Study of reproductive impairment of albino rat by calcium chloride mediated induction through injection in testis

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Abstract

Experiment using the injection of Calcium chloride (CaCl₂) in testis of experimental animals, it has been found that, the general growth pattern of the body has not been hampered in significant way. Besides this, the weight of testis and other accessory sex organs like epididymis, seminal vesicle and prostate has been reduced in significant manner. The sperm count and sperm motility have also been reduced significantly in dose dependent manner relating the consecutive changes in serum testosterone level taking place significantly in two differently dose treated groups of experimental animals in comparison with control group of animals.

Key words: Calcium Chloride, prostate, testosterone, sperm motility, seminal vesicle.

1. Introduction

This experiment deals with the effect of intra testicular injection of CaCl₂ in the context of search of new agent which can enhance the sterilization process in male reproductive system. In recent days several experiments are going on in search of these kinds of agents and also in other animals. Fluoride administration hampers the reproductive function of male rabbit and its effect is proportional to the duration of fluoride exposure (Kumar, Sood, Arora, Singh & Beena, 2010). Atrazine exposed males suffered from depressed testosterone, suppressed mating behavior, reduced spermatogenesis and decreased fertility (Tyrone & Hayes et al, 2010). Intra testicular 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, Samanta & Ghosh, 2005). Ciprofloxacin has the toxicological effects on reproductive system in male rats (Arash et al, 2008). Single intra testicular injection of calcium chloride in male stray dogs is effective on male reproductive system and may be used as substitution of surgical castration (Jana & Samanta, 2007). Chemo-surgical blockage of sperm transport with intra-epididymal injection of calcium chloride causes reduced sperm output without depressing libido in rams (Bowman, Senger, Koger, Gaskins & Hillers, 1978). So, the aims and objectives of the present study is also to find out the effect of intra testicular injection of calcium chloride on few related parameters of male reproductive system.

2. Materials and Methods

2.1. Experimental protocol
Adult 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 ºC) 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 intra testicular 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 0.5 ml 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 0.5 ml 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 (Das, 2011). Solution was prepared with distilled water of 0.5 ml/100 gm of body weight containing 10 mg 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 20 mg 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. Experimental parameters and their measurements

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 ºC for enzyme assay. Testis of each rat was dissected out and weight was taken after removing the adipose tissues. Besides this, the weight of epididymis, seminal vesicle and prostate were also taken. After sacrifice, the cauda portion of equal length was cut and it was kept in 1 ml diluents at 37 ºC. 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 (Majumder & Biswas, 1979). Serum testosterone was measured in ELISA method.

2.4. Statistical analysis

Finally results were compared with the respective controls with the help of student's 't' test (Das & Das, 2005) to generalize the effect of intra testicular injection of CaCl$_2$ on reproductive system of male albino rat model.

3. Results

3.1. Body weight

Insiginificant gain of body weight has been observed in dose treated groups of animals in comparison with the control groups of animals.

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.
3.2. Testicular weight

Testicular weight has been reduced significantly (p<0.001) in both the experimental groups in comparison with those animals of control 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.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Group-I</th>
<th>Group-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testicular weight</td>
<td>0.77±0.042</td>
<td>0.39±0.037</td>
<td>0.30±0.025</td>
</tr>
</tbody>
</table>

3.3. Epididymal weight

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.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Group-I</th>
<th>Group-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epididymal weight</td>
<td>110±5.01</td>
<td>79±2.72</td>
<td>70±2.88</td>
</tr>
</tbody>
</table>

3.4. Weight of seminal vesicle

Weight of seminal vesicle has been reduced significantly in two dose treated groups of animals (p<0.01) in comparison with control animals.

*Figure 4. Comparison of seminal vesicular weight (mg %) between controlled and CaCl\(_2\) treated rats*

values are mean ± SEM, n=12 rats in each group.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Group-I</th>
<th>Group-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt. of seminal vesicle</td>
<td>139±5.23</td>
<td>109±4.98</td>
<td>88±2.62</td>
</tr>
</tbody>
</table>

3.5. Weight of prostate

Weight of prostate in experimental animals has been reduced significantly (p<0.05) in comparison with control group of animals.

*Figure 5. Comparison of weight of prostate (mg %) between controlled and CaCl\(_2\) treated rats*
values are mean ± SEM, n=12 rats in each group.

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<thead>
<tr>
<th></th>
<th>Control</th>
<th>Group-I</th>
<th>Group-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt. of prostate</td>
<td>133±5.21</td>
<td>106±4.91</td>
<td>81±2.39</td>
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</tbody>
</table>

3.6. Sperm count

The count of sperm has severely been reduced (p<0.001) in the calcium chloride treated groups in comparison to control group.

*Figure 6. Effect of CaCl₂ on sperm count in control and treated groups*

Values are mean ± SEM (million/ml), n=12 rats in each group.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Group-I</th>
<th>Group-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm count</td>
<td>68.3±4.010</td>
<td>56.4±2.325</td>
<td>48.8±2.268</td>
</tr>
</tbody>
</table>

3.7. Sperm motility

In this present study the motility of the sperm has been significantly (p<0.001) reduced in experimental groups.

*Figure 7. Effect of CaCl₂ on sperm motility in control and treated groups*

Values are mean ± SEM ( %), n=12 rats in each group.

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<thead>
<tr>
<th></th>
<th>Control</th>
<th>Group-I</th>
<th>Group-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm motility</td>
<td>64.7±2.02</td>
<td>51.4±1.91</td>
<td>48.3±1.31</td>
</tr>
</tbody>
</table>

3.8. Serum testosterone

Serum testosterone level has been significantly reduced (p<0.001) in two dose treated groups in comparison with control group animals.

*Figure 8. Effect of CaCl₂ on serum testosterone concentration in male albino rats*

Values are mean ± SEM (ng/ml), n=12 rats in each group.

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<thead>
<tr>
<th></th>
<th>Control</th>
<th>Group-I</th>
<th>Group-II</th>
</tr>
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<tbody>
<tr>
<td>Testosterone</td>
<td>1.99±0.142</td>
<td>1.20±0.189</td>
<td>1.01±0.099</td>
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</tbody>
</table>

4. Discussion

Intra testicular injection of calcium chloride shows no significant change in general body growth pattern. Jana et al (Jana & Ghosh, 2002) showed same type of result in their experiment. Present study shows significant reduction in epididymal weight and testicular weight after injection of calcium chloride in testis. Similar results were found in experiment of Dixit (Dixit, 1977) and Koger (Koger, 1978).
Calcium Chloride injection in testicles of experimental animals affects other accessory sex organs like seminal vesicle and prostate significantly in both the dose treated groups in comparison with control group of animals.

After injection of Calcium Chloride in two dose treated groups, the sperm count and sperm motility have been significantly changed in dose dependent manner. 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 & Karmakar, 2015; Das & Karmakar, 2015).

Testosterone level has been reduced significantly in two dose treated groups after direct injection of calcium chloride in testicles with different doses. Lower level of serum testosterone in treated groups may be explained by the inhibition of $\Delta^1,3\beta$ HSD and $17\beta$ HSD, two androgenic key enzymes after treatment of testicular CaCl$_2$ injection (Kausik, Kazi, Debasis, Chhanda & Debidas, 2009; Murono & Payne, 1979).

5. Conclusion

Impairment has taken place after calcium chloride injection in two dose treated groups animals with two different doses. So it may be used as sterilizing substance in future.

References