INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

CHEMOSTERILIZATION INDUCED BY INTRATESTICULAR INJECTION OF CALCIUM CHLORIDE ($CaCl_2$) - A TOOL FOR POPULATION CONTROL

Soumendra Nath Karmakar* and Shyamal Kanti Das

Post Graduate Department of Physiology, Krishnath College,
Berhampore, Murshidabad, West Bengal, Calcutta, India.

ABSTRACT

Present study evaluates the effect of intratesticular calcium chloride ($CaCl_2$) injection on reproductive system of male albino rats. The effect has been observed on various reproductive parameters. Besides insignificant increment in body weight, the testicular weight and epididymal weight as accessory sex organ have been decreased significantly in dose treated groups. Sperm count and sperm motility have also been reduced significantly in experimental animals as the serum concentration of testosterone has also been drastically reduced. In response of that, FSH has been increased in both dose treated groups while serum concentration of LH is increased only in high dose treated groups. On the contrary metabolic parameters such as serum glucose and protein remain unaltered significantly but altering the serum cholesterol level significantly only in high dose treated group. Metabolic marker enzymes such as SGPT and SGOT have not been changed significantly in dose treated groups comparing to their respective control group. Histological structure of testis has been altered in experimental animals showing disintegrated seminiferous tubules and hampered spermatogenesis.

Keywords: Chemosterilization, androgenesis, intratesticular injection and spermatogenesis.

1.INTRODUCTION

Economic growth of a society largely depends on many factors. Population size is a major regulating factor of it. Besides this, large scale development also depends on the number of population. As the scientific improvement generally goes towards the necessity of a society, nowadays various scientists show their interest to keep on their experiment to search out new tools to combat against the problem related to overgrowth of population. Since many years, surgery was mainly employed as an effective permanent method of sterilization. But this method has not been supported by all socio economic groups of our society. Due to some reasons like its costliness, post operative hazards, expert surgeons, surgical equipments and infrastructure, this method has discarded by vast population of society. In this context it can be mentioned that more than one third of American couples use sterilization[1]. This trend was observed in Great Britain also, over 20% of men at the age group of around 40 years have undergone sterilization operation[2]. In Spain and Italy sterilization rates are very low[3]. On this background scientists are now interested to search few chemicals as parallel method to surgery. Experiments have been employed with various chemical with different types of application on varieties of animals. During 60 days injection in cat testis, it was showed complete necrosis and replacement by fibrous tissue with very low sperm count[4]. Intratesticular injection of calcium chloride on male cat is a well tolerated and extremely effective method of chemical sterilization[5]. Fluoride administration hampers the reproductive function of male rabbit and its effect is proportional to the duration of fluoride exposure[6]. Atrazine adversely affects amphibian larval development. Atrazine exposed males suffered from depressed testosterone, suppressed mating behavior, reduced spermatogenesis and decreased fertility[7]. Intratesticular injection of calcium chloride in Black Bengal goats (C. hircus) is effective and ecological for male sterilization without chronic stress and may be implied as simple alternative method of surgical castration[8]. Ciprofloxacin has the toxicological effects on reproductive system in male rats[9]. Single intratesticular injection of calcium chloride in male stray dogs is effective on male reproductive system and may be used as substitution of surgical castration[10]. Oral administration of retinoic acid receptor antagonist reversibly inhibits spermatogenesis in mice with a failure of spermatid alignment, sperm release and loss of germ cells into lumen[11]. Chemo-surgical blockage of sperm transport with intra-

epididymal injection of calcium chloride causes reduced sperm output without depressing libido in rams[12]. Excess chromium exposure is associated with various pathological conditions including reproductive dysfunction[13]. Polychlorinated biphenyl (Aroclor-1254) induced in leydig cell steroidogenesis and antioxidant system[14]. Administration of an inhibitor of the testis specific isozyme of the bromodomain and extraterminal subfamily of epigenetic reader proteins called bromodomain testis-specific protein (BRDT) exerts reversible suppression of spermatogenesis in mice without obvious side effects[15]. Fluoride contamination of drinking water can disrupt male gametogenesis and steroidogenesis and induce testicular oxidative stress[16]. It was suggested that lithium chloride administration may have association with significant adverse effects on testicular activities[17]. The aim of this observation is to evaluate the effect of intratesticular injection of $CaCl_2$ on male albino rat and its potency as chemo-sterilizing agent.

2.MATERIALS & METHODS

2.1.Animal selection, care and grouping

Adult (90±10 days) male albino rats of Wistar strain were taken for this experiment. Animals were maintained as per National guidelines and protocols. Animals were housed in clean polypropylene cages and were maintained in a controlled environmental temperature (22±2˚c) in an animal house under a photoperiod of 12 hours of light and 12 hours of darkness with free access to water. Animals were fed on standardized normal diet (20% protein) which consists of 70% wheat, 20% gram, 5% fish meal powder, 4 % dry yeast powder and 1% oil and water ad libitum. Rats were equally divided into three groups (n=12). Initial body weights of all the rats were recorded. Animals of group-I were treated as control group and this group was treated with single intratesticular injection of 0.5 ml normal saline/100 gm/rat in both testes. Animals in Group-II were treated with single intra testicular injection of 10 mg $CaCl_2$/100 gm body weight/rat in 0.5ml distilled water equally injected in both testes. Group III, treated with single intra testicular injection of 20 mg $CaCl_2$/100 gm of body weight/rat in 0.5ml distilled water equally injected in both testes. Group II and group III were treated as low dose group and high dose group respectively.

2.2.Preparation of calcium chloride solution

Calcium chloride solution was prepared according to the method of Das S K[18]. Solution was prepared with distilled water of 0.5 ml/100 gm of body weight containing 10mg of pure calcium chloride. This solution was injected in the animals of low dose treated group (group-II). Another dose of calcium chloride was prepared in the same manner which contained 20mg of calcium chloride for injecting in the animals of high dose treated group (group-III). The animals of all the groups were treated for 30 days.

