THE TOXIC EFFECT OF LITHIUM CARBONATE ON THE REPRODUCTIVE SYSTEM OF IMMATURE MALE MICE

ABSTRACT:
The present study was performed on pregnant female mice, by intraperitoneal injection of Camcolit (antipsychotic drug) at a daily dose of 25 mg/kg B.W. for 31 day. The treatment started 10 days after conception, during gestation and through postnatal weaning of the male offspring on neonatal development of the mouse till they reach 21 days, to describe the indirect effect of low therapeutic dose of lithium intake on reproductive organs of immature male mice through the injections of mother. Controls were given equimolar amounts of saline. Treatment of mothers by Camcolit (lithium carbonate) caused no mortality or deformed embryos. However, the treatment led to increase of baby body weight significantly accompanied by alteration in testosterone parameters. Also, degenerative changes in the testis, epididymis and vas deference indicated by a reduction in spermatogenesis are detected. The vacuolation of Leydig cells and the atrophy of Sertoli cells extensions between germ cells were detected. The body weight, testosterone level, spermatogenetic activity, and histology of testis, epididymis, vas deferens, were analyzed. In conclusion, the present study provides some morphological, hormonal and histological results suggesting adverse effects of chronic lithium administration on the reproductive system of immature male mice. Since some of these effects may not necessarily be reversible, these results raised concerns in the administration of this antipsychotic drug on a chronic basis usually recommended for prophylactic purposes.

KEY WORDS:
Lithium, reproductive system, immature male mice, testosterone.

CORRESPONDANCE:
Awatef Mohamed Ali Mostafa
Zoology Departments, Faculty of Science,
Alexandria University, Egypt.
E-mail: Awatefalii@yahoo.com

INTRODUCTION:
The therapeutic and adverse effects of drug-drug interactions have been well recognized over years, particularly when co administration of drugs are necessary for multiple symptoms control. Both the fetus and the newborn are sensitive to various drugs and combined drug administration often worsen such adverse interactions (Messiha, 1989). Lithium salt is most efficient in long-term preventive treatment and it also has an anti-suicidal effect (Shastry, 2005). Although psychotropic drugs have not been tested or approved by the food and drug administration for use during pregnancy, some women continue to take these medications while they are pregnant, particularly during mood and anxiety disorder during childbearing years. The relative risks/benefits of drug therapy for these women must be weighed with each patient and treatment limited to those situations in which risks to mother and fetus from the disorder are presumed to exceed the risk of drug treatment (Cohen & Rosenbaum, 1998).

Iqbal et al. (2001) reported that women treated with anti-manic drugs then become pregnant are commonly considered to be at high risk for fetal complication during the pregnancy or during lactation. The potential teratogenic effect of lithium has suggested its contraindication during pregnancy (Pillts, 1983). Mroczka et al. (1983) reported that lithium toxicity occurs much more frequently in male rats than in females. Lithium predominantly caused developmental effects when female animals were exposed during the first part of pregnancy, before and/or during organogenesis, exposure before conception or after organogenesis also had effects (Warkany, 1988). Further, decreased reproductive and developmental abilities as litter size, live pups have been reported in lithium chloride exposed pregnant rats (Sachzer et al., 1992).

Cohen et al. (1994) concluded that in pregnant women the teratogenic risk of first-
trimester lithium exposure was lower than previously suggested. Lithium may be used if absolutely necessary during the second and third trimesters, but should be discontinued or the dosage must be reduced by 50% several weeks before the birth (Iqbal et al., 2001).

Lithium chloride given to the nursing mothers had a delayed toxic effect on the endocrine organs (Messiha, 1989). However, Ghosh et al. (1991) showed that the normal testicular activity in immature rat was affected by chronic administration of lithium and appeared as adverse effect on testicular gametogenic function. Decreased reproductive and developmental abilities have been also reported in lithium chloride exposed pregnant rats (Sechzer et al., 1992). Teixeira et al. (1995) concluded that chronic treatment of dams with lithium with the same dose used in the prophylaxis of bipolar disorders aggravated the delay in physical and behavioral development of pups. This is produced by stress associated with limited water intake and handling. Pinelli et al. (2002) reported that lithium has adverse effects on the fetus and newborn infant, but data suggested normal behavioral patterns in childhood.

