Original Article

Lithium carbonate inducing disorders in three parameters of rat sperm

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Abstract

Background: Lithium has a significant impact in reducing the symptoms of bipolar mania but in long periods of use with therapeutic doses can cause several disorders in various organs including the reproductive system. In this study, the effect of lithium on the sperm concentration and motility and forms of abnormal cells has been examined.

Materials and Methods: Male Wistar rats under the 48-day treatment with lithium carbonate at doses of 10, 20, and 30 mg/kg bw/day were kept in standard conditions. At the end of this period, sperm cells isolated from the cauda epididymis were counted, motility was estimated, and stained with smear papanicolaou stain.

Results: In lithium-treated groups, the rate of spermatogenesis and sperm quality were reduced and was seen in a dose-dependent manner.

Discussion: Lithium alters intracellular signaling pathways such as inositol phosphate metabolic cycle and cyclic adenosine mono phosphate (cAMP) system and adenosine triphosphate (ATP) synthesis. It also interferes in the division of sex cells to produce mature sperm and showed changes in the sperm cell membrane, function, and structure.

Key Words: Lithium carbonate, morphology, motility, sperm concentration

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INTRODUCTION

Infertility is one of the essential health problems and of which 30% is due to male factors.[1] Diagnosis of reproductive system abnormalities and assessment of male fertility potential are of special importance, and common methods for determining male fertility potential include analysis of semen factors such as determination of sperm concentration, percentage of viable sperm, and percentage of sperm with normal motility and morphology using Papanicolaou staining.[2]

Among male infertility factors are primary factors like disorder in sperm transport from testicles to tubes and secondary factors such as side effects of drugs, hormones and their metabolites, toxins, urinary tract infections, and some diseases like diabetes, inflammation, and some surgical operations. In

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the twenty-first century, environmental pollution, radioactive radiation, inappropriate nutrition, noise pollution, addiction to tobacco, alcohol, coffee, and drugs are main factors of progressive disorder in reproductive processes.[3]

Over the last 50 years, researchers evaluating male fertility have paid attention to a branch of medicine named "Andrology" and its discipline "Seminology." This discipline concerns about study of sperm content of patients on the basis of factors related to a normal fertile man who has adequate sperms with normal motility and morphology (ability to reach the ovule and fertilize it).[4] Sperm maturation in humans is the result of intracellular processes and their interaction with epithelium of epididymis while passing through it. During this passage, special glycoproteins are replaced in the sperm membrane that supplies its immunological protection and stability of plasma membrane. In the female genital tract, removal of those glycoproteins causes a process known as capacitation that enables fertilization of ovules.[5] As mentioned before, spermatozoa that are present in epididymis spend their developmental stages and will change significantly as compared to those existing in testicles that have no progressive motility and their flagellar movements are minor fluctuations. At the beginning of epididymis, flagella gain active motility from the point joining the head. The first 15-16 μm has more motility than other parts. In this mode, sperm is seen with progressive developmental and irregular movements. At the end of epididymis, flagellar movements, especially the first 18-19 μm, is developed to forward progressive and regular movements.[6]

Besides epididymis and normal developmental process of sexual cells taking place in testes, changes such as an increase in osmolarity, reduction of pH, activity of mitochondrial plates in the middle of flagellum, structure of outer mitochondrial membrane, compact fibers of oxonium, an increase in concentration of calcium ions, and cAMP in sperm cell are involved in motility of sperm.[7] According to the previous studies, motility is known as one of the important parameters of qualitative evaluation of sperm cell as an impaired motility extremely affects male fertility negatively and reduces fertilization of ovules.[8] Apart from sperm motility, morphology of sperm is influenced by cytotoxic agents that target testicular tissue. An increase in abnormal forms of sperm reduces chances of fertility.[9]

In this study, the focus is on the lithium ion (+Li) among the factors affecting sperm parameters negatively and reducing male fertility. The lithium ion is the effective substance of drugs known for treating manic depression that is often used as carbonate and chloride salts and long-term use of its therapeutic doses causes certain structural and functional disorders in different body organs despite its strong effect on reduction of disease's symptoms.[10,11] In this study, sperm concentration of the liquid extracted from the end of epididymis and percentage of motility and morphology of spermatozoa were examined under the influence of lithium carbonate.

MATERIALS AND METHODS

Animal house and the course of treatment

Wistar adult male rats with a mean weight of 230 g were divided into three experimental groups and one control group, each with six rats. They were kept and treated at temperature of 23-25°C and a humidity of 60-75% for 48 days under 12h/12h light/dark cycles.