2.3.Animal treatment, sacrifice and measurement of parameters

After completion of 30 days of treatment, final body weights of all the rats were taken and the rats were anaesthetized one after another with anaesthetic ether and blood was collected directly from hepatic portal vein and allowed to coagulate. Clear serum was collected and stored in 20˚c for enzyme assay. Testis of each rat was dissected out and treamed off adipose tissues and weights were taken. One testis from each rat was processed for histology and 5µ thick sections were taken and stained with haematoxylene and eosin for further observation. After sacrifice, the weight of cauda portion of epididymis of equal length was taken. Then it was cut and kept in 1ml diluents at 37˚c. After scattering it, sperms were dispersed into the buffer solution and it was taken for the count of sperm and its motility through the process of Majumder and Biswas[19]. Serum Glutamate Pyruvate Transaminase (SGPT) and Serum Glutamate Oxaloacetate Transaminase (SGOT) were measured of all the control and experimental animals through the process of Kind and King[20]. Serum glucose was measured using the standard kits. The serum protein was estimated by Biuret method with a standard curve of BSA. Hormonal level like testosterone, FSH and LH in serum of all the animals were estimated with the help of ELISA method. Serum cholesterol was also measured with the help of standard kit.

2.4.Statistical analysis

Finally results were compared with the respective controls with the help of student's 't' test (Das 2005)[21] to generalize the effect of intratesticular injection of $CaCl_2$ on reproductive system of male albino rat model.

3.RESULTS

3.1.Body weight

Body weight is a common but meaningful parameter. Effect of any drugs or such things is generally reflected through this parameter. So it is obvious to measure body weight in this present study. Experiment showed that intra testicular injection of calcium chloride did not hamper the general growth

of the animals inferenced by insignificant change in body weight gain between all the experimental groups.

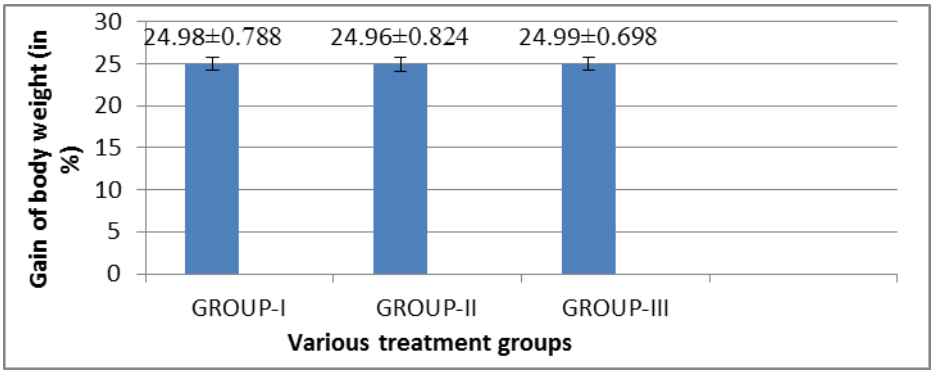


Fig. 1: Comparison of net gain of body weight of rats treated with $CaCl_2$ of different doses and respective controls. Values are mean±SEM (in %), n=12 rats in each group

3.2.Testicular weight

Testicular weight is an important parameter to measure in this study because direct injection of calcium chloride has been given to testis of experimental animals. Testicular weight has been reduced significantly ($p<0.001$) in both the experimental groups in comparison with those animals of control group.

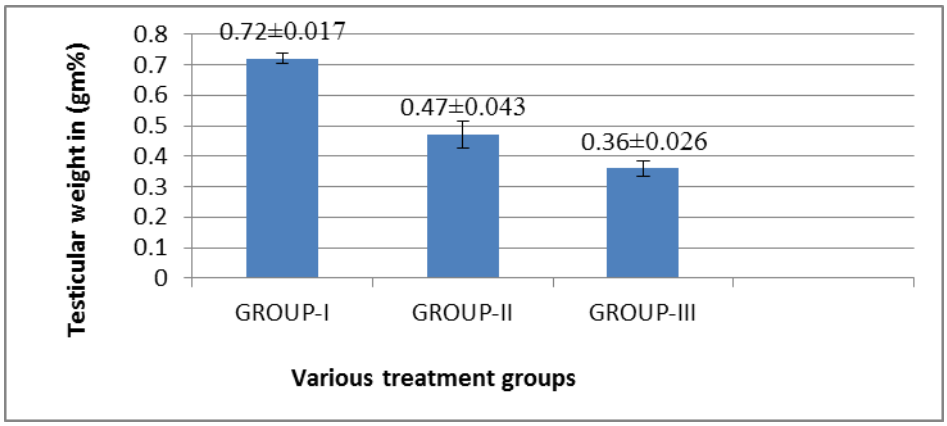


Fig. 2: Comparison of testicular weight (gm%) between controlled and $CaCl_2$ treated rats, values are mean ± SEM, n=12 rats in each group

3.3.Epididymal weight

Epididymal weight is measured as an important parameter to observe the effect of calcium chloride on accessory sex organ. The weight of epididymis is reduced significantly ($p<0.001$) in the animals of both the experimental groups compare to that of control group.

