

CHAPTER - FIVE

SUMMARY AND CONCLUSION

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5.0 Introduction

The world is facing with a critical problem^{of} ever increasing population. In order to check the population explosion, large number of socio-medical remedies have been tried and are being tried presently wide variety of contraceptive methods have been made available such as surgical termination of pregnancy, many mechanical devices and use of drugs for fertility control in man. Most of the earlier contraceptives tried were naturally occurring synthetic drugs. For a long time human female was a target of trial, but due to more complexity in the reproductive physiology of females as compared to that of males, now a days, the male have become main target of new inventions. Many contraceptives are also being tried with experimental male animals to produce a safer and effective measure to achieve male sterility, which should be an effective reversible male contraceptive. A critical review of available literature showed that several naturally occurring and chemical substances have been tried as potential a spermatogenesis, but they are not suitable for human use due to their toxic side effects on physiology of different organs. Therefore scientists are in search of an effective male contraceptive of plant origin without side effects.

The present investigation was undertaken with an idea of exploring Oscimum sanctum (Tulsi) for its effect on alterations in male reproductive tract. This study was carried out with reference to alterations in total body

weight, wet weight of organs, tubular diameter and alterations in histoarchitecture of male reproductive organs in albino rats.

O.sanctum commonly called as “Tulsi” or “holybasil” is a well known sacred plant of Hindus. The leaves of O.sanctum contain few essential oils and have some pharmacological properties like antifungal and antibacterial, antiulcerogenic, antipyretic, expectorant, stimulant, radiation protective, diaphoretic, antispermatogenic, and also used to cure fever. In a view of the fact that O.sanctum has been reported to possess antifertility activity by some workers in this field, the present study was undertaken to elucidate the effect of O.sanctum on reproductive tract of ^{male} albino rats.

5.1 Materials and Methods.

Fresh leaves and soft stems of O.Sanctum were selected from nearby areas. Fresh leaves were washed, ground and mixed with wheat flour to the extent of 10% and fed to the experimental animals along with pelleted diet and water orally. The control group of animals were also maintained which received only pelleted diet and water. The experiment was carried out for 120 days with an interval of 24 days. Alterations in total body weight, organ weight and histological alterations in testes, caput and cauda epididymes, seminal vesicles, prostate gland and Cowper's gland were recorded. Three males were sacrificed at an interval of 24 days from each

control and experimental groups upto 72 days. The histological alterations were studied by routine Haematoxyline-Eosine (H & E) technique. Changes in tubular diameter of testis and epididymes were recorded with the help of oculometer.

After 72 days treatment, the administration of Tulsi leaves was stopped upto 120 days of experiment to check recovery if any in all the male reproductive organs of albino rats.

5.2 Observations

5.2.1 Alterations in body weight: -

The body weights of all the control and experimental animals were seen to increase slowly during the period of experiment.

5.2.2 Wet weights of organs:-

O. sanctum showed a significant decrease in the wet weights of all the tulsi treated experimental animals in general.

5.2.3 Alterations in histoarchitecture of organs

1) Testes: -

Tubular diameter of seminiferous tubules in testes was decreased considerably in experimental rats.

Histologically, a number of alterations were noticed at different stages of the treatment. Tunica propria and basement membrane of seminiferous tubules get thickened and detached at the end of treatment.

O. sanctum induced number of spermatogenic elements in the seminiferous tubules except spermatogonia. The spermatocytes and spermatids were affected severely and under went degenerative changes such as vacuolisation in cytoplasm and nucleus of spermatogenic cells, appearance of ill defined spaces in between the cells and origin of multinucleated giant cells in the lumen of the seminiferous tubules. Large amount of cellular debris was found in the lumen of tubules which contained degenerated spermatids and spermatozoa. Sertoli cells were very little affected. Leydig cells were also affected which resulted into the functioning of other accessory sex organs in male albino rats. The over all seminiferous tubular elements were shrunken resulting into increased oedomatous interstitium. Thus O. sanctum showed direct aspermatogenic effect on testis.

2) Epididymes: -

Tubular diameter of both caput and cauda epididymal tubules was reduced in O. sanctum treated rats than those of control groups.

Histologically similar alterations in both caput and cauda epididymes were observed. O. sanctum affected the basal lamina, secretory epithelium, luminal sperms and interstitial connective tissue. Basal lamina was affected at later periods of treatment. Epithelial cells showed degenerative changes like vacuolisation in cytoplasm, appearance of pycknotic nuclei and reduction in the height of epithelium and stereocilia present towards the adluminal surface. The number of luminal sperms went

on decreasing with the duration of dose in caput epididymes but the population of denatured sperms and spermatids along with cellular debris accumulated in the lumen of cauda epididymal tubules after 48 days treatment which might have induced a little increase in its weight.

3) Seminal Vesicle: -

Histologically, the overall picture of seminal vesicle in O.Sanctum treated rats was distorted. The plant caused patchy degeneration of different parts of seminal vesicle. Muscular coat was reduced considerably, lamina propria was degenerated followed by degeneration and resorptive changes in the secretory epithelium which ultimately reduced the amount of luminal secretion leading to formation of empty lumina in the seminal vesicles. This may affect the pH and contents of seminal plasma in rats leading to high mortality of spermatozoa and functional sterility in males.

4) Prostate gland: -

Histology of O.sanctum treated experimental rats showed considerable atrophy and degeneration of prostatic alveoli, alveolar epithelium and interstitial stroma. This may lead to decreased volume of semen with functional sterility in male albino rats.

5) Cowper's gland: -

Histoarchitecture of O.sanctum treated Cowper's gland in experimental animals also showed degeneration of alveoli, degeneration of

secretory epithelium with increase in the interstitial connective tissue and decrease in eosinophilic secretion in the lumen of alveoli.

