CHAPTER 6

SUMMARY & CONCLUSION
In the present study an approach has been made to investigate the abortive effect of the crude bark powder (CBP) of the plant *Dysoxylum alliarium* traditionally used by the women belongs to ‘Adi’ tribe of Arunachal Pradesh to control the population size of their domestic pets. *Dysoxylum alliarium* which is locally known as ‘Situ Payu’ among the Adi people, is a wild tall plant grown in the tropical rain forests of north east India. The investigation has started considering the information that the traditional use of the bark of the *Dysoxylum alliarium* contains compound(s) that can modulate the reproductive function in female by way of abortion. Thin layer chromatographic separation of the methanol extract CBP showed that the bark powder contains certain compounds which have similarity with estradiol-17β, which may affect the reproductive functions of the female rat.

The study has been carried out in vivo using female albino rats during cyclic and pregnant stage. Respective control females were maintained along with the CBP treated females in both the cyclic and pregnant stage. The bark of the plant was collected from the hank of mighty Siang river of East Siang district of Arunachal Pradesh. The dried bark powder was administered to the female albino rats through oral route during the cyclic stage and the early gestation period to determine the threshold dose. A dose of 500mg/kg/day was determined as the threshold dose by the trial and error testing of the bark powder to the females. The effects of CBP on ovarian and uterine histoarchitecture were studied using the normal cyclic females. The objective of the present investigation was to determine the abortifacient effect of females. Therefore, the threshold bark powder of *Dysoxylum alliarium* in rat during early pregnancy. Therefore, the threshold dose of CBP was administered the pregnant females in six different regimen from day 1 to day 15 of gestation. During this period the affect of the CBP has been studied on implantation sites, protein profile, and histological architecture of ovary and uterus as well as in the
expression of certain vascular endothelial growth factor (VEGF) in the uterus. Two hepatic marker enzymes for toxicological evaluation namely SGOT and SGPT were studied during the CBP treatment period of gestation. Moreover, histological studies of liver tissues have been carried out on day 15 of gestation following CBP treatment of 15 consecutive days from day 1 of gestation. Administration of the CBP on pregnant females from day 1 of gestation showed adverse effect on the process of implantation from day 6 to day 8 of gestation. The CBP at a dose of 250mg/ kg/day from day 1 of gestation reduced the number of implantation sites than the control, though it was not statistically significant. However, the administration of CBP in a dose of 500mg/kg/day from day 1 to day 6, 7 and 8 of gestation showed a greater reduction in the implantation sites compared to that of control. The females treated with CBP at a dose of 500mg/kg/day from day 1 to day 15 of gestation failed to give birth of the pups at the end of the gestation period, while the control females delivered the normal pups.

The CBP was administered to the adult cyclic females for three consecutive cycles to study the effect of CBP on structural organizations of reproductive organs: ovary and uterus. Oral administration of the CBP to the cyclic females at a dose of 500mg/kg/day induced noticeable structural changes of the uterus and ovary than that of control cyclic females. An increased in the number of antral and preantral follicles in the ovary of CBP treated rats was observed. However, the majority of the follicles found to be degenerated at different stages of development. Statistical analysis showed that the average number of degenerated antral follicle was 5.3 ± 0.33, which was significantly higher than the average degenerated follicle number 3.0 ± 0.57 of the control (data not shown) females. Nuclear pyknosis was observed in the nucleus of granulosa and theca cells of CBP treated ovary. This ovarian structural change of the CBP treated females have been accompanied with structural changes in the uterine
tissues. Extensive proliferation and branching of uterine luminal epithelium with endometrial hyperplasia were observed in the CBP treated rats’ uteri. However, total stromal tissue area was found to be decreased and also appeared loose in compared to control females’ uterine structure. With the decreasing stromal tissue area, number of endometrial gland has been found to be decreased in the CBP treated rat uteri.

