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Research article

POTENTIAL EFFECT OF CALCIUM CHLORIDE ($CaCl_2$) AS CHEMICAL CASTRATIVE AGENT ON MALE ALBINO RAT

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ABSTRACT: Present study deals with the efficiency of calcium chloride ($CaCl_2$) as chemical castrative agent. Intratesticular injection of $CaCl_2$ has been given to the animals of two experimental groups in different doses. After completion of 30 days, animals were sacrificed. There was no significant difference of weight gain between control and experimental animals but the weight of testis and epididymis was significantly reduced. Reduction of sperm count and sperm motility was also significant in experimental animals compare to that of their respective control. SGPT and SGOT were measured as metabolic marker and were found insignificant between control and experimental animal groups. Some changes were also observed in histological structure of testis of experimental animals compare to the histological structure of control animal showing hampered structural organization of testicular characteristics.

Key words: Chemical castration, calcium chloride, intratesticular injection, metabolic marker and spermatogenesis.

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INTRODUCTION

Castration means the process by which the sterilization does occur. In case of male sterilization possibly the production of sperm called spermatogenesis is basically hampered. Impotency or sterility when imposed through a chemical agent is called chemical castration. Chemical castration is a means executed to control the unprogramed growth of population. In this present century, population explosion is a major threat to civilization. To combat with this problem there are several methods to prevent the population explosion by means of contraception. Having a mixed type of population in our nation, all types of contraceptive measures are not quite feasible to be utilized as a protective weapon to control the over growth of population. Besides various types of physical contraceptive measures there is also surgical method which can provide the actual assured way to stop the threatening growth of population. But this method is not implemented properly due to its costly and painfulness nature and after all due to its post operative complications. In this context it can be mentioned that more than one third of American couples use sterilization (Parker W and Segal S.,1998). This trend was observed in Great Britain also, over 20% of men at the age group of around 40 years have undergone sterilization operation (Murphy M., 1995). In Spain and Italy sterilization rates are very low (Riphagen F and Fortney J., 1998). So this present study is a simple search for new chemical agent which has the potentiality to create male impotency or sterility. In this connection review has been done to find some chemicals having their effect on male reproductive system. During 60 days injection in cat testis, it was showed complete necrosis and replacement by fibrous tissue with very low sperm count (Jana K and Samanta PK., 2011). Fluoride administration hampers the reproductive function of male rabbit and its effect is proportional to the duration of fluoride exposure (Kumar N et al., 2010). Atrazine adversely affects amphibian larval development. Atrazine exposed males suffered from depressed testosterone, suppressed mating behavior, reduced spermatogenesis and decreased fertility (Tyrone B.Hayes et al.,2010). 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 (Jana K et al., 2005).

Ciprofloxacin has the toxicological effects on reproductive system in male rats (Arash khaki DVM et al., 2008). 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 (Jana K and Samanta PK., 2007). 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 (Sanny SW et al., 2011). Chemo-surgical blockage of sperm transport with intra-epididymal injection of calcium chloride causes reduced sperm output without depressing libido in rams (Bowman TA et al., 1978). The present study has its objective to evaluate the effect of intratesticular injection of calcium chloride on male reproductive system of albino rats.

MATERIALS AND METHODS

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.

Preparation of calcium chloride solution

Calcium chloride solution was prepared according to the method of Das SK(2011). 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.

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 cauda portion of equal length was cut and it was 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 GC, Biswas R. (1979). 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 PR et al (1980). Finally results were compared with the respective controls with the help of student's 't' test (Das D and Das A., 2005) to generalize the effect of intratesticular injection of $CaCl_2$ on reproductive system of male albino rat model.

RESULTS

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.

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.