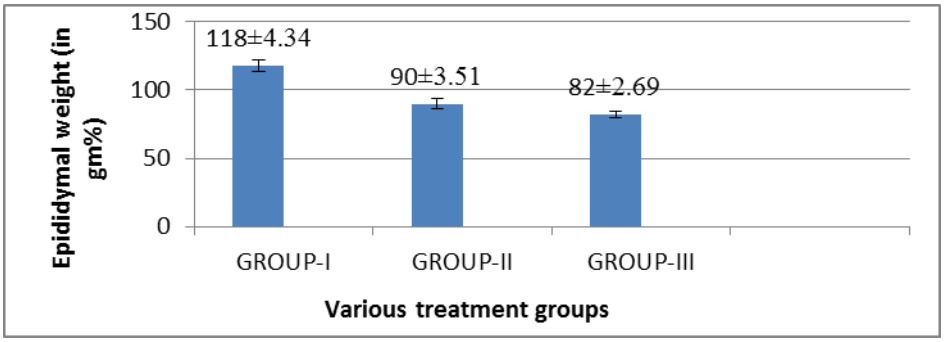


Fig. 3: Comparison of epididymal weight (mg%) between controlled and $CaCl_2$ treated rats, values are mean ± SEM, n=12 rats in each group

3.4.Sperm count

Sperm count has been done to observe the effect of calcium chloride directly on male reproductive system. The count of sperm has drastically been reduced ($p<0.001$) in the calcium chloride treated groups in comparison to control group.

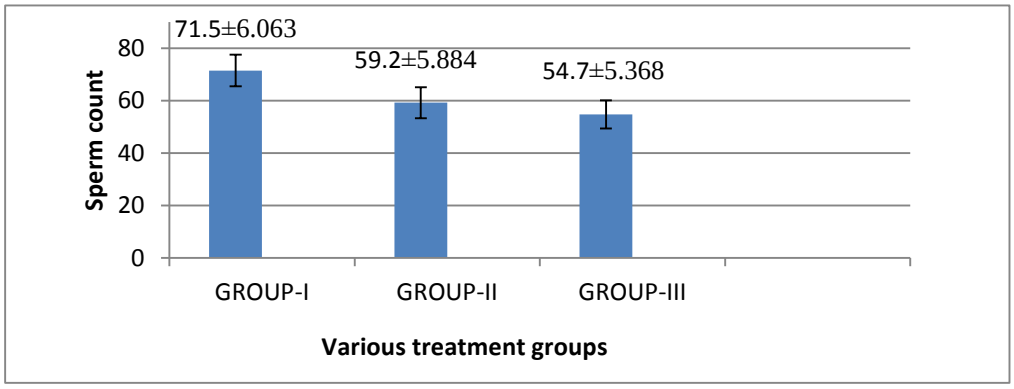


Fig. 4: Effect of $CaCl_2$ on sperm count in control and treated groups, Values are mean ± SEM (million/ml), n=12 rats in each group

3.5.Sperm motility

Sperm motility is also an important parameter to judge the efficacy of a particular drug used in experiment. In this present study the motility of the sperm has been significantly ($p<0.001$) reduced in experimental groups.

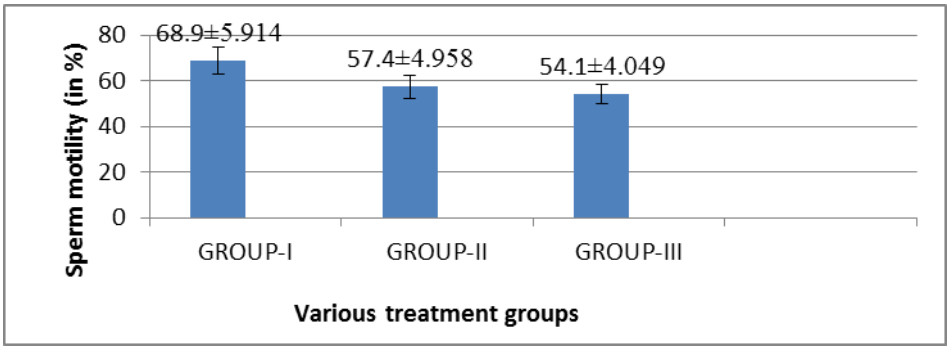


Fig. 5: Effect of $CaCl_2$ on sperm motility in control and treated groups, Values are mean ± SEM (%), n=12 rats in each group

3.6.Serum glucose

Present study clearly shows that there is no significant change in serum glucose concentration between the group-II and group-III animals compared with that of their respective group-I animals.

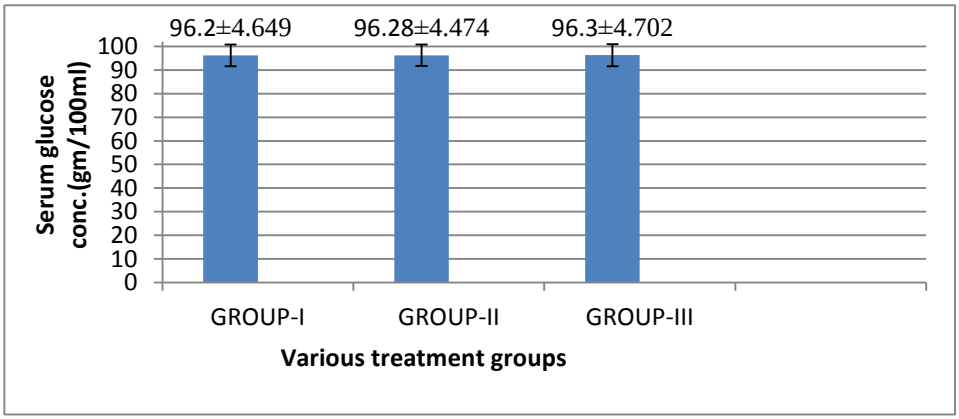


Fig. 6: Effect of $CaCl_2$ on serum glucose concentration in control and treated groups, Values are mean ± SEM (gm/100ml), n=12 rats in each group

3.7.Serum protein

Protein is a general parameter of the blood which has important role mainly in energy production and backbone formation. Present observation indicates that there is no significant change in serum protein level between control animals (group-I) and $CaCl_2$ treated animals (group-II & group-III).

