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The Effects of Lithium Carbonate Nanoparticles During Lactation On Transaminases and Liver Tissue of Neonatal Male Rat's Progeny After Maturity

Safieh. Golkhani[1,2], Akbar. Vahdati[2*], Mehrdad. Modaresi[3], Mohammad.Amin Edalatmanesh[2]

[1]Department of Biology , College of Science, Fars Science and Researches Branch, Islamic Azad University,Fars, Iran
[2] Department of Biology, College of Science, Shiraz Branch, Islamic Azad University, Shiraz, Iran.
[3]Animal Sciences Department, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran.

ABSTRACT

Lithium carbonate salt (Li2Co3) was used initially to treat gout. After that, it was used as a psychiatric drug for the treatment of manic phase of bipolar disorder. This research was carried out to study the effects of lithium carbonate nanoparticles on liver enzymes of AST, ALT, and ALP and liver tissue of neonatal rats in lactation period. Forty virgin female rats in the weight range of 180-200g and age range of 90-100 days old were divided into four groups: control, placebo, and two experimental groups. Control group received no treatment. Placebo group received 0.5cc of normal saline every day, during lactation. Two experimental groups received $1.26_{mg/kg}$ and $1.92_{mg/kg}$ nanoparticles in semi colloid form (1000_{ppm}) intraperitoneal every day during lactation. At the end of the experiment, which lasted for 24 days, blood samples were taken from heart of neonatal rats(10 rats in each) and the amount of ALT, AST and ALP enzymes were measured. Also liver tissue slides were prepared and studied histologically. According to results, lithium carbonate nanoparticles increased ALT, AST and ALP enzymes and reduced the number of hepatocytes significantly ($p<0.05$). It is probable that lithium carbonate nanoparticles affect liver cells and increase liver enzymes because of their physicochemical properties which is sign of dysfunction and histology disorders of liver.

Keywords: *Lithium carbonate nanoparticles, liver transaminases, liver tissue, lactation, male progeny, neonatal.*

Corresponding author: Avahdatim@yahoo.com

INTRODUCTION

Researches show that nanoparticles technology is growing fast. Recent developments in this area can lead to new applications (Amiri et al. 2008).
Lithium cannot be found freely in nature but enter the body through some foods, mineral waters and vegetables. The amount of lithium daily intake is about 2 mg. Compounds of this element are used frequently in automotive industries, battery making, welding, soldering and ceramics. The most frequently used compound of it is lithium carbonate (Sarbishegi et al. 2003). Salt form of lithium carbonate (Li_2Co_3) was used initially to treat gout. After that, it was used as a psychiatric drug for the treatment of manic phase of bipolar disorder. As well as other therapeutic applications Lithium carbonate can be used to treat cluster headaches, skin disorders, depression, schizophrenia, attention deficit disorder, and aggression (Sharma and Igbal. 2005, Sarbisheghi et al. 2003).
Lithium is easily absorbed by the intestine after oral intake. This element makes weak bonds with plasma proteins. Lithium is distributed throughout the body by water, and excreted from kidneys by rate of 1/5 creatinine clearance. Half-life time of lithium is about 20 hours (Katzung et al. 2010). Lithium is distributed in the human body almost uniformly. It can be found in tissues such as brain, kidney, thyroid, bones, liver and muscle cells.
Lithium is distributed widely in central nervous system and is associated with a number of neurotransmitters. It can reduce norepinephrine releasing and increase serotonin synthesis (Csutora et al. 2005). About 95% of lithium carbonate is excreted via urine, 1% in the feces and 4-5% via sweat (Ahmad shah et al. 2014).
Mechanism of lithium is not well determined. Lithium controls various enzymes which are in charge of recycling phosphoinositides of neuron membranes. This may lead to discharge the source of secondary peak of phosphatidylinositol phosphate (PIP2) which it reduces the production of inositol triphosphate (IP3) and Di-acetyl-glycerol (DAG). These secondary peaks are important in amine neurotransmission including those which are mediated with central adrenoreceptors and muscarinic receptors (katung et al. 2010).
Lithium carbonate nanoparticles are from products of nanotechnology. It has been reported that lithium reduces growth and development of fetus and infants (Opresko, 1995,

Iqbal et al. 2001). Lithium is accompanied by various side effects such as thyroid problems, impaired regulation of urine osmolality, allergic reactions and gastrointestinal symptoms. Also, many reports about polyuria complications have been published (Miroliyayi et al. 2013).