Lithium carbonate solution and used doses

The experimental groups were treated with 10, 20, and 30 mg/kg bw/day doses of lithium carbonate lithium provided by Tehran Darou Co. (with serial no. 8808), which was taken orally for a 48 day of spermatogenesis process. In each gavage, 0.5 mL of lithium carbonate solution (prepared out of sterile distilled water in order to balance daily normal water consumption) was given to the rats.

Sampling and evaluation of sperm parameters

The rats were anesthetized with 10% ketamine solution, 24 h after termination of treatment course. Their testicles and genital ducts were exposed by dissecting the skin of the lower abdomen in order to remove the distal part of epididymis. The removed epididymal tissue was cut into small pieces in 1 cc of Ham's F-10+ albumin solution with a ratio of 1 to 9 (provided from Isfahan's Royan Research Institute). The resulted sperm solution obtained was used to study sperm parameters including percentage of sperm motility, total sperm count, and determining percentage of normal and abnormal sperms, and diagnosing the type of abnormality. The sperms in the solution maintain their normal motility for 6 h and survive up to 12 h.

Total sperm count obtained from the cauda epididymis

The sperm motility was inhibited by adding 50 μL of 10% formalin to the sperm solution, and then, total number of sperm cells was estimated in 1 mL of the sperm solution prepared on a neubauer slide with a magnification of ×10.

Determining the percentage of motile sperms

After several times of sampling, the solution containing epididymal pieces was placed in the laboratory

environment and shaken gently for 20 minutes in order to extract the sperms. One drop of the sperm solution was placed on the slide, and the percentage of sperm motility with progressive movement was estimated using a light microscope with a magnification of ×10, then with ×40 in 10 fields, and the percentage of sperms with normal and abnormal morphology in the fields was recorded.

Papanicolaou staining for morphological analysis of sperm cells

In this method, the sperm fluid was fixated by adding 100 μL of 10% formalin, and then, a smear was made on the slide and Papanicolaou staining was carried out sequentially. The slide was immersed in 70% methanol for 15 min and rinsed by running tap water and then dipped in acid alcohol for 1 s and again rinsed in running tap water. After staining with OG-6 (orange) for 2-4 min, the slide was dipped in distilled water for 1 s and immersed in alcohol 1 for 1 s and then put in alcohol 2 for 2 s. Then, it was stained with EA-50 for 2-4 min. Once again, the slide was dipped in distilled water for 1 s and immersed in alcohol 1 for 1 s and then put in alcohol 2 for 2 s and air dried. Finally, the slide was immersed in xylene for 10 min and again air dried.

Morphology of the sperms in terms of normal and abnormal shape of the head and tail was analyzed in the prepared slides and mean data were recorded.[12]

Statistical analysis

Finally, the results of cell count were statistically analyzed by one-way ANOVA and Duncan post hoc tests using SPSS software and are shown in Table 1 and Figures 1-3.

RESULTS

The analysis by one-way ANOVA test showed that mean percentage of motility, number, and percentage of normal and abnormal sperm cells were not equal in different groups. The status of experimental groups and the control group to each other was determined using the Duncan *post hoc* test as shown in Table 1 and Figures 1-3.

Table 1: Comparison of mean percentage of motility, number, and percentage of normal and abnormal sperm cells in cauda epididymis

Group	Percentage of sperm motility±SD	Percentage of normal sperms±SD	Total number of sperms in cauda epididymis±SD
Control	96.00±0.89	97.33±1.21	$2.19\times10^{8}\pm9715966.24$
Lithium carbonate 10 mg/kg BW	67.66±1.21	87.50±0.55	$1.42\times10^{8}\pm3881580.43$
Lithium carbonate 20 mg/kg BW	48.17±3.43	88.00±0.89	$1.21\times10^{8}\pm5750362.31$
Lithium carbonate 30 mg/kg BW	38.50±2.59	70.83±3.43	$1.12\times10^{8}\pm3488074.92$

DISCUSSIONS

Studies have shown that men using lithium for a long time suffer from complications such as reduced steroidogenic activity and reduced efficiency of spermatogenesis process.[13] In a study on a species of Viscacha (*Lagostomus maximus maximus*), a 35-day injection of therapeutic doses of lithium showed destruction of Leydig cells followed by reduction of plasma testosterone level and remarkable reduction of sperm motility and viability in sperm solution.[14] Lithium