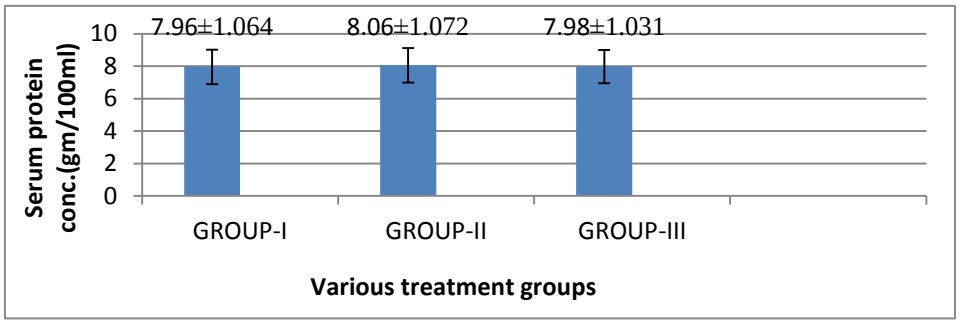


Fig. 7: Effect of $CaCl_2$ on serum protein concentration in control a nd treated groups, Values are mean ± SEM (gm/100ml), n=12 rats in each group

3.8.Serum cholesterol

Cholesterol is another important parameter which is used in the energy production in general. But it also has an important role in steroidogenesis. So measurement of this parameter is important when reproductive experiment is concerned. This study establishes that there is no significant difference between control (group-I) and low dose (group-II) animals but significant difference ($p<0.05$) is found between control (group-I) and high dose (group-III) animals.

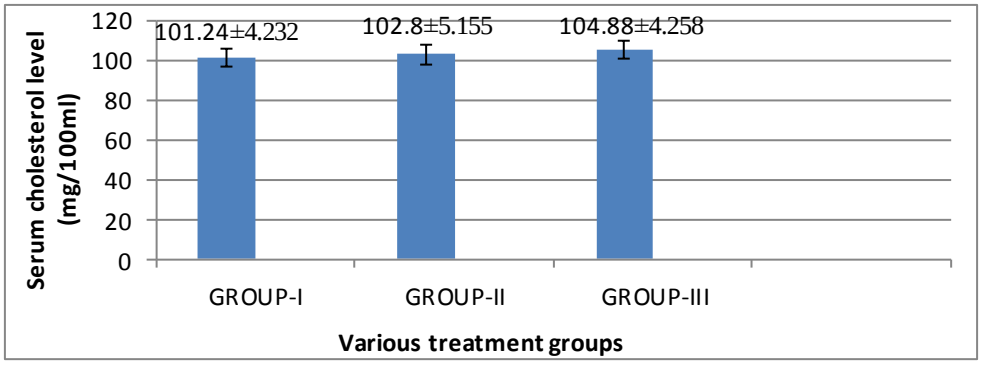


Fig. 8: Effect of $CaCl_2$ on serum cholesterol level in control and treated groups, Values are mean ± SEM (mg/100ml), n=12 rats in each group

3.9.SGPT & SGOT

SGPT and SGOT are generally measured as metabolic marker to observe the side effect of any drug. Present study declares no significant change of SGPT and SGOT level in serum of experimental animals compare to that of their respective control.

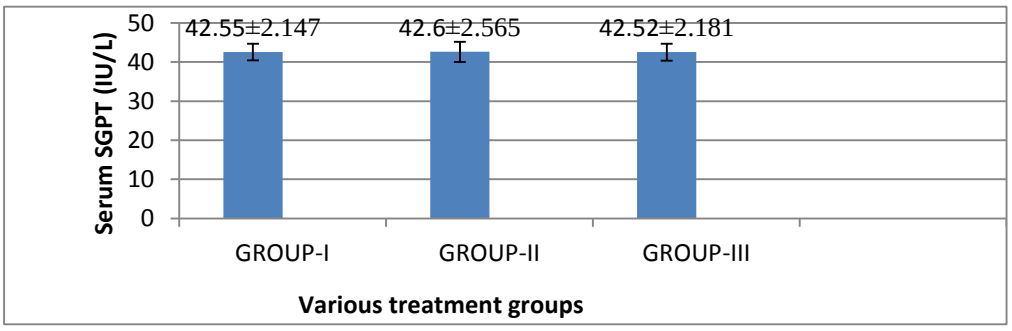


Fig. 9: Effect of $CaCl_2$ on SGPT activity in male albino rats, Values are mean ± SEM (IU/L), n=12 rats in each group

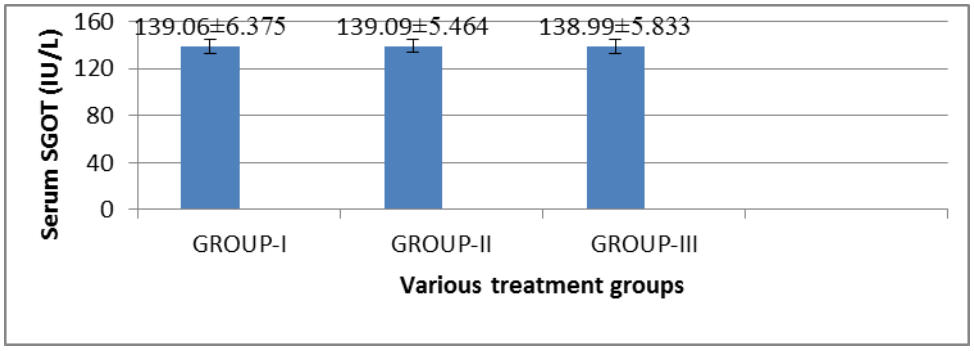


Fig. 10: Effect of $CaCl_2$ on SGOT activity in male albino rats, Values are mean ± SEM (IU/L), n=12 rats in each group

3.10.Testosterone

Testosterone is main reproductive hormone for male. It has important role in spermatogenesis. In present study this hormone is being measured to show the efficacy of the drug used in this experiment. Intratesticular injection of $CaCl_2$ showed its efficacy reducing the serum testosterone concentration significantly ($p<0.001$) in low dose (group-II) and high dose (group-III) treated animals when compared to control animals (group-I).