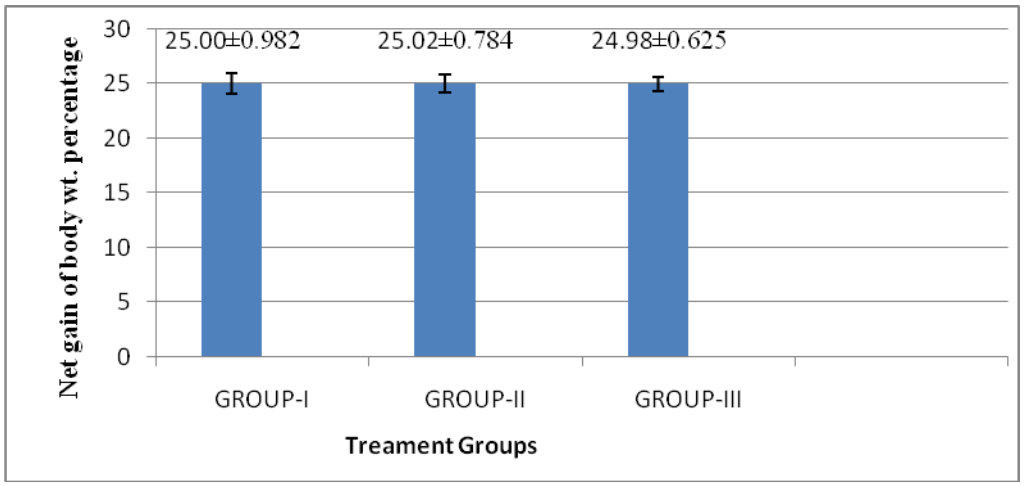

Figure-1: Comparison of net gain of body weight of rats treated with $CaCl_2$ of different doses and respective controls. Values are mean±SEM (in %), n=12 rats in each group.

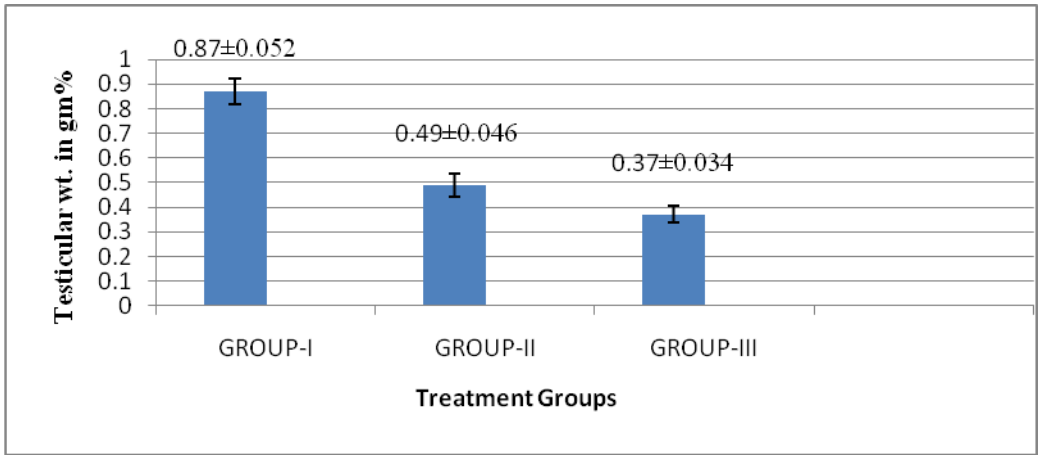

Figure-2: Comparison of testicular weight (gm%) between controlled and $CaCl_2$ treated rats, values are mean ± SEM, n=12 rats in each group.

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.