Most of the existing literatures are reports on the biochemical adverse effect of sub chronic lithium carbonate on neonate mice, less is known about the toxic effects of lithium on the male reproductive system. The present investigation was undertaken to examine whether chronic lithium treatment could lead to morphological alteration in immature male reproductive organs or not.

MATERIAL AND METHODS:

Twenty sexually mature females of Swiss albino mice (Mus musculus) weighing 25-30 g were obtained from king Fahad center for research. The mice were mated with adult males of comparable age. The females were separated from the males immediately after conception (Upouse et al., 1984) and were housed as groups; four females each.

The gestating females were divided into two groups (experimental and control). The experimental ones received ip (25 mg/ lithium carbonate dissolved in saline/kg B.W./day), this dose is equivalent to the low human therapeutic dose (Show and Bastrup, 1966). The control group had access to saline injections.

The duration of maternal lithium treatment persisted throughout 10 days after conception and nursing periods up until the end of lactating period (i.e. the experiment extended for 31 days). Animals maintained under standard laboratory condition in special cages (14h light / 10h darkness) at 25 ± 3°C with constant humidity 40-60%, standard pellet diet and continuous water supply were provided during the entire experimental schedule.

RESULTS:

During the experiment, all animals were survived. This means that the doses administered, the laboratory conditions were

Lithium Carbonate has an unimpeded formula of (Li₂CO₃), molecular weight of 73.89. It is provided as tablets of 250 mg by Norgin Compan and under the international trade (Camcolit). Paget and Barnes (1964) formula was applied to determine the equivalent low dose of Camcolit.

The offspring weight was recorded after birth and at the end of experimental period.

At the termination of the respective experimental periods, 24 controls and 24 experimental immature male babies were taken 24 h after the last injection of mothers.

Six animals from each group were sacrificed by decapitation, the testis, epididymis and vas deferens were excised and cut into small pieces to allow good fixation in Bouin’s fluid. Dehydration, clearing and paraffin embedding were followed according to Bancroft and Gamble (2002). Sections were stained with haematoxylin and eosin and Toluidin blue (Pantin, 1964).

The diameter of twenty five cross sections of round shaped seminiferous tubules randomly selected from each testis of each mouse using a calibrated eye piece (x 400) ocular micrometer.

Primary spermatocytes were counted in 50 randomly chosen seminiferous tubules at each mouse testis. The mean value of the primary spermatocytes was taken as the index of spermatogenic activity.

The diameters of twenty five Leydig cell nuclei from 5 to 7 section of each mouse testis were measured using a calibrated eye piece (x 1000) ocular micrometer.

The diameter of twenty five cross sections of round shape ductules epididymis randomly selected from each mouse epididymis is done using a calibrated eye piece (x 400) ocular micrometer.

Six animals from each group were slightly anaesthetized, 24 hours after the last injection, of their mothers. They bleed within two minutes by eye using a heparinized syringe, plasma samples were separated by centrifugation, frozen and stored at -85°C until all samples have been collected for the determination of testosterone levels by radioimmunoassay (RIA) according (Chang et al., 1995).

