CHAPTER-FIVE
SUMMARY

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5.0 Introduction

The study of the reproducive system has assumed special importance in our country as well as in the world, because of the problems of an ever increasing population. In order to check the population explosion, various sociomedical measures have been taken up. Contraceptive measures to prevent pregnancy are widely employed for the controlled reproduction and a planned family all over the world. Conception is prevented by a number of ways. Many chemicals have been used to supress spermatogenesis for the control of fertility in the laboratory animals but were unsuitable for human use due to toxic side-effects. Attention has therefore been focused on plant material.

The present investigation was undertaken with a view to study alterations in the male reproductive system of rat after the administration of Plumbagin. The study was carried out with reference to alterations in body weight, wet weight of organs, tubular diameter, histology, a lysosomal enzyme-acid phosphatase and a nonlysosomal enzyme-alkaline phosphatase in the male reproductive system of albino rat.

Plumbagin, an active principle from the root extract of the plant Plumbago zeylenica was administered intraperitoneally to the experimental rats. The control rats received vehicle Tween 80 + saline. All the rats were 100 days old, weighing about 120 to 140 g each. The experiment was carried over a period of 72 days with an interval of 12 days. The histological changes were studied by routine Haematoxylene-Eosine (H-E) technique. Changes in tubular diameter of testis and epididymis were measured with the help of occulometer. The enzymatic studies of acid phosphatase and alkaline phosphatase were carried out by employing the bioassy techniques.

5.1 Alterations

5.1.1 Alterations in body weight

The body weight of the experimental rats was elevated slightly during the experiment as well as at the end of the experiment.

5.1.2 Alterations in testis

i) Wet weight - Plumbagin caused a significant reduction in the wet weight of testis. The reduction was gradual and duration dependent.

ii) Seminiferous tubular diameter - Seminiferous tubular diameter decreased in the experimental rats.

iii) Histology - The Plumbagin treatment caused definitive changes at testicular level. A number of alterations were noticed at different stages of the treatment. The tunica propria and basement membrane of seminiferous tubules got thickened and appeared collasped towards the end of the treatment. The thickened tunica propria and basement membrane possibly become a barrier for the entry of nutrients into the seminiferous tubules leading to the testicular alterations. Plumbagin seemed to affect most of the germinal epithelial cells. Practically all spermatogenic cells except the spermatogonia were affected. Spermatocytes were found to be highly susceptible. Formation of clear spaces between the germinal epithelium and vacuolization in the cells were noticed. Spermatogenesis of damaged spermatids led to the formation of damaged spermatozoa. Alterations in seminiferous tubules included appearance of giant cells. The cellular debris formed in the lumina thus contained the spermatogenic elements which were sloughed off from the germinal epithelium. Sertoli cells were little affected. Leydig cells were affected to some extent. The cells were shrunken and the cytoplasm was scanty.

At the final stage of the treatment majority of seminiferous tubules were empty. The aspermatogenesis caused by Plumbagin seems to be due to the direct effect on the cellular components of the seminiferous tubules along with an occassional effect on the Leydig cells.

iv) Acid phosphatase - The acid phosphatase exhibited an increase in the activity at the end of experiment as compared to the control activity.

v) Alakaline phosphatase - The enzyme activity was decreased after the Plumbagin treatment.

5.1.3 Alterations in epididymis

i) Wet weight - A significant reduction in the wet weight of caput and cauda epididymis was observed. This decrease seems mainly due to the reduction in the number of spermatozoa in the epididymal lumina.

ii) Tubular diameter - Tubular diameters of both caput and cauda epididymis were gradually decreased in Plumbagin treated animals.

iii) Histology - The Plumbagin administration seemed to affect basal lamina, epithelium, luminal contents and interstitium of epididymal tubules. The basal lamina was thickened and eosinophilic. The height of the epithelial cells and stereocilia was also reduced. The alterations in the epithelium consisted of oval spaces, vacuolization and reduction in size. Epithelium of the cauda epididymis showed distinct infoldings at the 72nd day of the treatment. Majority of the tubular lumina were devoid of sperms. The lumina of both caput and cauda contained broken fragments of sperms, cellular debris, giant cells and immature germ cells. It appears that the cellular debris is mainly derived from the damaged testes. The interstitium was widened.

iv) Acid phosphatase - At the end of the experiment the acid phosphatase activity increased both in caput and cauda epididymis.

v) Alkaline phosphatase - There was a decrease in caput epididymis while there was an inrease in the cauda epididymis.