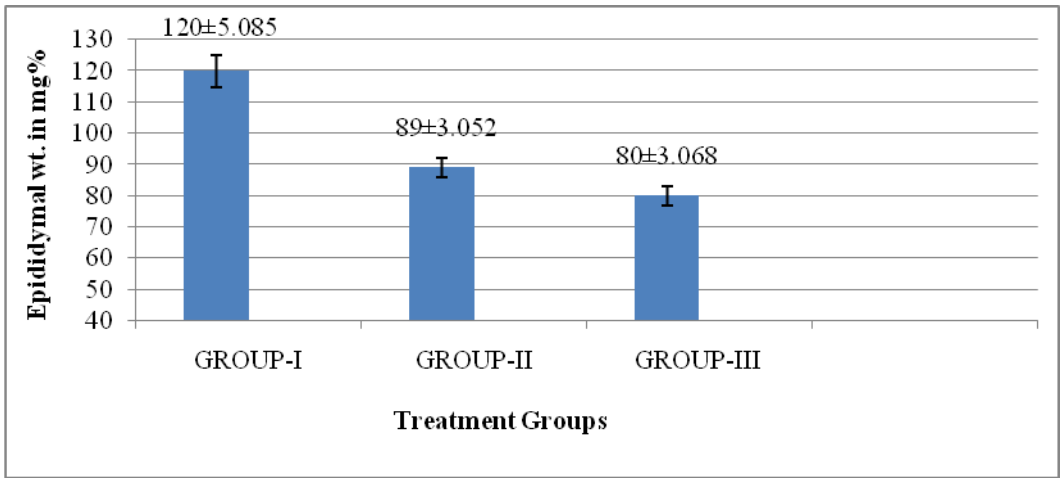

Figure-3: Comparison of epididymal weight (mg%) between controlled and $CaCl_2$ treated rats, values are mean ± SEM, n=12 rats in each group.

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.

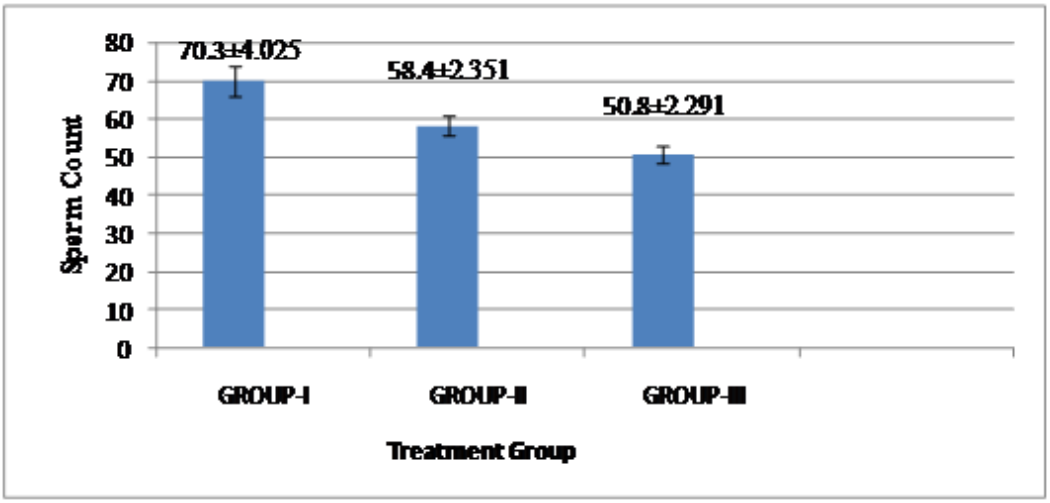

Figure-4: Effect of $CaCl_2$ on sperm count in control and treated groups, Values are mean ± SEM (million/ml), n=12 rats in each group.

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.

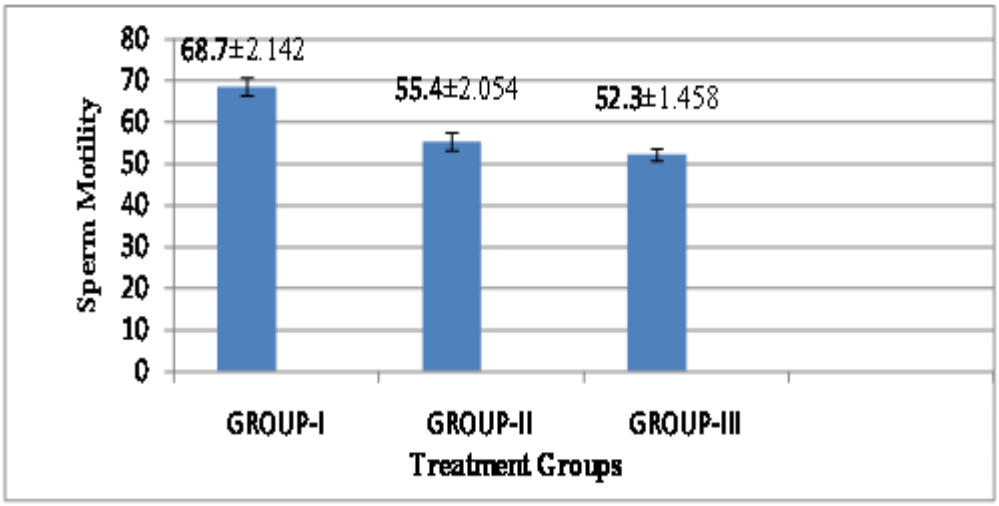

Figure-5: Effect of $CaCl_2$ on sperm motility in control and treated groups, Values are mean ± SEM (%), n=12 rats in each group.