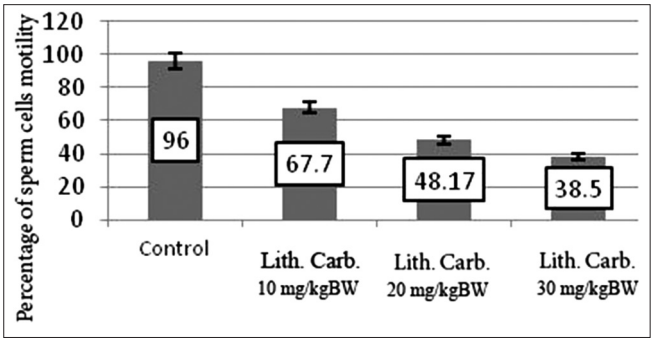

Figure 1: Percentage of sperm cell motility in groups receiving lithium shows a dose-dependent reduction

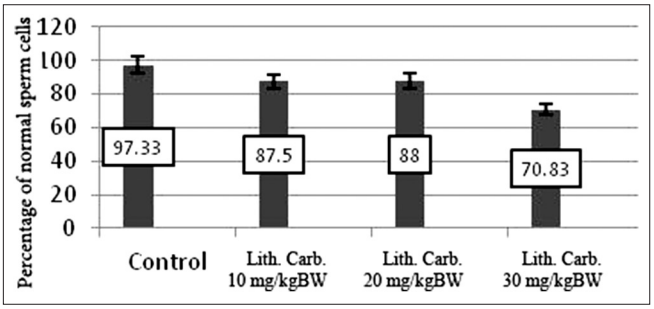

Figure 2: Percentage of normal sperm cells obtained from cauda epididymis is reduced in treatment with lithium

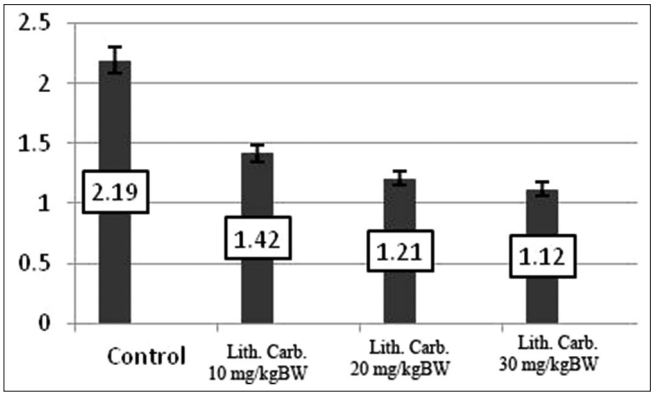

Figure 3: Number of sperm cells in the cauda epididymis is reduced in experimental groups treated with lithium

reduces the activity of hypothalamus-pituitary-gonad axis, and spermatogenesis-stimulating hormones. It also affects testicles directly that may result in profound complications. Since the lithium ion can pass through the blood-testis barrier, it affects developing sexual cells and disrupts maturation and release of spermatozoa out of seminiferous epithelium by stopping cell differentiation and growth cycle and consequently reduces the number of the total sperm count.[11,13] The results of this study also confirm the role of lithium in reduction of the produced sperm, number of motile sperms, and motility of sperm's tail and production of abnormal sperms. Papanicolaou staining showed sperms with abnormal tail. These results agree with the results of the previous studies on the effect of lithium on the function of male reproductive system. Some probable reasons for verifying results of this study will be mentioned in the following parts [Figures 4-7].

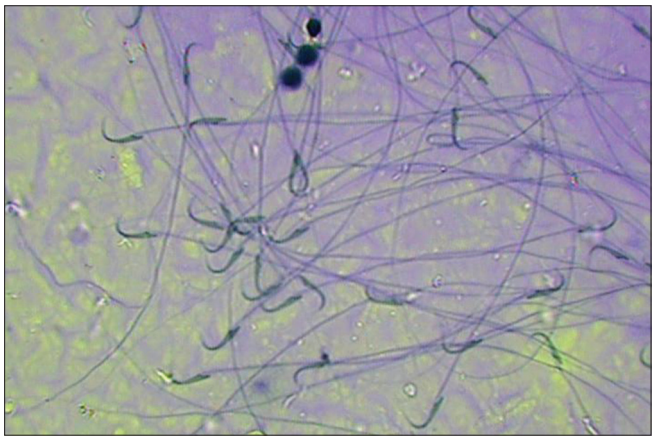

Figure 4: Papanicolaou staining of sperm cells of the control group that are seen to have normal head and tail, under a light microscope with magnification of ×100

