CHAPTER V

SUMMARY AND CONCLUSION

The present study established the estrogenic nature of the root extract of *Careya arborea* Rox. The study identified a few active principles in the methanolic root extract after a thorough chemical analysis using sophisticated techniques. As this plant has a very old history of traditional use as contraceptives in the NE region of India, it obviously demanded a proper investigation. From the viewpoint of human use to control fertility it was very important to evaluate their antifertility activity and the actual cause and nature of this type of activity. Local information and knowledge obtained on bioactive phytochemicals used by different communities and population groups in different localities in the Regions have not been addressed properly because of the weak interaction with the people of those remote areas. As India’s NE region is one of the Biodiversity Hot-spots in the World, it is now high time to explore the bioactivity of these indigenous medicinal plants so that a new horizon may be opened up in the field of research in this area. Keeping this in mind, in the present investigation, the fertility/antifertility activities of the root extract of *Careya arborea* Roxb. were determined *in vivo* in albino mice of either sex.

The summary of the present studies was:

1. The methanolic root extract of the plant did not show any sign of acute toxicity up to the dose level 5000mg/kg bw in adult mice.
2. The present study provides a direct indication of the antifertility activity of the root extract of the plant *Careya arborea* Roxb. The crude methanolic root extract of the plant exerted strong antifertility activity at the dose level 1000mg/kg bw up to the end of the 2nd mating (22 days after 1st mating). However, at the dose level 500mg/kg bw, the plant showed 100% antifertility activity by the end of the first mating. The effects exerted by the root extracts were reversible.

3. In the animals treated with higher dose of the root extract (500mg/kg bw), estrus phase was prolonged, but in the animals treated with lower dose of the root extract (250mg/kg bw) the diestrus phase was prolonged. The changes induced by higher dose of the root extract were comparable with the changes induced by 17β-estradiol at the single dose 10μg/kg bw, though the intensity of the changes was higher in the latter. Changes in the normal reproductive cyclicity were the indicative of hormonal changes inside the body induced by the root extract.

4. In the treated animals, pregnancy was affected. The duration of gestation period, litter size (number of litter produced) and birth weight of litters were reduced significantly. Though the gestation period and the litter size were decreased after exposure to the root extract, but it could not conclude as adverse effect, as the birth weights of the litters were increased.

5. In the female offsprings of the extract-exposed mother, there was a delay in the opening of the vagina that was associated with a delay on the onset of estrus. In contrast, the female offsprings of *E2* exposed mothers exhibited an early vaginal opening associated with an early onset of estrus. The female offsprings of the root extract-exposed
mothers exhibited normal estrous cycle, whereas in the female offsprings of E\textsubscript{2} exposed mothers, the estrus phase of the cycle was prolonged. On mating, all these female offsprings from both the root extract and E\textsubscript{2} exposed mothers mated normally and delivered healthy pups of normal size and shape. As the female offsprings of the extract exposed mothers did not reveal any adverse effects in their reproductive performance, therefore it was confirmed that the bioactive compounds of the plant were neither bioaccumulated nor biomagnified inside the body and could not transfer from one generation to the next.

6. Uterine glycogen content was decreased significantly in both adult cyclic female mice and ovariectomized mice after exposure to the root extract and E\textsubscript{2}. On the other hand, uterine protein content was increased significantly in both adult cyclic female mice and ovariectomized mice after exposure to the root extract and E\textsubscript{2}. The increased level of uterine protein that was associated with the hyperplasia of the endometrium and hypertrophy of the luminal epithelium might prevent the attachment of the blastocyst. The fall of optimum levels of glycogen in uterus might cause inadequate nourishment to both ova and spermatozoa resulting in failure of implantation.

