CHAPTER EIGHT
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8.1. Summary and Concluding remarks

Various plant preparations have been tried to induce changes in the reproductive system and thereby causing infertility. However very few of them have been the subject of scientific investigation.

The present investigation was undertaken with a view to study the changes in male reproductive system of albino rats. after the administration of Agnus castus berries extract, leading to possible infertility. The study was carried out with reference to body weight, organ weights and histological changes in testes and accessory reproductive organs.

Agnus castus berries mother tincture (hereafter called Agnus extract) was administered intraperitoneally to the male albino rats of proven fertility for five weeks. Control rats during this period received only vehicle (Double distilled water). Histological alterations were studied by routine Haematoxylene - Eosin technique. All the studies were carried out at an interval of a week over a period of 5 weeks.

1) Body weight :

Agnus castus extract treatment decreases weights of the body treated animals.

2) Alterations in testis :

A) Wet weight :

Agnus caused reduction in the wet weight of testis. This reduction was more or less gradual.

B) Histology :

It was found that Agnus extract affected testes and induced aspermatogenesis. The tunica propria and basement membrane was thickened, which probably become a barrier for the entry of nutrients into the seminiferous tubules. The diameter of the tubules reduced. The tubules lost their shape towards the end of the treatment. All spermatogenic cell types except spermatogonia were affected. Primary and secondary spermatocytes were highly susceptible cell types to the treatment. The damage noted in the spermatids was seems to be of secondary nature. Damaged spermatids gave rise to damaged spermatozoa. Multinucleated giant cells were formed. The cells from the germinal epithelium, because of the treatment, sloughed off from their places and appeared in the lumina of tubules. There appeared many spaces, vacuoles in the germinal layers because of the sloughed off cells.

These cells alongwith spermatozoa formed cellular debris, which in the course of further treatment disintegrated and lumina became practically empty. Sertoli cells showed vacuolization. Leydig cells were affected occasionally showing some atrophy, in the final phase of the treatment. The aspermatogenesis induced by Agnus extract seems to be due to progesterone like substance. It affect directly on the gonial elements of seminiferous tubules and secondaly its antiandragenic nature, revealed by Leydig cell atrophy, causing reduction in the androgen level and thus affecting the prccess of spermatogenesis.

3) Alterations in epididymis :

A) Wet weight :

Both caput and cauda epididymis showed reduction in the wet weights. It is mainly due to decrease in the number of spermatozoa in the lumina of epididymis.

B) Histology :

Administration of Agnus affected epididymal histo-architecture. The epithelium showed reduction in the height. Tabular lumina were without normal sperms and contained

cellular debris. The cellular debris consisted of spermatocytes, spermatids, giant cells, cytoplasmic masses and degenerating as well as agglutinated spermatozoa. The cellular debris seems to be derived mainly from damaged testes and probably alterations in epithelial secretory activity. In majority of tubules the lumina became empty towards the end of the treatment. Fate of the cellular debris is not known, but it seems that it is expelled through the remainder of the duct system. The basal lamina thickened and interstitium was widened.

4) Alterations in Vas deferens :

A) Wet weight :

Agnus administration resulted into reduction in the wet weight of vas deferens.

B) Histology :

Agnus extract affect the mucosa and caused reduction in the height of epithelium and its folds. Stereocilia of the epithelial cells decreased in height. Epithelium detached from lamina propria at certain places. Because of the

decrease in mucosal folds the lumen get widened. The lumen contained large number of broken fragments and cellular components which were received from damaged testes. Normal spermatozoa were not observed in lumen towards the conclusion of the treatment. The advential remained unaltered. Muscularis also did not show significant change.

5) Alterations in Seminal vesicles :

A) Wet weight :

Agnus treatment resulted in gradual reduction in the wet weights of seminal vesicles. The decrease in weight seems to be mainly due to depleted secretory activity of the organ.

B) Histology :

The height of the epithelial cell (Mocosal epithelium) reduced. The mucosal folds which were highly arborized reaching up to the centre of the lumina in controls, got reduced in height and arborization. Epithelium showed signs of degeneration. The secretion was reduced; few lumina were without any secretion. Lamina propria and muscular coat showed changes only in the second phase of the treatment.

All these changes in seminal vesicles seems to be due to direct action of the extract accomponied by probable depletion in the androgen level.

6) Alterations in Prostate gland :

A) Wet weight :

Prostate gland showed decrease in the weight after the treatment of Agnus extract.

B) Histology :

Epithelial cells lining the acini showed signs of degeneration and reduction in height. Secretory activity of the cells also affected hence there was reduction in secretion. Acinar lumina showed scanty or in some no secretion at the end of the treatment. Decreased secretion of prostate must have also affected volume of semen; which might have been resulted into decrease in the fertility.

7) Fertility :

The fertility tests reported here showed that the fertility of the Agnus treated rats was reduced to 31 % only.

8.2. PLAN OF FUTURE WORK :

Agnus castus berries extract definitely, shown to be induced alterations in the male reproductive organs. This present investigation opens several avenues for further research on the effects of Agnus on the male reproductive system. Some ideas for such work are listed below :-

1) In the present investigation only histological alterations are studied enzymes (Lysosomal and non-lysomal), mucosubstances, lipids and proteins play important role in physiology of reproduction. Optimum level of these metabolites is necessary for the normal process of reproduction and fertility. Therefore it is planned to study alterations in these metabolites in the reproductive system of Agnus extract treated rats in future work.

2) Agnus extract seems to interfere occasionally in the structure and functioning of Leydig cells. From such changes the idea of possible depletion in androgenic level is projected. This conclusion is drawn from some indirect observations. Therefore a direct investigation of bioassay of androgen in treated rats is highly desired to confirm or modify the above conclusion. In the present investigation only histological studies are carried out with reference to

the Leydig cells. A histochemical study of steroid dehydrogenases in the Leydig cells of the treated rats will give a better information on their androgenic state.

3) The pituitary and adrenal glands are the sources of gonadotropins and sex hormones. No attention is paid to the alterations in these glands. Hence a study of pituitary and adrenal glands in Agnus treated rats is necessary.

4) Agnus induced aspermatogenesis. Whether the spermatogenesis returns to normal when the treatment is stopped ? This aspect of reversibility has to be investigated.

5) Mode of administration of Agnus extract in the present investigation, was of intraperitoneal injection. The most acceptable and desirable mode of administration of drug, inducing infertility, should be oral. Hence it is necessary to find, whether the Agnus extract shows same aspermatogenic potencies if administered orally as is evidenced in intraperitoneal injection.

6) The Agnus extract induced azoospermia, causing alterations in testes and epididymes. To confirm azoospermia epididymal sperm count should be noted. Therefore sperm count is to be studied.

While concluding the present M.Phil. dissertation; the author would like to state that the present work is by no means complete. He is fully aware of his short comings. Though maximum efforts were made to complete this dissertation, some aspects of male reproduction were not explored due to certain limitations of existing laboratory conditions and stipulated time limit.

The author feels gratified that he has made a detailed histological study of male reproductive organs during administration of Agnus castus extract and made original contributions which have yet not been reported. The author wishes to carry out an extensive research project which will include the above mentioned shortcomings.