5.3 Concluding remarks

The results based on all the observation of O.sanctum treated experimental rats confirmed the antifertility effect of O.sanctum in albino rats. It induces testicular a-spermatogenesis probably by arresting at spermatocyte and spermatid level. Which has resulted into decrease in the number of luminal sperms.

Degenerative changes in the spermatogenic elements led to appearance of ill defined spaces and vacuoles in the cells of seminiferous tubules. Arrest of spermatogenesis is probably due to arrest of mitosis which resulted into formation of pycknotic nuclei and multinucleated giant cells in the lumen of seminiferous tubules.

Degeneration of Leyding cells at later periods of treatment might have caused decrease in testosterone levels which evoked degenerative and antifertility effects on accessory reproductive organs.

Decreased number of spermatogenic cells and spermatozoa has led to loss in weight of all the reproductive organs of present study.

The reduction in fertility hence is attributed to direct effect of O.sanctum which inhibits the release of androgen stimulating hormones of FSH and LCSH . These changes evoked degeneration of testicular and accessory reproductive gland elements leading to infertility in males.

5.4 Recovery Tests

In the present investigation, the leaves of O.sanctum were administered upto 72 days in experimental animals and then administration was terminated upto 120 days of experiment. The body weights, tubular diameters and histological alterations in all the male reproductive organs under consideration were studied for confirming the recovery of alterations observed if any.

The body weights of all the experimental animals showed similar increase in values more than those of 72 days treated rats as well as their control group.

The organ weights of all the experimental animals interestingly showed reversible changes. The loss in weight of organs due to degenerative effects ^{was} stopped after termination of O. Sanctum treatment upto 120 days and the organ weights increased considerably to match a little with those of their control groups.

Histologically, after termination of treatment from 72 days upto 120 days, the structure of testes, epididymes and other accessory reproductive organs showed interesting recovering changes.

Testicular elements like seminiferous tubules showed increase in diameter of tubules normal development of spermatocytes and spermatids

with more viable sperms in the lumina. Leydig cells also became normal which ultimately may have caused recovery of remaining sex organs.

Epididymes both caput and cauda also showed regenerative changes in the epithelia, secretory cells, in the height of stereocilia which has increased to more or less like normal. The lumina of epididymal tubules which were empty in 72 days treated rats again became filled with normal functional sperms and maturing spermatids.

Seminal vesicles also showed regeneration of secretory epithelium with increased secretion in the lumen. The muscle layer also showed regeneration of damaged muscle fibres.

Prostate glands and Cowper's glands being most sensitive functional accessory male reproductive organs also showed a good deal of recovery changes after termination of the treatment from 72 days upto 120 days of the experiment.

This suggests that O.sanctum can be explored as a suitable and reversible male contraceptive agent.

5.5 Plan and scope for future work

The present investigation opens several avenues for further research on O.scimum sanctum leaf extract induced alterations in the testis and accessory male reproductive organs. Some ideas for such future work are listed below.

- 1) From the present investigation it seems that O.scimum sanctum leaves interfere occasionally in the structure and functioning of Leydig cells. From such changes the idea of possible depletion in androgenic level is projected in the present investigation. This conclusion is drawn from some indirect observations. Hence a direct investigation of bioassay of androgens 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 idea on the androgenic status.
- 2) In the present investigation, the of O.sanctum leaf induced alterations in testes and other accessory reproductive organs are tested for reversibility of the effect. These recovery tests are made only once at the end of experiment after termination of treatment from 72 to 120 days. Whether these recovery effects were graded should be checked at different intervals of time. These recovery tests shall be confirmed by biochemical and histochemical studies on several protein and enzyme contents of the organs.
- 3) The changes in seminal vesicles, prostate glands and Cowper's glands of O.sanctum treated rats can be explored further with respect to the amount of secretion contents biochemically as well as histochemically.

- 4) There is a scope for investigation of changes in the sperm count made from testes and epididymes of O.sanctum treated rats as compared to those of their control groups to check the percentage of sterility induced by the plant. The fertility potencies of the epididymal spermatozoa can be compared with those in the final ejaculate.
- 5) To get a general picture of possible changes in the physiology of other organs induced due to the administration of O.sanctum, further studies on biochemical, histological and histochemical alterations in liver, intestine and kidney etc of O.scimum treated experimental rats at different duration of dose a teronological study of various organs in the body.
- 6) In the present investigation, the histological alterations are studied only under light microscope. The observations can be explored by application of more enhanced sophisticated techniques like
i) Observation of testicular cell details of all the spermatogenic cell types by electron microscopy, ii) Separation of testicular mitochondria, microsomes, lysosome and nuclear reactions by ultracentrifugation and subsequent biochemical estimation of various components will be made. iii) Abnormalities in DNA of different cell types by cell fractionation and gel electrophoresis are also suggested, iv) Occurance of some new proteins and disappearance of some necessary proteins for spermatogenesis etc. can be studied further.

- 7) It is known that the fixed oils extracted from Oscimum sanctum contain unsaturated fatty acids like palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid. Very little literature is known about the chemical composition of leaf extracts of O.sanctum and its effect on male reproductive tract of albino rats. Now in the present investigation it is observed that feeding leaves of O.sanctum show antifertility effect on male reproductive tract of albino rats but the various component of leaf extracts should be isolated and their action on various male reproductive organs can be studied further.
- 8) Finally, O.sanctum induced sterility in males should be confirmed by fertility tests by mating the pretreated males with normal females of proven fertility. It will be interesting to study the reproductive behaviors of male animals like grooming, pursuit, mount, Intromission and ejaculation.