NORGESTREL) AND "NORACYCLINE" (CONTAINING ETHINYLESTRADIOL AND LYNESTRENOL) ON METHYLCHOLANTHRENE-INDUCED CERVICAL CARCINOGENESIS IN MOUSE

In the present study three different doses of OVRAL and NORACYCLINE were used which are as follows:

**Ovral**

a) Dose $D_1$ (1/2000th of a pill) containing 0.025 ug of ethinylestradiol and 0.25 ug norgestrel;

b) Dose $D_2$ (1/200th of a pill) containing 0.25 ug of ethinylestradiol and 2.5 ug norgestrel.

c) Dose $D_3$ (1/20th of a pill) containing 2.5 ug of ethinylestradiol and 25 ug norgestrol.

**Noracycline**

a) Dose $D_1$ (1/2000th of a pill) containing 0.025 ug of ethinylestradiol and 0.5 ug lynestrenol;

b) Dose $D_2$ (1/200th of a pill) containing 0.25 ug of ethinylestradiol and 5 ug lynestrenol.
c) Dose $D_3$ (1/20th of a pill) containing 2.5 ug of ethinylestradiol and 50 ug lynestrenol.

O.C pill treatments were given for 30, 60 and 90 days, starting from the date of thread insertion, through oral route.

Placement of sterile cotton thread impregnated with bees-wax containing ~300 ug of MCA produces cervical tumors in 0.0%, 8.6% and 26% of the control mice (treated with vehicle only) respectively in 30, 60 and 90 days.

Modulation by "Ovral" Treatment

When animals, inserted intracervically with carcinogen containing threads, are treated with dose $D_1$ (1/2000th of a pill) and $D_2$ (1/200th of a pill) for 90 days, the tumor incidence drops down to 3.3% ($P < 0.05$) and 3.4% ($P < 0.05$) respectively. Treatment at these two dose levels for 60 days also shows a trend of reduction in tumor incidence. Treatment of the pill at $D_1$ and $D_2$ dose levels for 30 days did not show any effect on tumor incidence. Administration of the highest dose $D_3$ (1/20th of a pill) for 30, 60 and 90 days, on the other hand actually causes enhancement in the tumor incidence which is however not significant. All the three doses of the pill used in this study enhance the
incidence of hyperplasia significantly. Incidence of dysplasia does not show any definite correlation with exposure to different doses of the pill.

Modulation by Noracycline Treatment

Noracycline treatment at dose levels $D_1$ (1/2000th of a pill), $D_2$ (1/200th of a pill) and $D_3$ (1/20th of a pill) for 30, 60 and 90 days, shows different effect on cervical tumor incidence. When animals, inserted intracervically with carcinogen threads, are treated with dose $D_1$ for 90 days the tumor incidence drops down to 3% ($P < 0.05$). On the other hand administration of the highest dose $D_3$ for 60 and 90 days enhances the tumor incidence respectively to 54% ($P < 0.005$) and 63% ($P < 0.05$). However $D_2$ dose treatment for 60 and 90 days does not show any significant result but gives a trend of tumor inhibition. $D_1$ and $D_2$ dose treatment for 30 days do not show any effect while $D_3$ dose treatment for 30 days gives a tumor enhancing effect. $D_1$ dose treatment for 60 days shows a trend of tumor inhibitory action. Like Ovral, all the three doses of the Noracycline also enhance the incidence of hyperplasia significantly. Incidence of dysplasia does not show any definite correlation with different treatments.
3. MODULATORY INFLUENCE OF INJECTABLE CONTRACEPTIVE STEROID "MEDROXYPROGESTERONE ACETATE" (MPA) ON METHYLCHOLANTHRENE-INDUCED CERVICAL CARCINOGENESIS IN MOUSE

Methylcholanthrene (~300 ug) plus bees-wax-impregnated thread, when placed inside the canal of uterine cervix of virgin, female, adult mice, produces cervical tumors in 0.0%, 10% and 30% of mice respectively in 30, 60 and 90 days. Intramuscular treatment of MPA at the dose level of 50 ug/5th day, to the carcinogen thread inserted mice for 30, 60 and 90 days starting from the day of thread insertion produces cervical tumors respectively in 0.0%, 13.3% and 60.5% (P < 0.05) of mice. A significant increase in hyperplasia was also observed in the present study.