5.1.4 Alterations in vas deferens

i) Wet weight - The Plumbagin administration caused a significant reduction in the wet weight of vas deferens.

ii) Histology - The Plumbagin administration caused changes in muscularis, mucosa and luminal contents. It induced reduction in musculature with empty spaces in it. Mucosa showed eosinophilia and mucosal folds of epithelial layer were reduced. Epithelial cells and stereocilia were reduced in height and got degenerated. Lumen was large and consisted of a large number of broken fragments and cellular components which were sloughed off from the testis during the course of Plumbagin treatment. The lumen was without any normal spermatozoa.

iii) Acid phosphatase - The acid phosphatase exhibited a decrease in the activity.

iv) Alkaline phosphatase - There was a decrease in the alkaline phosphatase activity.

5.1.5 Alterations in seminal vesicle

i) Wet weight - Plumbagin caused a decrease in wet weight of seminal vesicle.

ii) Histology - Muscular coat was reduced. Lumina propria was degenerated.

The height and arborization of mucosal folds got reduced. The epithelium showed eosinophilia. It got degenerated and showed a number of vacuoles. Majority of lumina were empty while some showed a very little secretion.

iii) Acid phosphatase - At the end of the experiment the acid phosphatase activity was decreased.

iv) Alkaline phosphatase - There was an increase in the alkaline phosphatase activity.

5.1.6 Alterations in prostate gland

i) Wet weight - Plumbagin caused a reduction in wet weights.

ii) Histology - The Plumbagin administration induced reduction in the epithelial cell height and secretion. It also induced vacuolization and degeneration in the epithelial cells. Almost all acini were without any secretion. Interacinar tissue was widened.

iii) Acid phosphatase - The activity increased after the treatment of Plumbagin.

iv) Alkaline phosphatase - The activity exhibited an increase after the Plumbagin administration.

5.1.7 Alterations in Cowper's gland

i) Wet weight - The present work demonstrated reduction in the wet weights of Cowper's gland.

ii) Histology - Plumbagin induced changes in the histological structure of Cowper's gland. It caused reduction in cell height and degeneration of the

epithelium. Interacinar connective tissue was increased, muscles were more thickened and eosinophilic. The secretion was also reduced.

iii) Acid phosphatase - At the end of the experiment the acid phosphatase activity was increased.

iv) Alkaline phosphatase - The alkaline phosphatase activity exhibited a decrease in Plumbagin treated rats.

5.1.8 Fertility

The fertility test indicated that Plumbagin induced 100 % infertility in the experimental male albino rats.

5.2 Concluding remarks

The results of the present study show that Plumbagin inhibits spermatogenesis as well as alters the functions of epididymes, vas deferenses, seminal vesicles, prostate and Cowper's glands.

Decrease in the tubular diameters of seminiferous tubules and epididymal tubules is due to the lack of germinal epithelial cells as well as spermatozoa.

The testes exhibit variable degrees of spermatogenic arrest mainly at the spermatocytes stage. The damaged spermatocytes, may be giving rise to the damaged spermatids. The spermeogenesis of the damaged spermatids may be leading to damaged spermatozoa. Disorganization, sloughing of germinal epithelial cells and giant cell formation are the common features of the damaged tubules. The Sertoli cells are affected little. The Leydig cells are occassionally altered.

After the Plumbagin treatment the cellular heights of epididymes, vas deferenses, seminal vesicles and prostate glands are decreased. The lumina of epididymes and vas deferenses are devoid of the spermatozoa. A decrease in the wet weights of the testes and accessory reproductive organs is also evident.

The most obvious explanation for the aspermatogenic effects of Plumbagin administration would be that Plumbagin had a direct effect on the testes, primarily on the germinal epithelial cells of the seminiferous tubules with lesser effect on the Leydig cells. Occasional atrophy of Leydig cells probably results in the less secretion of androgens further enhancing the aspermatogenesis.

Loss in the wet weights of different organs corresponds to the absence of spermatozoa as well as less secretion. The decrease noticed in the epithelial cell heights of epididymis, vas deferens, seminal vesicle and prostate is suggestive of sensitiveness of androgen dependent structures towards the Plumbagin treatment. These are suggestive of lesser androgen level due to Plumbagin.