7. The total amounts of uterine DNA and RNA contents were increased at 24hrs after exposure of the root extract for single day only; but at 24hrs after continuous exposure for 14 days, the total amounts of uterine DNA and RNA contents were decreased significantly in the uterus of both adult cyclic female mice and ovariectomized mice. The changes were analogous with the changes induced by E\textsubscript{2}. The increased level of uterine DNA and RNA contents after single day exposure indicated the estrogenic nature of root
extract. The low level of uterine DNA and RNA contents after continuous exposure might not be adequate to meet the degree of requirement for translation process necessary to facilitate implantation.

8. The total serum cholesterol was reduced significantly in both adult cyclic female and ovariectomized mice after exposure to the root extract and E_2. By decreasing serum total cholesterol, the plant exerted its beneficial effects on the estrogen related heart diseases.

9. Exposure to the root extract impaired the normal histoarchitecture of the ovary. The ovary appeared to contain less number of mature graffian follicle and corpora lutea. Degeneration of corpus luteum with frequent hemorrhage and presence of cystic follicle were also observed in some animals. The changes were comparable with the changes induced by E_2.

10. The root extract also evidently induced prominent histological changes in the uterus of adult cyclic mice. The endometrium was thickened with the hypertrophy of the luminal and glandular epithelium. Focal hyperplasia and hyperchromation were also observed. The endometrial glands showed degenerative changes leading to the functional aberrations of the uterus. The changes were equivalent with the changes induced by E_2.

11. Pronounced morphological changes were observed in the uterus of ovariectomized mice after exposure of the root extract and the changes were analogous with the changes induced by E_2. The structural changes include folded lumen, enlarged endometrial glands and hyperplasia of the luminal epithelium.
12. In the cervix of adult cyclic female mice, glandular development was greater after exposure of the root extract and \( E_2 \) in comparison with the control. Hyperplasia of the squamous epithelium with increased keratinization was evident in these animals. The vaginal epithelium showed increased thickness and keratinization after exposure to the root extract. Changes were comparable with the changes induced by \( E_2 \).

13. Exposure to the root extract resulted in the thyroid follicular hypertrophy in adult cyclic female mice and was similar with the thyroid follicular hypertrophy induced by \( E_2 \). Histological changes of the ovary, uterus, cervix, vagina and thyroid contributed together to exert the antifertility activity of the plant.

14. At the dose level of 500mg/kg bw, the root extract induced mild degenerative changes in the liver. Karyomegaly was the most frequent encountered abnormality in all the treated mice. Focal necrosis, karyorrhexis and karyopycnosis were also restricted in some areas. The changes were similar with the liver tissue of the \( E_2 \) treated animals. Reduction in the activity of the enzyme SDH, as demonstrated by the intensity of the Nitro-BT reaction in cryostat sections of the liver tissues from the treated animals, was also evident. However, the enzyme LDH, G6-PDH and G6-Pase revealed no changes in their activities. The changes were similar with the liver tissue of the \( E_2 \) treated animals. As the plant induced mild degenerative changes in the liver, caution should be taken for its application.

15. The uterotrophic bioassay in both immature female mice and adult ovariectomized mice showed positive response. In immature mice, the root extract at the concentrations of 50mg and 100mg were able to induce early onset of estrus that was persistent. The
total numbers of cornified cells were increased with the increase in days of treatments. The uterine wet-weight also increased in these animals in comparison with the controls. These changes were comparable with the changes induced by $E_2$ at the concentration 5µg/kg bw. In ovariectomized mice, the root extract were also able to induce estrus that was persistent at the concentrations of 250mg and 500mg/kg bw. Total numbers of cornified cells were increased with the proceeds of the days of treatments. The uterine wet-weight was increased in these animals in comparison with the controls. Morphological size of the uterus was also increased. These changes were comparable with the changes induced by $E_2$ at the concentration 10 µg/kg bw though the intensity of the changes was higher in $E_2$ treated animals. The positive uterotrophic response in immature and ovariectomized mice clearly indicated the estrogenic nature of the root extract.