Body weight, tubular diameters, Leydig cell nuclear diameter, spermatogenic activity, ductules epididymis diameter and testosterone were analyzed by using the program SAS (Institute Inc. Cary, NC, USA) where t-test was used to assess the significance of changes between control and treated mice (Daniel, 1991).
appropriate. No deformities or discolorations were observed in fetuses from control or experimental animals.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Body weight</th>
<th>Diameter of seminiferous tubules</th>
<th>Primary spermatocytes count</th>
<th>Nuclear diameter of Leydig cells</th>
<th>Diameter of epididymis ductules</th>
<th>Testosterone level</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.04±1.26</td>
<td>116±0.19</td>
<td>22.76±8.68</td>
<td>0.62±0.19</td>
<td>56.25±0.28</td>
<td>0.42±0.011</td>
</tr>
<tr>
<td>Experimental</td>
<td><strong>11.17±1.09</strong></td>
<td><strong>159.57±0.29</strong></td>
<td><strong>13.76±5.90</strong></td>
<td><strong>0.48±0.20</strong></td>
<td><strong>16.88±0.05</strong></td>
<td><strong>0.13±0.010</strong></td>
</tr>
</tbody>
</table>

Each value is given as the mean with standard deviations. ** Significantly different from control group at < 0.0001.

All parameters corresponding to a mean value of 6 animals, except the testosterone level corresponding to a mean value of another 6 animals.

Transverse sections of the immature control mice testis contain large numbers of small and rounded seminiferous tubules with a stare lumen (Fig. 1A) and its diameter is around 116 ± 0.19 µm (Table 1). The tubules show normal columnar radiation of the germ cells where basal spermatogonia are observed next to the limiting membrane of the tubules. These spermatogonia are differentiated into dark type spermatogonia (large cells, usually surrounded by a clear space and displaying dark irregular clumps of chromatin within round nucleus) and light spermatogonia (have spherical nucleus with pale chromatin). The spermatogonia are followed by primary spermatocytes which have a larger number with mean value 22.76 ± 8.68µm (Table 1) and secondary spermatocytes with small central heterochromic nuclei around the empty stare lumen. Seminiferous tubules at this age are almost devoid of spermatides and spermatozoas (Fig. 1B). Spermatocytes are outlined by a thin layer of Sertoli cell cytoplasm which bordered by the limiting membrane of the seminiferous tubules and had large triangular nuclei (Fig. 1B). The seminiferous tubules are wrapped in a clearly defined sheath separating them from the surrounding interstitial tissue which loaded with Leydig cells (Fig. 1B&C). Leydig cells are small, inconspicuous cells with strongly basophilic cytoplasm and spherical nuclei with diameter 0.62 ± 0.19 µm (Table 1). Both of the seminiferous tubules and the interstitial cells are surrounded by a connective tissue sheath called tunica albuginea (Fig. 1A).

At the end of the experiment, there was a noticeable increase in the experimental body weight; 11.17 ± 1.09g (P<0.001) compared to the control body weight; 8.04 ± 1.26g (Table 1).
1*A) compared to control animals; 116.25 ± 0.74µm (Table 1). This may be due to the disruption in organization of various differentiating germ cells from spermatogonia to the secondary spermatocytes indicating irregularity mechanisms of spermatogenesis.

Degenerative cells were noticeable (Fig. 1*C) and it was difficult to differentiate between degenerating primary and secondary spermatocytes which characterized with clear spaces between cell membrane and Sertoli cell which lost its extensions (Fig. 1*B). This degeneration led to a significant reduction in the numbers of primary spermatocytes, as it reached 13.76 ± 5.90 (p<0.001) compared with control group 22.76 ± 8.68 (Table 1).

The present data established that Camcolit produced profound degenerative change in the disarranged Leydig cells where dark stained nuclear area were surrounded by vacuolated cytoplasm near dilated blood capillary (Fig. 1*B). The irregular nuclei with more intense heterochromatin became smaller and their nuclear diameters became less 0.48 ± 0.20µm when compared to control animals, 0.62 ± 0.19 µm, and the dense heterochromatin showed the inactive state which led to low secretion of testosterone in the experimental group 0.13 ± 0.01ng/ml (Table 1).

As shown in table 1, the diameter of control immature ductus epididymis reached 56.25±0.28 µm; it is lined with pyramid basal cells and high principal columnar cells with spherical nuclei, long cilia. No sperms were detected in their lumen (Fig. 2A&B).