4. EFFECT OF COMBINED ORAL CONTRACEPTIVE PILLS "OVRAL AND NORACYCLINE" AND INJECTABLE CONTRACEPTIVE STEROID "MEDROXYPROGESTERONE ACETATE" ON PHASE I AND II DRUG METABOLIZING ENZYMES AND ACID SOLUBLE SULFHYDRL LEVEL IN MOUSE LIVER

Three different doses (explained in the previous sections) of the pills used in this study show different effects on Phase I and II drug detoxifying enzymes and acid soluble sulfhydryl level in the liver of mouse. All the three doses of the pills were given for 15 days through oral
route. The lowest dose of Ovral, $D_1$, which is equivalent to the human dose almost did not show any effect on cytochrome $b_5$ (Cyt.$b_5$) and cytochrome P450 (Cyt. P450). However, the other two doses, i.e. $D_2$ and $D_3$, which are higher than the human dose, showed a significant decrease in Cyt. $b_5$ while a significant decrease in Cyt.P450 was observed only with dose $D_2$. Glutathione S-transferase (GST) activity shows a significant fall with the treatment of highest dose ($D_3$) of Ovral while $D_1$ and $D_2$ doses did not show any change. Acid soluble sulfhydryl (SH) level was found to be elevated with all the three doses but failed to show statistical significance at higher dose $D_3$. Noracycline with a different progestogen component showed a trend different than that observed with ovral treatment in modulating the drug metabolizing enzymes and SH levels except Cyt.$b_5$, Cyt.P450 and SH groups with $D_2$ dose treatment, Cyt. P450 with $D_1$ dose treatment and GST activity with $D_3$ dose treatment. Noracycline treatment at $D_1$ and $D_2$ levels shows significant decrease in Cyt.$b_5$ while dose $D_3$ causes a significant enhancement. $D_2$ dose treatment of Noracycline shows a significant decrease while $D_3$ dose treatment shows a significant increase in Cyt.P450. All the three doses of Noracycline cause a significant decrease in GST activity. Only $D_2$ dose of Noracycline showed a significant enhancement of SH level.
A significant decrease in microsomal protein was observed with $D_1$ dose treatment of Ovral and $D_3$ dose treatment of Noracycline. $D_1$, $D_2$ and $D_3$ dose treatments of Ovral as well as $D_2$ and $D_3$ dose treatments of Noracycline show a significant enhancement in cytosolic protein.

MPA treatment at the dose level of 50 ug every 5th day for 30 days through intramuscular route decreases the Cyt.b$_5$ content significantly ($P < 0.01$) while Cyt.P450 remains unchanged. No significant alteration is observed either in GST activity or SH level. Cytosolic protein shows significant enhancement ($P < 0.01$).

The results obtained in the present study show the possible modulatory influence of contraceptive steroids, its different doses and combinations on the process of chemically induced cervical carcinogenesis as well as on different phase I and II hepatic drug metabolizing enzymes and acid soluble sulfhydryl level in mouse. It is not clearly understood how steroids and their different combinations achieve the tumor inhibitory or enhancing actions on the process of chemically-induced cervical carcinogenesis.

Effect of steroids on tumor incidence could be either due to its direct, net estrogenic or progestogenic action on
target cell or/and by modulation of the carcinogen metabolism either by altering the drug metabolizing enzyme system or competitive inhibition.

The ultimate effects of various combinations of estrogens and progestogens present in the hormonal contraceptives seem to be mediated by their overlapping antagonistic and synergistic biological effects in the metabolic pool of the animal or human body.

In the present study when the lower two doses (i.e. $D_1$ and $D_2$) of OC pills were given, the tumor inhibitory potentials of progestogen component (i.e. norgestrel present in Ovral and lynestrenol present in Noracycline), seem to be synergized by ethinylestradiol, while at higher dose level (i.e. $D_3$) the tumor inhibitory potential of progestogen component of the pills seems to be antagonized by ethinylestradiol which on its own has been reported to have tumor inducing and promoting action.

Different doses as well as combinations of oral contraceptive steroids in the present study display a wide range of modulatory effect on phase I and phase II drug metabolizing enzymes and sulfhydryl level in the liver of mouse. However the injectable contraceptive steroid "MPA"
brings about a decrease in Cyt.b5 only and a significant enhancement in cytosolic protein.

The overall findings of the present study suggest that oral and injectable contraceptive steroids have the potentials to modulate the process of chemically induced cervical carcinogenesis in mouse, depending upon the doses, combination and duration of use. It is interesting to note that the lower doses combination confer a protective effect.

Besides this the contraceptive steroids can successfully alter the drug metabolizing enzyme systems and acid soluble sulfhydryl level which can affect the susceptibility of an individual to the action of different xenobiotic chemicals including carcinogens.

Furthermore, experimental studies dealing with the exact mechanism of tumor inhibitory or enhancing action of contraceptive steroids as well as epidemiological studies, controlling every possible confounding variables, will give a better understanding of contraceptive steroids and their association with cervical cancer risk.