16. The male reproductive health was affected by the exposure of the root extract. There was a marked reduction in testis and epididymis weight after 14 days of treatment in comparison with the control. Histological observations of testes in the root extract treated animals reflected the reductions in the sperm concentration inside the seminiferous tubules. The leydig cells were also atrophied and degenerated in some areas of all the treated mice. These changes were comparable with the changes induced by $E_2$. By decreasing the weight of testis and epididymis, by decreasing sperm concentration inside the seminiferous tubules and causing atrophy of leydig cells, the plant exhibited its antispermatogenic effect.
17. After exposure to the root extract, there was a reduction in the food intake of adult male, female and ovariectomized mice. However, their body weights remain unaffected. In contrast, after exposure to $E_2$, both food intake and body weight were decreased significantly. However, in immature mice, body weights were increased significantly in comparison with the controls. Reduction in food intake indicated the loss of appetite that might be indicative of disturbances in the physiology of digestion. As there were no changes in the body weights after exposure of the root extract, therefore its administration was safe.

18. The root extract of the plant also affected the reproductive behavior of the adult animals of either sex and the ovariectomized animals. The adult male showed a reduction in the mounting and lordosis behavior, whereas the adult female rapidly accepted the mounts by the male. Lordosis was also increased in these adult female animals. In the ovariectomized animals, receptivity and proceptivity were restored towards the end of the treatment. However in the $E_2$ treated animals these changes were observed much earlier than the extract treated animals. Furthermore in the $E_2$ exposed ovariectomized mice, lordosis was observed in 50% of the animals. The sexual behavior observed in the treated animals may not be readily observable in humans.

19. Based on the rodent uterotrophic assay, the degree of the estrogenic activity of the root extract in vivo was in the order of $E_2 >$ root extract.

20. There was no significant difference between the control and the vehicle control animals for all variables.
21. Analysis of the root extracts of the plant *Careya arborea* Roxb. showed presence of phenolic compounds. The phenolic compounds were identified as: hydroquinone, resorcinol, syringic acid, vanillic acid; 4-hydroxy-3- (p-hydroxyphenyl)-5,7-dimethoxy-coumarin; 3-(o-hydroxyphenyl) coumarin; 3', 4,4', 7-tetra methoxy-, trans-2, 3, cis-2, 4- (+)-3-flavanol and 2-methoxy dibenzofuran. Presence of these phenolic compounds might be responsible for the antifertility activity of the plant.

**CONCLUSION**

The studies described in this Thesis confirmed the antifertility effects of the root extract of the plant *Careya arborea* Roxb. From the results of the present investigation, it has been confirmed that the root extract influenced virtually every aspect of mammalian reproduction in albino mice of either sex via effects on the morphology and physiology of reproductive organs and sexual behavior. The effects exhibited by the root extract were dose dependent and were analogous with the effects induced by E2. The present study established the estrogenic activity of the root of the plant *Careya arborea* Roxb. Chemical analysis of the root extract for the first time confirmed the presence of a few estrogenic chemicals in this plant.

**FUTURE STUDIES**

The present studies have established that the root of the plant, *Careya arborea* Roxb. induced strong antifertility activity and it has a potential source of many phenolic compounds. However, a number of important issues remain to be investigated. There should be a thorough investigation on the estrogenic nature of the individual phenolic compound present in root portion. The other parts of the plant might have hormonal
activity. Moreover, the other hormonal activities (like estrogenic, antiestrogenic, androgenic and progestogenic etc.) of the root portion have to be investigated. As the herbal preparations are safe, comparing with the synthetic steroidal contraceptives, the plant Careya arborea Roxb. may open up a new era in the field of phytoestrogen research that may lead to discovery of novel estrogens in plants and herbs of NE region of India. However, more bioassay-guided in vivo and in vitro assays and elucidation of the detailed molecular mechanism of the action of these phenolic compounds are yet to be investigated. Establishment of a bioactivity-screening Centre in the North Eastern Region of India will certainly help this type of research in this region of Biodiversity Hot-spots of the World.

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