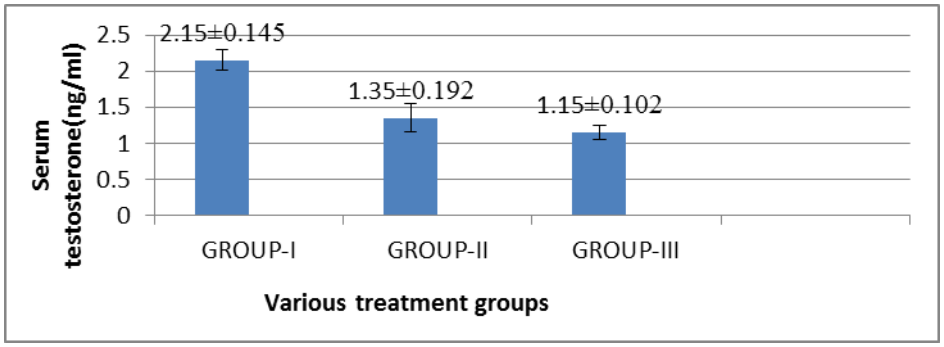


Fig. 11: Effect of $CaCl_2$ on serum testosterone concentration in male albino rats, Values are mean ± SEM (ng/ml), n=12 rats in each group

3.11.FSH & LH

These two hormones are released from pituitary gland and play important roles in spermatogenic activity. FSH and LH act for spermatogenesis after influencing on sertoli cell and leydig cell respectively. FSH has been altered in both low dose and high dose (group-II and group-III) animals significantly ($p<0.001$) but in case of LH, significant ($p<0.02$) change has taken place only in high dose (group-III) treated animals.

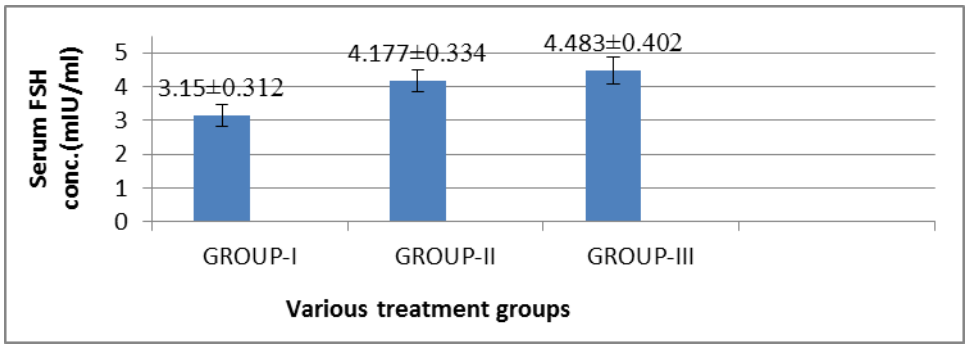


Fig. 12: Effect of $CaCl_2$ on serum FSH concentration in male albino rats, Values are mean ± SEM (mIU/ml), n=12 rats in each group

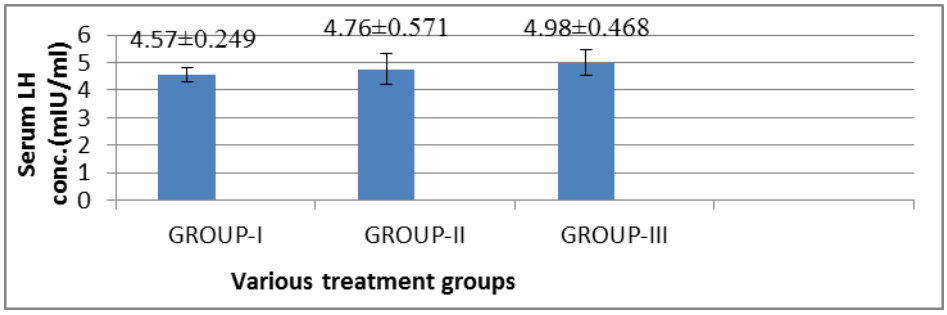
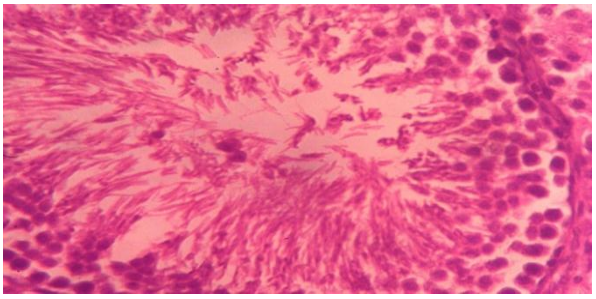


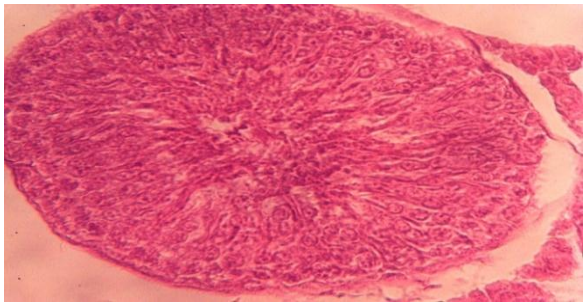
Fig. 13: Effect of $CaCl_2$ on serum LH concentration in male albino rats, Values are mean ± SEM (mIU/ml), n=12 rats in each group