A general increase is observed in the acid phosphatase in the testis and accessory reproductive organs except vas deferens. This increase is competent with the lytic and degenerative alterations observed in testis and accessory reproductive organs.

Elevation of acid phosphatase of epididymis is indicative of change in sperm disposal and resorptive function as a result of which the epididymal function gets altered. Increased acid phosphatase activity of the testes, epididymes, prostate and Cowper's glands is suggestive of onset of cell damage since acid phosphatase activity is intimately associated with lysosomes.

Alkaline phosphatase activity is decreased in the testis which possibly suggests interference in the transport of substances into the seminiferous tubules.

The decrease in the alkaline phosphatase activity in case of caput epididymis, vas deferens and Cowper's gland might be due to less secretary activity. In cauda epididymis towards the end of the experiment a slight increase in the alkaline phosphatase activity was observed. It seems that the Plumbagin treatment causes a slight increase which may be due to the stored and damaged spermatozoa.

Reduction in the fertility rate is due to the direct effect of Plumbagin on germinal epithelial cells and partly on the Leydig cells causing probably less androgen secretion which in turn would have caused reduction in the secretion of accessory glands leading to infertility.

In conclusion, it can be suggested that Plumbagin has gonadotoxic effects.

5.3 Plan of future work

The present investigation opens several research ideas for further research on the Plumbagin induced alterations in the male reproductive system of rat. Some ideas are listed below :

i) From the present investigation, it appears that Plumbagin causes a number of alterations in the male reproductive system. From such alterations the idea of a possible depletion in androgenic level is projected. This conclusion is drawn from some indirect observations. Hence a direct investigation of androgen level in Plumbagin administered rats is highly desired. In the present

investigation only histological studies are carried out with reference to Leydig cells. The histochemical as well as biochemical studies of steroid dehydrogenase in the Leydig cells of the Plumbagin administered rats will give a better information on their androgenic level.

ii) In an ideal contraceptive the idea of reversibility is most important. Aspermatogenesis was evident during the Plumbagin treatment, whether spermatogenesis returns back to normal when the treatment is stopped ? This question of reversibility has to be investigated.

iii) Plumbagin leads to aspermatogenesis, a depletion in the number of sperms, decrease in secretion of epididymis and other organs. Optimum level of secretion of accessory reproductive organs is needed for sperm maturation and survival. At the same time there is a necessity of optimum level of sperm number for the normal process of fertilization. The Plumbagin administration causes alterations in testes and epididymes probably leading to azoospermia. Confirmation of azoospermia is possible only with the epididymal sperm count. Hence it is necessary to study the sperm count.

iv) In the present investigation no attention is paid to the alterations in the pituitary and adrenal glands. These are the sources of gonadotropins and sex hormones. Hence a study of pituitary and adrenal glands in Plumbagin treated rats is necessary.

v) Mucosubstances, lipids as well as proteins play an important role in the physiology of the reproduction. Optimum level of these metabolites is essential for the normal process of reproduction and fertility. So it is planned to study alterations in mucosubstances, lipids and proteins in the reproductive system of the Plumbagin treated rats in future work.

vi) In the present investigation only one lysosomal enzyme-acid phosphatase is studied. Other lysosomal enzymes like β-glucuronidase, esterase, ATPase, succinic dehydrogenase, hyluronidase, etc. also play a significant role in the process of reproduction. In the present investigation the enzymes are studied only biochemically. A study of other lysosomal enzymes by applying biochemical and histochemical techniques will throw more light on Plumbagin alterations in the enzymes.

vii) No attention is given to the side effects of Plumbagin. Hence there is much scope for further studies in this matter too.

viii) The black and white microphotography gives idea of only histological alterations. The coloured photography would have given better results especially of nuclear and cytoplasmic stainings.

The present work is limited and aim was to study effects of Plumbagin on the male reproductive system. The aim is achieved partially with the histological and biochemical studies. 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, stipulated time limit and paucity of financial grants.

The dissertation was carried out for the degree of M.Phil. which is time bound. The author is also conscious of the fact that this work is in no way compelete here, and wishes to carry out an extensive research project which will be including the above lacunae.