To investigate the effect of the CBP in ovary and uterus during pregnancy, the histoarchitecture of the CBP treated ovary and uterus at gestation period had been studied. During this period, the uterine histology was carried out from day 4 to day 8 of gestation, while ovarian histology has been studied only on day 8 of gestation. Administration of CBP from day 1 to day 8 of gestation induced structural alterations in the ovarian histology. Multiple numbers of preantral and antral follicles were observed in the CBP treated females’ ovary. As noted earlier, the uterine histology was carried out in the implantation sites and/or inter sites of the uterine horns during this period of gestation to determine the pregnancy interrupting effect of the CBP. Oral administration of CBP for four consecutive days from day 1 to day 4 of gestation to the pregnant females induced structural changes in uterine tissues. The cells of the hypertrophied endometrial surface epithelia appeared multinucleated and ciliated in contrary to the thin columnar epithelial cells (LE) uniformly lined the uterine lumen (UL) of the control females. The endometrial glands of the control day 4 pregnant females showed distinct epithelial cell layers with a lumen, whereas the CBP treated glands showed the degeneration loosing nuclei of the epithelial cells and dissolution of the cellular membrane. The Day 4 of gestation which, has been considered as the window of embryo implantation in rodents is the most critical period for successful implantation of zygote. Alternations of the normal structure can be a challenge for the normal progress of
Implantation. Likewise the day 4, administration of CBP induced structural changes of the normal uterine histoarchitecture of day 5 and day 6 pregnant rats. Endometrial surface epithelial cell layers has been desquamated from the basal lamina in parts of the luminal epithelium on day 5 treated females. The decidual cells of the decidual zones became vacuolated and the stroma appeared loose in structure. The number of endometrial glands appeared to be more in comparison to that of the control rat uterus. However, the glands showed cellular degeneracy. Similarly, the luminal epithelium and glandular epithelium of day 6 CBP treated rats showed degenerated cells with vacuole formation following CBP treatment. Administration of CBP from day 1 to day 7 of gestation hindered the normal development of fetal maternal unit as well as the growth of the blastocyst. The embryo (blastocyst) has been failed to become elongated losing its normal development. The normal rodent embryo of the day 1-7 pregnant females became elongated developing the giant trophoblast cells with clearly stained nucleus. The treatment of CBP from day 1 to day 8 of gestation showed an impairment in the normal development of the embryo and as well as the degeneration of cells of decidual zones.

During the investigation a study had been carried out localize the expression of vascular endothelial growth factor-C (VEGF-C) in the uterine tissues especially in the fetal-maternal interface during this periimplantation period. The study of the VEGF was made to investigate whether the CBP can exert its abortifacient effect by modulating the expression of the growth factor. The expression pattern of the growth factor was studied in the pregnant uterine tissues during the gestation period of day 4 to day 8. In the control rat uterus on day 4 of pregnancy the growth factor VEGF-C is strongly expressed in the endometrial surface epithelium, secondary decidual zone and myometrial zone of serosa. Moderate stained for VEGF was
noticed in the undifferentiated stromal zone near the inner myometrium layer of the control
day 4 female and strong positivity for VEGF observed in the both primary and secondary
decidual zones. However, after the administration of CBP from day 1 to day 4 of gestation
intensity of the growth factors expression was appeared lesser in the uterine epithelium and in
the decidual zones. On the day 5 of gestation of control females the apical part of the
epithelial cells of the endometrial surface epithelium showed strong expression of VEGF. The
strong expression of the VEGF also observed in the blood vessels of the myometrial zone and
the compact decidual zone below the endometrial epithelium. Administration of CBP affects
the expression pattern of VEGF in uterine tissues on day 5 of gestation than that of control.
Unlike control tissues, intense expression of the VEGF appeared in the cytoplasm at apical
and basal part of the epithelial cells, as well as its basal lamina. However, lesser intensity of
the VEGF expression showed in the desquamated uterine luminal epithelial cell layers. The
embryo and the decidual zones of the primary and secondary decidua zones showed intense
expression of the growth factor (VEGF) on day 6 of gestation in control rats. An alternation in
the expression of VEGF has been observed in the fetal maternal unit on day 6 of CBP treated
females' gestation. Expression pattern of VEGF in the vacuolated decidual cells and in the
myometrial vascular plexus was lesser than the respective control of the day 6. On the day 7
of pregnancy, control females' embryo exhibited expression of VEGF in a lesser intensity
than the decidual cells. While the myometrial region with the dilated vascular plexus showed
a stronger expression of the growth factor. The decidual cells of both the primary and
secondary decidual zones expressed the growth factor. On the contrary the expression of
VEGF has been restricted only in certain decidual cells and degenerating trophoblast cells
surrounding the embryo in the CBP treated females. The vascular plexus of the myometrial
region lost the expression of VEGF indicating vascular degenerate on in the treated fetal-maternal unit. The fetal maternal unit on control females' on day 8 of gestation in showed expression of VEGF in the embryonic tissues, the decidual zones, growing vascular plexus in the myometrial region and the uterine luminal epithelium in intersites. Administration of CBP from day 1 to day 8 of gestation results with the decreased in the intensity of VEGF expression by the embryonic cells. Vacuole formation in the cytoplasm of the decidual cells lead to lost their normal characteristics and at the same time the cells also showed decreased expression of the growth factor. However, the uterine luminal epithelium in intersite region of the uterus showed the positive stain for VEGF expression.