SGPT and 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.

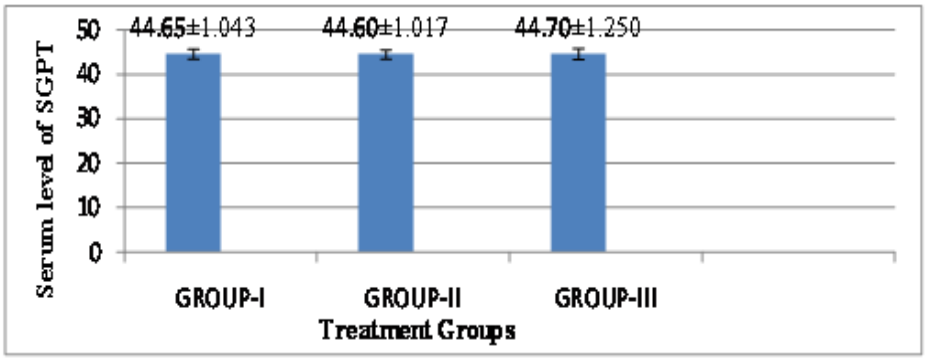

Figure-6: Effect of $CaCl_2$ on SGPT activity in male albino rats, Values are mean ± SEM (IU/L), n=12 rats in each group.

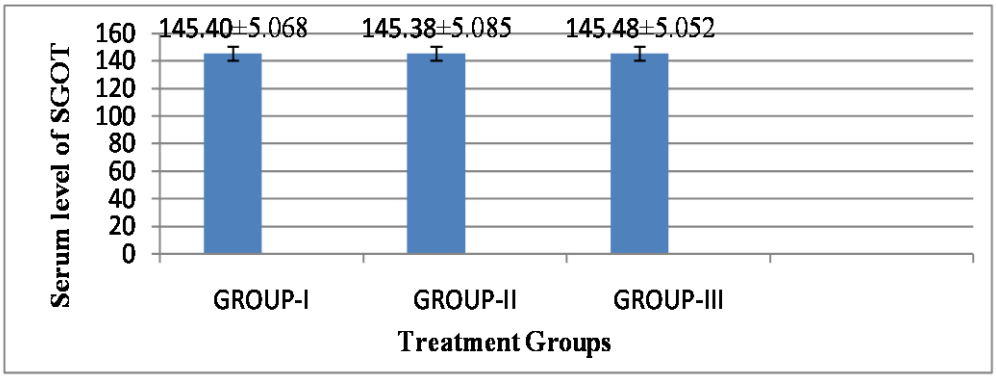

Figure-7: Effect of $CaCl_2$ on SGOT activity in male albino rats, Values are mean ± SEM (IU/L), n=12 rats in each group.

Histological study

Some changes are found in the histological structure of treated animals with $CaCl_2$ in comparison with animals of control group.