3.12.Histological study

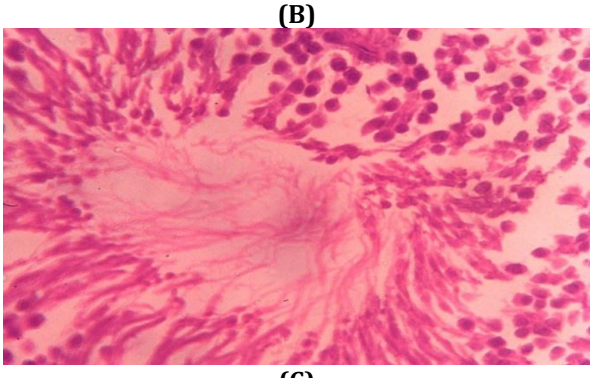
Histological structure has been changed in both the low dose treated group and high dose treated group in comparison with the animals of control group. Changes have taken place basically with disintegration of seminiferous tubules. It has also been found that reduction in accumulation of spermatozoa has taken place in the animals of both the treated group.



(A)



(B)



(C)

Fig. 14(A): H/E stained section of control group (group-I) testis (10 X 40x).
(B):H/E stained section of low dose (group-II) of $CaCl_2$ treated testis (10 X 40x).
(C): H/E staines section of high dose (group-III) of $CaCl_2$ treated testis (10 X 40x) showing an increase in luminal area, reduced spermatozoal mass and disorganized cellular orientation

4.DISCUSSION

After experiment on two sets of animals with different doses, it has been observed that intratesticular injection of $CaCl_2$ hampers few reproductive parameters without alteration of normal functioning of other systems. $CaCl_2$ injection in testis with specific dose has shown no significant influence on general growth pattern of low and high dose treated animals. In accordance it can be stated that Ghosh et al (2000)[22] and Jana et al (2002)[23] showed same type of result in their experiment.

It has been clearly observed that in the present study the weight of the testis and epididymis as accessory sex organ have been reduced significantly. So it can be said that the intratesticular injection of $CaCl_2$ has direct effect on the growth pattern of sex organs. This observation of present study has been well supported by the experimental results of Dixit (1977)[24] and Koger (1978)[25].

Sperm count and sperm motility are considered as the important reproductive parameters. It has been reported that the decrease in sperm count and motility are valid indices of male infertility in laboratory animals[26,27]. However, sperm motility is often used as a marker of chemical-induced testicular toxicity[28]. Current observation expresses the effect of intratesticular $CaCl_2$ injection as the tubular disintegration takes place and consequently sperm count and sperm motility both have been decreased significantly.

To show the effect of $CaCl_2$ on metabolic activity, SGPT and SGOT were measured in present study. SGPT is thought to be concerned with the integrity of the mitochondria[29] and it is also abundant in liver and acts as a marker of metabolic activity[30]. Observation of present study indicates no such significant changes in serum level of SGPT in experimental animal groups when compared to that of the control group. So it is clear that there is no stress or degenerative changes in mitochondria after application of $CaCl_2$ intratesticularly. On the contrary, it is known that SGOT is abundant in cardiac cells and $CaCl_2$ has harmful effect on cardiac tissue when given intravenously[30]. SGOT has integrity with lysosomes[31] and adrenal corticoids stimulates SGOT activity[32]. No significant change is found in serum SGOT level in experimental animals when compared with control group.

Glucose and protein are major parameters which generally play vital role as main metabolic indices. These two factors contribute to energy production and the normal metabolic functioning of the body. Besides this protein helps to form the basic structure of the body. No significant change in these two parameters and the normal metabolic functioning of the body. Besides this protein helps to form the basic structure of the body. No significant change in these two parameters in present study indicate that no metabolic impairment taken place there after testicular injection in two different doses after being compared with their respective control animals.

From this study it has been noted that, serum cholesterol has been altered significantly only in high dose treated animals (group-III). This slight increment may be due to lack of steroidogenesis[33,34]. Because from the flow chart (fig:15), it is prominent that cholesterol has significant role in testosterone bio-synthesis.

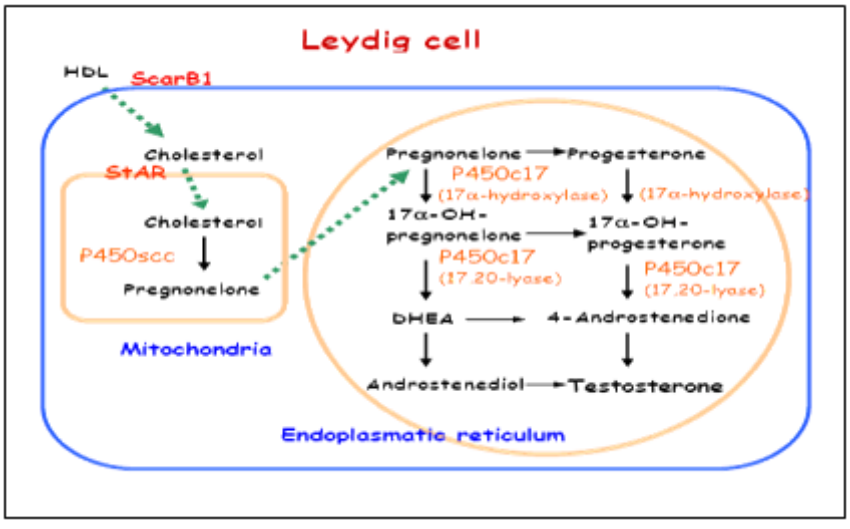


Fig. 15: Testosterone bio-synthesis from cholesterol

Earlier it has been stated that, sperm count has been hampered after testicular $CaCl_2$ injection. It can be correlated by the significant decreased in serum testosterone level in two treated groups (group-II and group-III) of animals with different doses because testosterone is one of the important regulators of the spermatogenesis[35]. Lower level of serum testosterone in treated groups may be further explained by the

inhibition of Δ^5,3β HSD and 17β HSD, two androgenic key enzymes after treatment of testicular $CaCl_2$ injection[36,37]. This decreased serum concentration of testosterone has been supported by earlier study[23]. Direct effect of testicular injection on androgenesis has been proved effective on increased level of serum FSH and LH concentration. It is found in the present study that FSH has been increased significantly in both experimental groups (group-II and group-III) but in case of LH significant increment takes place only in high dose treated animals (group-III). This increment may be due to feedback effect of testosterone on hypothalamo-pituitary axis[38,39](Fig:16).This result has been supported by few previous works on male reproductive system with different herbal product[40,41].