In the experimental group, the atrophic epididymis with cellular disorganization, reduction of epithelial height, lose of stereocilia, alterations in nuclear size and disarrangement of the nuclei were observed (Fig. 2*A&B). Lithium- administration for 31 days resulted also in a significant (p<0.001) decrease in the diameter of ductus epididymis; 16.88 ± 0.05 µm, when compared to those of the saline-injected control animals (Table 1).
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Fig. 2. Cross section of control immature mice epididymis (H&E):
A) Connective tissue (CT), ductus epididymis (DE) and empty lumen (L) (x 400).
B) Pyramid basal cells (BC), principal columnar epithelial (CC) with spherical nucleus and long cilia. Blood vessel (BV) (x 1000).

Fig. 2*. Cross section of treated immature mice epididymis (H&E):
A) Less diameter ductus epididymis (DE), congestion (arrow) and dividing stage (*) (x400).
B) Principal columnar epithelial (CC) with short cilia, less diameter ductus epididymis (DE), small lumen (L) and congestion (arrow) (x1000).

The vas deferens of the control immature mice is lined with pseudo stratified columnar epithelium, followed by inner and outer longitudinal muscle layers in-between circular muscle layer (Fig. 3A). The pseudo stratified epithelium have oval nuclei and ciliary tuft (Fig. 3B). However, the vas deferens showed large lumen cavity had lost its ability for staining with disruption of muscular layers in the experimental group (Fig. 3*A). The vas deferens also had low epithelial cells with vacuolated cytoplasm and hyperchromic nuclei (Fig. 3*B).

Fig. 3. Cross section of control immature mice vas deferens (H&E):
A) Showing lumen (L), smooth muscles layers (*) and blood vessels (BV) (x100).
B) Pseudo stratified columnar epithelium with stereo cilia (Sc), muscular layer (•) and Lumen (L) (x 1000).
DISCUSSION:

In the present study, intraperitoneal injection of lithium carbonate was used. Pharmacokinetic study showed that lithium is absorbed rapidly from the peritoneal cavity (Giles and Bannigan, 1997). The immature animals were used in the present experiment as the hypothalamic pituitary axis of it are more sensitive in the immature than in the mature animals (Swerdoloff et al., 1968).

Lithium, like other drugs, circulating in maternal blood, would pass into the alveolar cells of the mammary gland and would be excreted into the milk (Messiha, 1986), which was delivered to the experimental neonate by breast feeding. Krachler et al. (1999) indicated that lithium followed concentration gradients mechanism for transport from the mother to the newborn exists. Infants, breast fed by mother treated with lithium, had serum concentrations 10 to 50% of the mother’s level, causing most clinician disorders (Herfindal and Gourley, 2000).

During the present experiment, no deformities or discolorations were observed in fetuses of all groups as indicated early by Mroczka et al. (1983). They reported that mice maintained on 2000 mg of lithium chloride starting from 6-8 weeks of age showed no adverse effects, but their reproduction capacity was reduced. In the contrary there were lithium teratogenic effects in the developing fetuses obtained from pregnant albino rats which treated with intragastrically (7mg Li2CO3/kg B.W./day) during 0-10 day of gestation (Sharma and Rawat, 1986). Villanueva et al. (1989) also stated that the administration of lithium should be avoided during pregnancy, at least during the first trimester due to lithium teratogenic potential.

The present investigation indicated that, there was an increase in the body weight of (21-day) old mice, as the result of ingesting 25 mg Li2CO3/kg/day compared to the control ones. This agrees with Ncir et al. (2009) who reported that lithium treatment, at dose of 15.08 mg/kg body weight for 28 days, was found to induce weight gain. Also, Messiha (1986) observed a little body weight gain of nursing dams mice as a result of pre and post maternal ingestion of lithium chloride in drinking water. Otherwise, Ghosh et al. (1991) reported that body weight of lithium treated animal did not differ from that of control.