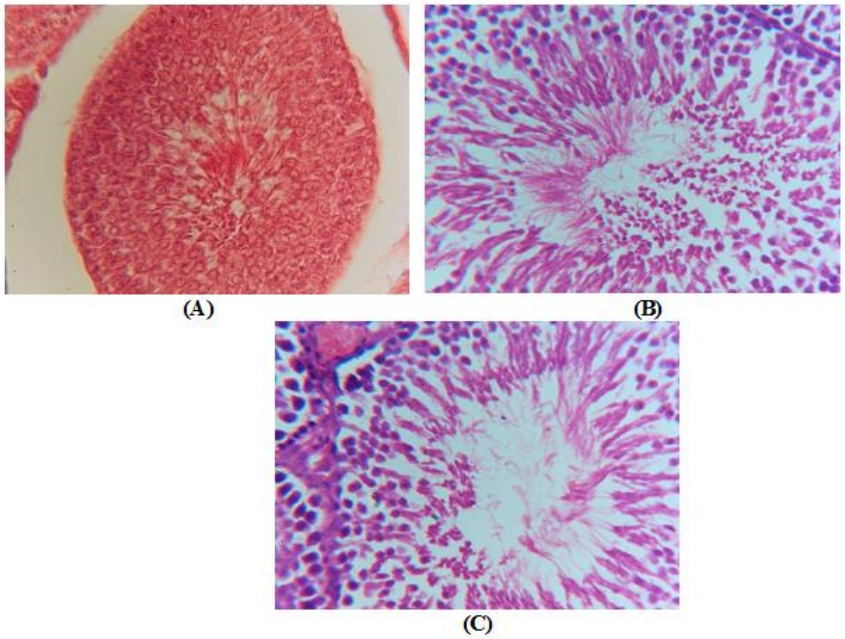

Fig: 8 (A): H/E stained section of control group (group-I) testis (10 X 40x).
(B): H/E stained section of low dose (group-II) of $CaCl_2$ treated testis (10 X 40x).
(C): H/E staines section of high dose (group-III) of $CaCl_2$ treated testis (10 X 40x) showing an increase in luminal area, reduced spermatozoal mass and disorganized cellular orientation.

DISCUSSION

It is obvious from the present observation that intratesticular injection of $CaCl_2$ hampers few reproductive parameters without altering the normal functions of some other systems. $CaCl_2$ injection in specific dose has shown no significant change in body weight in experimental animal groups comparing the control group. Ghosh et al (2000) and Jana et al (2002) showed same type of result in their experiment. So, it is clear that intratesticular injection of $CaCl_2$ does not hamper the normal growth pattern. The weight of testis and epididymis has significantly decreased as observed in the study of Dixit VP (1977) and Koger LM (1978). Reduction of weight of these sex organs indicates that intratesticular injection of $CaCl_2$ has direct effect on reproductive ability.

It has been reported that the decrease in sperm count and motility are valid indices of male infertility in laboratory animals (Working PK, Chellman GJ., 1993 and Lemasters GK, Selevan SG., 1993). However, sperm motility is often used as a marker of chemical-induced testicular toxicity (Bitman J, Cecil HC., 1970). Present study clearly depicts the drastic reduction in sperm count as well as sperm motility which indicates castrative potency of $CaCl_2$ injection. This may be due to decreased serum testosterone level which can affect the spermatogenesis evidenced by previous experimental result on male reproductive system by different herbal product (Das SK, Karmakar SN., 2015 and Das SK, Karmakar SN., 2015). It can be further explained by the linear relationship between sperm count and testosterone concentration (Fig: 9).

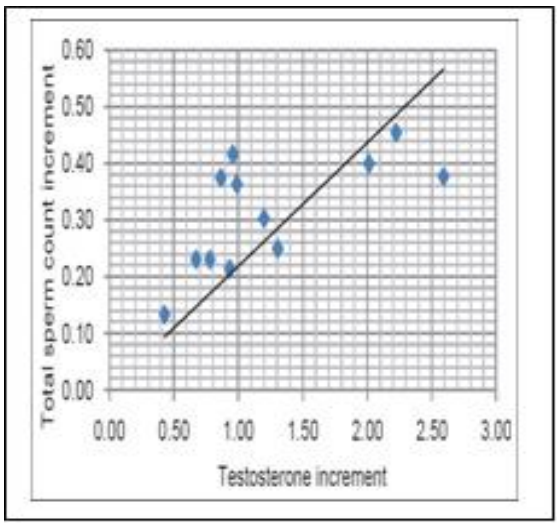

Fig-9: Linear relationship between sperm count & testosterone

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 (Wilkinson JM.,1976) and it is also abundant in liver and acts as a marker of metabolic activity (Das D.,2000). 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 (Das D.,2000). SGOT has integrity with lysosomes (Lee D et al.,1979) and adrenal corticoids stimulates SGOT activity (Forsham PH.,1968). No significant change is found in serum SGOT level in experimental animals when compared with control group.

Some changes are found in the histological structure of testis of experimental animals. The degenerative nature of germinal epithelium is found which may hamper the spermatogenesis as well as epididymal sperm count (Ghosh D et al.,1991) which is also reflected in the histological structure of $CaCl_2$ treated animals when compared to that of their respective control group.

CONCLUSION

In conclusion it can be stated that intratesticular injection of $CaCl_2$ has its potential to hamper the reproductive ability of rats without any side effect. Histological structure was also changed leading to impairment of testicular function like spermatogenesis. So $CaCl_2$ may be used as castrative agent in near future.

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