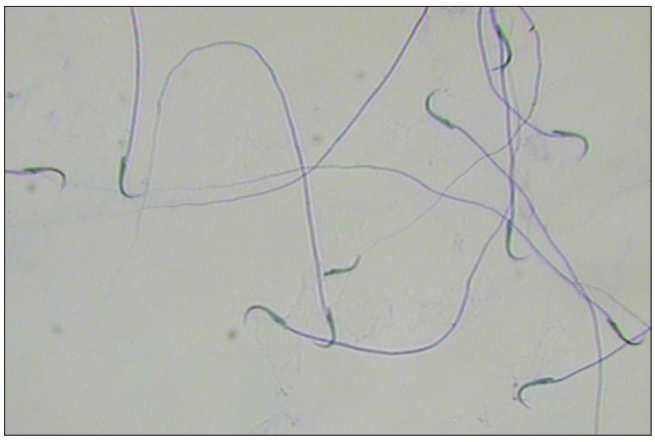

Figure 6: Papanicolaou staining of sperm cells of the group treated with 20 mg lithium carbonate under a light microscope with magnification of ×100 showed more frequent abnormal tails and total sperm density was reduced

Lithium and Cyclic adenosine mono phosphate

Cyclic adenosine mono phosphate (cAMP) molecule binds to cAMP-dependent protein kinase enzyme, stimulates dynein protein phosphorylation and slippage of microtubules of flagellum,[15,16] and increases flagellar beat frequency.[17,18] More evidence show that the lithium ion reduces sperm motility through inhibiting the activity of cAMP second messenger system, disrupting activity of adenylate cyclase enzyme, and reducing cAMP's concentration.[19]

Lithium and cytoplasmic calcium

Adhesion of cortical exocytotic vesicle membranes, hyperactivation induction, capacitation, acrosome reaction, several chemotaxic and thermotaxic pathways that are activated in aggregation, orientation, and increased motility of sperms toward the fertilization area and also the onset of sperm motility dependent on calcium and its release from intracellular reservoirs

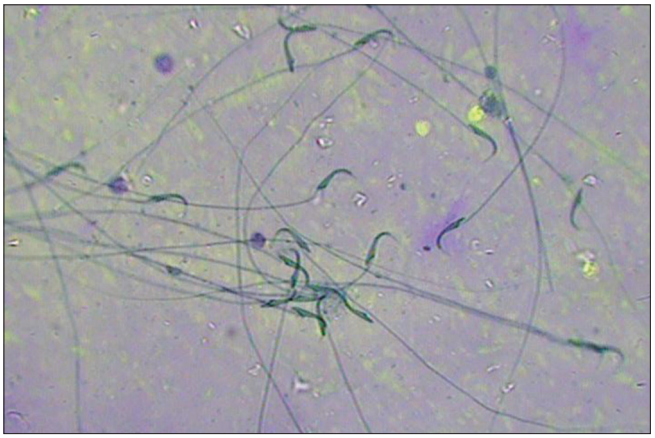

Figure 5: Papanicolaou staining of sperm cells of the group treated with 10 mg lithium carbonate showed some sperms with abnormal tails (curl-like shape) under a light microscope with magnification of ×100

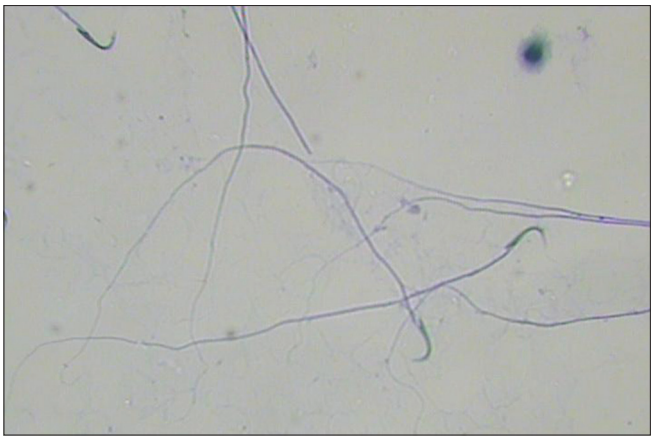

Figure 7: Papanicolaou staining of sperm cells of the group treated with 30 mg lithium carbonate under a light microscope with magnification of ×40 showed abnormal curling tails with abnormal sperms, which was more than that of 10 mg and 20 mg doses, and the least sperm density was observed using 30 mg dose

enhance these processes.[20] Moreover, reduction of cytoplasmic free calcium in developing sexual cells stops transfer of membrane exocytotic vesicles added to the cytokinetic area that causes meiosis to slow down or stop.[21] In this study, percentage of sperm cells with normal motility at all three doses of lithium, especially at the highest doses, showed a significant reduction revealing interference of lithium in normal motility of sperms that may be due to reduced cAMP signaling and low release of calcium into cytoplasm.