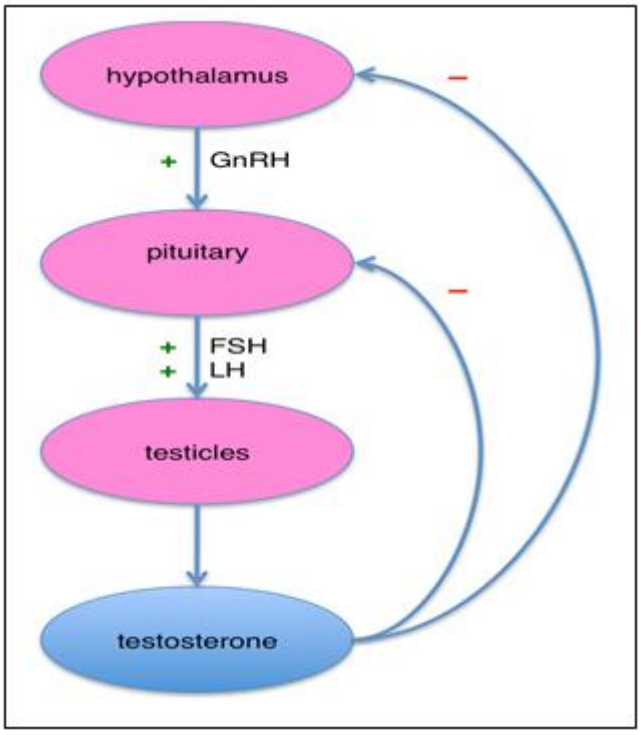


Fig. 16: Hypothalamo-pituitary axis

Histological structure of testis has been found hampered in experimental animals. The degenerative nature of germinal epithelium is found which may hamper the spermatogenesis as well as epididymal sperm count[42].The distribution of sperms and other germ layers has been found ill defined.

CONCLUSION

From the above observation it can be concluded that, intratesticular injection of $CaCl_2$ has its major role on male reproductive system impairing various key reproductive parameters including testicular histology keeping other general parameters intact. So it can be declared that, intratesticular $CaCl_2$ injection has potent sterilizing activity.

ACKNOWLEDGEMENT

Authors are grateful to all respected teachers and other support stuffs of K.N College, Berhampore, Murshidabad, W.B.

CONFLICT OF INTEREST

Authors have no conflict of interest regarding this research paper and its publication.

REFERENCES

1. Parker W and Segal S. Prevalence in contraceptive use in the United States. Family Planning Perspective. 1998;30:4.
2. Murphy M. Sterilization as a method of contraceptive. Recent trend in Great Britain and their implications. J Bio Social Sc. 1995;27:31.
3. Riphagen F and Fortney J. Contraception in women over forty. J. Bio. Social Sc. 1998;20:127.