The present histological study illustrated thin tunica albuginea in the experimental immature mice testes. This was reported by (Sabry and Abdelmigid, 1988) in toad treated with testosterone for one week.

The present work indicated the presence of several abnormal tubules exhibited irregular shape. This agrees with Sasso-cerri et al. (2001) in rat testis. Also these tubules were surrounded by an increase number of flattened myoid cells as in (Sabry and Abdelmigid, 1988). For instance the tubular myoid cells are functionally very important for the development of the spermatogenic process, and together with Sertoli cells are responsible for the formation of basal lamina (Dym, 1994).

In the present study, there was a significant increase in the diameter of experimental seminiferous tubules. However, Banerji et al. (1999) showed that lithium administration resulted in a significant reduction in the diameter of seminiferous tubules in parakeet.

In several mammalian species such as rats, mice, hamsters, rams and boars, one spermatagonium stem cell went through approximately 10 mitotic divisions before differentiation into a spermatocyte (Franca et al., 2005).

In the present treated mice, the degeneration process in the testes was increased. The same was reported before in the viscacha after lithium treatment (Perz-Romen et al., 2000). One main feature of degenerating spermatocytes was the formation of clear vacuolated spaces between spermatocytes and Sertoli cell as reported by Sharpe (1994). This lead to a significant reduction in primary spermatocytes numbers (Ghosh et al., 1991; Banerji et al., 1999).

The mammalian Sertoli cells are proliferated actively during the fetal period (Orth, 1993) and the development of the male reproductive tract and the secondary sex characteristics are regulated by steroids.
secreted by fetal Leydig cells (Merchant-Larios et al., 2001; Tilmann and Capel, 2002), which are stimulated by adrenocorticotropic hormone (O’Shaughnessy et al., 2003). The balance between androgen and estrogen is very important for the normal development of the male genital tract (Sharpe and Frank, 2002). Overall, Sertoli and Leydig cell differentiation, as well as seminiferous cord formation, are the main structural changes that occurred genetically within the male gonads, and these events are crucial for male reproductive tract function (Merchant-Larios and Moreno-Mendoza, 2001; Tilmann and Capel, 2002).

The higher efficiency of spermatogenesis observed in some mammalian species is resulted from the combination of a higher Sertoli cell supporting capacity for germ cells and a greater number of Sertoli cells per gram of testis (Franca et al., 2003). In the present study, Sertoli cells lost its extensions between the germ cells, so defects of Sertoli cell that provide support and nutrition for the spermatogenic cells resulted in loss of spermatogenic cells and might lead to the destruction of this tissue and infertility according to Monsees et al. (2000) and Yano and Dolder (2002).

In the present work, disrupted Leydig cells with less nuclear diameters in lithium treated animals was observed. This was true for birds received acute or chronic lithium administration (Banerji et al., 1999). Thakur et al. (2003) found that higher doses of lithium carbonate promoted Leydig cell degeneration. The number and size of Leydig cells were the best indication of the capacity of this cell to produce testosterone (Zirkin et al., 1980). Testosterone was very important for the maintenance of quantitatively normal spermatogenesis in most mammalian species (Sharpe, 1994).

The present work indicated that as lithium injections for the mother appeared to decrease the serum testosterone level for the baby. This result agrees with Ghosh et al. (1991), Thakur et al. (2003), and Nciri et al. (2009) by modulated Leydig cell function to reduce steroid production or promoted Leydig cell degeneration in treated animal.

The disruption in the maternal endocrine system and offspring hormone levels (Johansen and Ulrich, 1969) are occurred when plasma lithium concentration was below or within the therapeutic range (Ghosh et al., 1989).

In the present study, the degree of epididymis cellular disorganization, dark stain cytoplasm, reduction of epithelial height, and loss of stereo cilia, alterations in size, shape and arrangement of the nuclei were essentially identical to those reported for goat (Goyal et al., 1994).