Lithium and production of inositol

The most important therapeutic effects of lithium in neurons, i.e., inhibiting activity of inositol monophosphatase (IMpase) enzymes and inositol polyphosphate 1-phosphatase (IPPase) and prevention of inositol reproduction in its retrieval cycle (the main source of these lipids) may slow down or stop cytokinesis of sexual cells. Phosphatidylinositols in sex cells are the main molecules adjusting cytoskeleton, cytokinetic membrane, and direct signaling of aggregation of many types of essential molecules in cells. Induction of actin polymerization in cells and production of meiosis spindle and absorption of cleavage-activating proteins like septin to the cytokinetic area along with calcium are necessary for stability of cleave furrow and actomyosin contractile ring.[22] In this study, reduction of total produced sperm at three doses of lithium carbonate was obvious and the previous studies also showed the same disorder in cell division.

Lithium and nitric oxide

Endogenous NO is produced under normal conditions to cause and support sperm normal motility, and exogenous NO results in hyperactivation of sperm motility, which is commonly observed when sperm penetrates ovula. High concentrations of NO in sperm may reduce or inhibit viability and motility of sperms. Increasing expression of the NO pathway in sex epithelium in urinary-genital tract infections and intense immunological reactions in order to create cytotoxicity against microbes can make the surrounding tissues and sperm cells toxic, that is the main cause of infertility arising from genital tract abnormalities and infections.[23] At higher doses of lithium, high concentrations of NO are produced in the sperm cell that set out an oxidative stress reaction and membrane lipid peroxidation and consequently reduce sperm viability and motility.[24,25] Therefore, besides previous reasons, remarkable reduction of motile sperms and total produced sperm may be due to the effect of nitric oxide.

Lithium, adenosine triphosphate level of sperm, and Glycogen Synthase Kinase-3 enzyme

Factors, like lithium, which change the normal shape of mitochondrial membrane disrupt mitochondrial ATP formation.[26] Furthermore, lithium is considered as a strong ATP production blocker by inhibiting GSK3 enzyme in the glycolysis pathway (main pathway for ATP production). The strong GSK3 kinase adjusts phosphorylation of glucose metabolism enzymes and its inhibitory effect on severe reduction of flagellar movements is remarkable.[27,28] In this study, it was observed that there was a significant difference between the motility speed and interval of consequent beats of the normal sperms and sperms of rats receiving lithium. This finding confirms to the mentioned reasons. In addition to the above-mentioned reasons, lithium reduces the activity of hypothalamus, pituitary gland, gonad, and level of gonadotropins and subsequent reduction of intratesticular testosterone that is directly effective in development of sex cells[13,29] and also an excessive increase in expression of the signaling pathway of WNT/CTNNB1 in Sertoli and Leydig cells and aggregation of stable structure of the molecule CTNNB1 along with changes in gene expression of cell toward stopping cell cycle in M/G2 and apoptotic induction reduce the number of sex cells and testicular somatic cells.[30]

When lithium induces cholesterol aggregation in cell[31] and increases membrane cholesterol in a long period, it reduces fluidity of cell and disrupts the membrane-dependent functions including reduction of acrosome reaction index, reduction of sperm capacity, and an increase in abnormal sperms and due to the high cholesterol, cells in the physiologic solution get hypo-osmotic stress and the normal shape of their membrane changes that is visible at the sperm's tail.[32] On the basis of these information and results of this study, treatment with lithium severely reduces active progressive motility with linear acceleration in a straight direction, especially at the highest concentration, and the flagellar movements change into slow vibrations with intervals.[33] Moreover, abnormal sperm's tail and curled membrane in this area were observed in this study.

CONCLUSION

The results presented here support that lithium carbonate exposure can considerably reduce the sperm motility, normal sperms, and total sperm count during a spermatogenesis cycle in Wistar rats. This may justify for reduced fertility, while taking this drug for a long time. This study indicated the detrimental effect of lithium drug on fertility and thereby suggested the importance of monitoring patients' health undergoing this treatment.

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