4. Jana K and Samanta PK. Clinical evaluation of non surgical sterilization of male cats with single intra-testicular injection of calcium chloride. BMC Veterinary Research. 2011;7:39.
5. Baran A, Ozdas OB, Gulcubuk A, Hamzaoglu AI and Tonguc M. Pilot study:Intratesticular injection induces sterility in male cat.
6. Kumar N, Sood S, Arora B, Singh M and Beena. Effect of duration of fluoride exposure on the reproductive system in male rabbits. J Hum Reprod Sci. 2010;3(3):148-152.
7. Tyrone B Hayes . Atrazine induces complete feminization and chemical castration in male African clawed frogs(xenopus laevis). National academy of scienceswww.pnas.org. 2010;107(10):4612-4617.
8. Jana K, Samanta PK and Ghosh D. Evaluation of single intratesticular injection of calcium chloride for nonsurgical sterilization of male Black Bengal goats (Capra hircus):a dose dependent study.Animal reproduction science. 2005;86: 89-108.
9. Arash khaki DVM. Adverse effects of ciprofloxacin on testis apoptosis and sperm parameters in rats. Iranian Journal of Reproductive Medicine. 2008;6(2):71-76.
10. Jana K and Samanta PK. Sterilization of male stray dogs with a single intratesticular injection of calcium chloride: a dose dependent study. Contraception. 2007;75:390-400.
11. Sanny SW Chung, Xiangyuan Wang, Shelby S. Roberts, Stephen M Griffey, Peter R Reczek and Debra J Wolgemuth. Oral Administration of a Retinoic Acid Receptor Antagonist Reversibly Inhibits Spermatogenesis in Mice. Endocrinology. 2011;152(6):0000–0000.
12. Bowman TA, Senger PL, Koger LM, Gaskins CT and Hillers JK. Blockage of Sperm Transport Using Intraepididymal Calcium Chloride Injections in Rams. J ANIM SCI. 1978;46:1063-1065.
13. Chandra AK, Chatterjee A, Ghosh R and Sarkar M. Vitamin E-supplementation protect chromium (VI)-induced spermatogenic and steroidogenic disorders in testicular tissues of rats. Food Chem Toxicol. 2010;48(3):972-9.
14. Muruqesan P, Muthusamy T, Balusubramanian K and Arunakaran J. Studies on the protective role of vitamin C and E against polychlorinated biphenyl (Aroclor 1254)--induced oxidative damage in Leydig cells. Free Radic Res. 2005;39(11):1259-72.
15. Matzuk MM, McKeown MR, Filippakopoulos P, Li Q, Yu RN, Qi J, Knapp S, Bradner JE. Small Molecule Inhibition of BRDT for Male Contraception Cell. 2012;150(4):673-684.Matzuk MM, McKeown MR, Filippakopoulos P, Li Q, Ma L, Agno JE, Lemieux ME, Picaud S, Yu RN, Qi J, Knapp S, Bradner JE.
16. Sarkar SD, Maiti R and Ghosh D. Management of fluoride induced testicular disorders by calcium and vitamin-E co-administration in the albino rat. Reprod Toxicol. 2006;22(4):606-12.
17. Ghosh D, Chaudhuri A, Biswas NM and Ghosh PK. Effects of lithium chloride on testicular steroidogenic and gametogenic functions in mature male albino rats. Life Sci. 1990;46(2):127-37.
18. Das S K. Chemical castration of male albino rats by single intra testicular injection of calcium chloride. Ind J Physiol. & Allied Sci. 2011;65(1):25-30.
19. Majumder GC and Biswas R. Evidence for the occurrence of an ecto-(adenosine triphosphatase) in rat epididymal spermatozoa. J The Biochemical. 1979;183(3):737-43.
20. Kind PR, King EJ, Inverley H and Gowenlock AH. Method of practical clinical biochemistry. 899-900p.
21. Das D and Das A. Statistics in biology and psychology, Academic publishers, Kolkata, 4th ed. 117-26p. 2005.
22. Ghosh D, Maiti R, Tripathy N, Jana K and Samanta PK. Chemical castration by single intra testicular injection in rat. Proceeding of third congress of federation of Indian Physiological Societies. 2000.
23. Jana K, Samanta PK and Ghosh D. Dose dependent response to an intratesticular injection of calcium chloride for induction of chemosterilization in adult albino rats. Vet Res Com. 2002;26: 651-663.
24. Dixit VP. Action of monochlorohydrin on epididymis of dog. Ind J Of Expt Biol. 1977;15:233-235.
25. Koger LM. Calcium chloride in castration. Modern Veterinary Research Practice. 1978;59:119-121.
26. Working PK and Chellman GJ. The testis, spermatogenesis and the excurrent duct system. In: Reproductive toxicology and infertility. Scialli AR, Zinaman MJ, eds.ISBN, McGraw Hill, 1993; 55-76.
27. Lemasters GK and Selevan SG.. Toxic exposures and reproduction: a view of epidemiology and surveillance. In:Reproductive toxicology and infertility. Scialli AR, Zinaman MJ, eds. McGraw Hill, 1993;307–321.
28. Bitman J and Cecil HC. Estrogenic activity of DDT analoges and polychlorinated biphenyls. J Agr Food Chem. 1970;18:1108–1112.
29. Wilkinson JM. Principles and Practices of Diagnostic Enzymology. E Arnold Publishers. 1976;87-95.
30. Das D. Biochemistry, 10th ed. Academic Publishers Kolkata. 2000;132-134.

31. Lee D. Integrity of lysosomes on the isolated perfused rat live before and after exposure to dimethylsulphoxide. Cryobiology. 1979;16:18-23.
32. Forsham PH. The Adrenals. In: The Text Book of Endocrinology,(William R.H ed) (4th edn) W.B Saunders, Philadelphia, 1968;310.
33. Das UB, Mallick M, Debnath JM and Ghosh D. Protective effect of ascorbic acid on cyclophosphamide-induced testicular gametogenic and androgenic disorders in male rats. Asian J Androl. 2002;4:201-207.
34. Morris MD and Chaikoff IL. The originof cholesterol in liver, small intestine, adrenal gland, and testis of the rat: dietary versus endogenous contribution. J Biol Chem. 1959;234:1095-1097.
35. Sharpe RM, Maddocks S, Millar M, Saunders PTK, Kerr JB and McKinnell C. Testosterone and permatogenesis: Identification of stage dependent, androgen regulated proteins secreted by adult rat seminiferous tubules. J Androl. 1992;13:172-184.
36. Kausik Chatterjee, Kazi Monjur Ali, Debasis De, Chhanda Mallick and Debidas Ghosh. Induction of Chemosterilization by Single Intratesticular Calcium Chloride Injection in Stray Dogs. Research Journal of Animal and Veterinary Sciences. 2010;4:22-29.
37. Murono EP and Payne AH. Testicular maturation in the rat. In vivo effect of gonadotropins steroidogenic enzymes in hypophysectomized immature rats. Biol. Reprod. 1979;20:911-917.
38. Tillbrook AJ and Clarke IJ. Negative feedback regulation of the secretion and actions of gonadotrophins releasing hormone in males. Biol. Reprod. 2001;64:735–742.
39. Plant TM. Effects of orchidectomy and testosterone replacement treatment on pulsatile lutinizing hormone secretion in the adult Rhesus monkey (Macaca mulatta). Endocrinology. 1982;110: 1905–1913.
40. Das SK and Karmakar SN. Herbal castration by means of oral administration of green tea leaf extract (camellia sinensis l.) on male reproductive system. World Journal of Pharmaceutical Research. 2015;4(4).1162-1172.
41. Das SK and Karmakar SN. Effect of green tea (camellia sinensis l.) leaf extract on reproductive system of adult male albino rats. Int J Physiol Pathophysiol Pharmacol. 2015;7(4):178-184.
42. Ghosh D, Biswas NM and Ghosh PK. Studies on the effect of prolactin treatment on testicular steroidogenesis and gametogenesis in lithium treated rats. Acta Endocrinol. 1991;125:313-318.