Lithium- administration for 31 days resulted in a significant decrease in the diameter of ductus epididymis. This agrees with Kim et al. (2004) who explained the marked shrinkage in size of the epididymis by the reduction of testosterone concentration.

In the present work the disorganization of the muscular layers of vas deferens might be as a result of low level of testosterone. This agrees with Fawcett (1986) who reported that testosterone influenced the function of smooth muscle in the vas deferens.

In conclusion, the present findings suggested that experimental immature male mice testes, epididymis and vas deferens architecture and activity were affected by chronic administration of lithium, which might be of clinically important with regarded to the possible adverse effects of lithium. The underlying mechanisms involved and the eventual consequences of these effects on androgenesis and spermatogenesis remained to be elucidated in future experiments.

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REFERENCES:


التأثير السام للكربونات الليثيوم على الجهاز التناسلي لذكور الفئران القبر بالعثاء

 автомоби محمد علي، عبر طلعت فطان، فاطمة محمد عبد القادر

قسم علم الأحياء، كلية العلوم، جامعة الملك عبد العزيز

للمعالج ونعين في مستوى هرمون تستوستيرون. أدت التغيرات الحليبية في الخصية والبربخ والوسع الناقل إلى فلة الخلايا المدوية ونوعات الخلايا ليدج. بالنسبة لخلايا سرطاني، حدد صور لامتصاداتها بين الخلايا الجزيئية. ثم تحليل الفرائض التي تم الحصول عليها لزن الجسم، وقياس قطر الأورجان المندية وقياسات البربخ ومستوى هرمون استوستيرون ونشاط المتنوع، أظهرت أن هذه الفرق بين الفئران المختلفة لم تظهر الاندماج، حيث أن الفرق بين الفئران الفارين بالعثاء وفئران دون العثاء لا تكون انعكاسة مثالية للفرق بين استخدام كربونات الليثيوم لعلاج الإكتئاب بشكل مزمن.

كلمات المراجع: كربونات الليثيوم، الجهاز التناسلي، فئران ذكور غير بالعثاء، تستوستيرون.

المحللون:

أ.د. الأحمد سفيق الدهمي، قسم علم الحيوان، علوم الزواحف، جامعة الملك سعود.
أ.د. سهام بيومي سالم، قسم علم الحيوان، علوم طبّ النبات، جامعة الملك سعود.

تتناول هذه الدراسة تأثير عقار (كربونات الليثيوم) المستخدم في علاج الإكتئاب على التركيب النسيجي للجهاز التناسلي لذكور الفئران القبر بالعثاء من جنس الأليين. تم استخدام في الدراسة عشرون من ذكور الفئران الكهاني الأحمر (أبوين الأثري من الحقل) حيث تم جمع نシュليمات بحوللي وقمتصى كل منهم للتساوي في فسيم ضابط حيث تم محلل ملحمي وقمقص تخريبي حمست بيومياً لمدة 31 يوم بجرعة مقدارها (25 مليجرام لكل). من العقار تم الحصول على 24 ماء من موزين كل من الفئران المتعاطية والرجيحة بعد الوصول لسن الفطام تم الحصول على الجهاز التناسلي لتموزين النسخ من الإنجذب والأشكال في المجموعة الخاصة والختامية (المؤسسات، التي تم حلها) لبيان التأثير الفي من الجهاز العصبي الهياليني، من الفئران على الجهاز التناسلي لذكور الفئران القبر بالعثاء عن طريق قليل من الأحماض، مرتب التفاعلات المذكورة للحصول على طبقات تجميعية سمك 5 ميكرن وصغيب بالهيدروكسيلين والفيبروزيغ والفيبروزيغ للدراسة بالمجهر الضوئي. ثُمْ بكي الكربونات الليثيوم تأثيرها حادة مثل وفيات أو تشوه الأجنحة، ولكن وجدت زيادة كبيرة في وزن الجسم.