The uterine protein of the pregnant rats during the periimplantation period was studied by SDS-PAGE. The objective was to determine the effects of CBP on uterine protein profile during day4 to day8 of gestation. The studied showed variations in the protein expression pattern in the control and CBP treated female rats' uteri. The protein separated on the day 4 of pregnancy in control rats showed multiple number of protein bands in a wide range molecular weight ranging from 6.5kDa to 205kDa. The uterine proteins having molecular weight 60kDa has been found to express in the highest intensity, while the protein molecule with highest molecular weight (205kDa) expressed in the lowest intensity in the control day 4 pregnant rat uterus. Administration of the CBP from day 1 to day 4 of gestation showed expression of two high molecular weight proteins in between 205kDa and 66kDa molecular weight range in higher intensity than that of the control rats' uterine proteins. The protein molecule with a high molecular weight approximately 205kDa has found to be intensely express in the CBP treated rat uterus. At the same time two low molecular weight proteins having molecular weight approximately 35kDa and 17kDa respectively were expressed in very low intensity
following CBP treatment. On day 5 of gestation, two protein molecules with molecular weight 55kDa and approximately 66kDa showed intense expression with the regular expression of other protein molecules in control females. Another protein of approximately 125kDa, which has been stimulated to express in the CBP treated rats' uterus was not observed in the control day 5 pregnant rats. The protein molecule having molecular weight 29kDa expressed in doublets, but in lesser intensity following CBP treatment. On the day 6 of gestation, it has been observed that the protein molecules having molecular weight > 66kDa have been expressed in distinct bands in both the control and CBP treated females. In addition, administration of CBP induced expression of a high molecular weight protein (> 205kDa). A low molecular weight protein having molecular weight 29kDa expressed in lower intensity following CBP administration compared to that of the control females. The control females showed expression of low molecular weight proteins (< 55kDa) in higher intensity than that of the comparatively high molecular weight proteins (> 55 kDa) on day 7 of gestation. Protein molecule having molecular weight 66 kDa, showed a strong expression on day 6 of gestation in control females' uterus, expressed in lower intensity on control day 7 of gestation. Administration of CBP from day 1 to day 7 of gestation increases the expression intensity of high molecular weight proteins (> 205 kDa). The protein profile of both the control and CBP treated females on day 8 of pregnancy showed a total of eight protein bands found to be expressed in the control uterine tissues. However, administration of CBP stimulated the expression of new protein having molecular weight approximately 35kDa. Another protein molecule with molecular weight approximately 55kDa was observed to be up regulated following CBP treatment but appeared with less intensity on day 8 pregnant control rats' uteri. One newly synthesized low molecular weight protein having molecular weight
approximately 16kDa has been found to be expressed following CBP treatment in treated rat uterus.

Following the separation of uterine protein the expression of vascular endothelial growth factor (VEGF) in the pregnant rat uterus has been studied by western blot during day 4 to day 8 of gestation using anti VEGF antibodies. The data of the VEGF expression of the CBP treated uterine samples from day 4 to day 8 of gestation were compared with that of the controls. The expression pattern of the VEGF appeared almost similar in both control and CBP treated females in the day 4, 5 and day 6. However, the intensity of the bands increased in the CBP treated day 7 and day 8 females as compared to the control day 7 and day 8 females.

The biochemical analysis of hepatic enzymes (SGOT & SGPT) and the histological studies of liver tissues in the control and CBP treated female rats have been carried out to determine the toxic effect (if any) of the bark of the *Dysoxylum alliarium*. The results of the serum profile of SGOT and SGPT during the gestation period showed an increased in the enzymes level in the treated samples than the control as the day of CBP treatment increases. With the increasing days of gestation, the serum SGPT and SGOT levels in CBP treated females’ increases to a maximum level of 28.6 ±2.23/U/ml and 41.16 ±1.4U/ml respectively on day 8 of gestation than that of control values of 13.96 ±1.37U/ml (SGPT) and 20.41±1.12U/ml (SGOT). A long term treatment of CBP continuously for 15 days (day 1 -15 of gestation) increased the level of SGPT to a maximum level of 35.0 ±1.97U/ml (control 14.3±1.97U/ml) and SGOT to the level of 43.5 ±1.29U/ml (control 20.91±0.97 U/ml).

Administration of CBP consecutively for 15 days from day 1 of gestation showed mild effects on the histological structural organization of hepatic lobule. Infiltration of neutrophils
observed following CBP administration indicating cellular necrosis. In the present investigation, it was observed that the administration of crude bark powder of the *Dysoxylum alliarium* orally in pregnant and cyclic females exerted an anti reproductive effect on the reproductive performance of female albino rats. The CBP impaired the normal histoarchitecture of the reproductive organs as well as retarded the normal growth of the developing embryo. The structural anomalies of the reproductive organs possibly have effect on the normal endocrine function of the organs itself and so in the proper functioning of reproductive processes. The CBP also imparted its effect in the molecular level modulating the normal protein profile of the uterine tissues. Retardation of normal development, alternation of biochemical composition and hormonal imbalances certainly lead to the failure of implantation and/or untimely early abortion causing interception of normal of pregnancy. The nature of the potential active component present in the bark of *Dysoxylum alliarium* and its mechanism of action in the reproductive organs need further study.

***************