In addition to regulating the metabolic processes, liver should neutralize poisonous matters which enter the body (stranton and keoppen. 2017).

There are few studies about the effect of lithium carbonate in nano form. This study was carried out to study the transmission of different concentrations of lithium carbonate nanoparticles through mother's milk and its effect on liver transaminases.

MATERIALS AND METHODS

The study was carried out in animals' nest of Islamic Azad University- Falavarjan Baranch. Forty female virgin rats (Wistar race) in the weight range of 180-200g and age range of 90-100 days old were used. To adapt to new environment, rats were kept for 14 days in 22-26˚c, 40-60% humidity, and natural photo cycle, with free access to food and water. Also, 10 male Wistar rats were used for mating.

To menstrual synchronization, 100 micrograms of estradiol valerate was dissolved in 0.2 ml of olive oil and was injected intramuscularly into mice. After 42 hours, 50 micrograms of progesterone were injected intramuscularly (Hosseini et al. 2013). After six hours, vaginal smear was prepared. Vagina sampling was done using a swab moistened with physiological serum.

Immediately after spreading the sample on a slide, 96% ethanol was added to stabilize them and dried in the air. Then, slides were colored using Gimsa solution which was diluted at a ratio of 1 to 20 (Jamil et al. 2013).

According to the proportions and morphology of leukocytes and epithelial cells, estrus cycle stages were determined. So that in protestors stage nucleated epithelial cells were domirant, in estrous phase, horn cells without nuclei and during the next stageMatt Strauss, the same percentage of horn cells, epithelial cells and leukocytes were observed. In diestrus stage leucocytes were dominant (Hubscher et al. 2005, marcondes et al. 2002).

Microscopic observations showed that rats were synchronized in estrous stage. Then, female mature rats were divided into four-members' groups plus one male rat for one night. By observing vaginal plug day zero of pregnancy was designated, male rats were separated and ten rats were kept in each cage to spend pregnancy time. After birth, samples were divided into four groups (10 rats in each) including control, placebo, and two experimental groups. Control group received no treatment. Placebo group received 0.5cc of normal saline every day, during lactation(24 days). Two experimental groups received 1.26mg/kg and 1.92mg/kg nanoparticles in semi colloid from (1000ppm) intraperitoneal every day during lactation(24 days). Nanoparticles of lithium carbonate (Bgatova et al. 2014). were prepared from Nanozino Company (Iran). Male and female progeny were separated from day 24 which is the end of lactation period and kept for 2 months which is maturity time. After that, male progeny were anesthetized by intraperitoneal injection of $0.7_{mg/kg}$ of ketamine10% and blood samples were taken from heart(10 rats in each). Gel tubes were used to fast separation of serum. Samples were centrifuged for 15 minutes with 3000 cycle per minute. After that ALP, AST, and ALT enzymes were measured using auto analyzer machine (Alpha classic). Also, liver samples were kept in formalin 10% immediately after biopsy. Then, slides were prepared for light microscopy study by dehydration, clarification, parraffinization, molding, preparing tissue sections by microtome (with a thickness of 5 micrometers, and coloring by eosin – hematoxylin method.

Obtained date were analyzed using SPSS program, one-way analysis of variance and Tukey mean comparison at 5% probability level.

RESULTS

Liver enzymes

The amount of aspartate amino transferase (AST) in lithium groups was significantly more than placebo and control groups ($p<0.05$) during the neonatal period (graph1).

Alanine amino transferase (ALT) in lithium groups was significantly more than placebo and control groups ($p<0.05$) during the neonatal period (graph2).

Graph 3 shows that the amount of Alkaline phosphatase (ALP) in lithium groups was significantly more than placebo and control groups ($p<0.05$) in neonatal period.

Hepatocytes

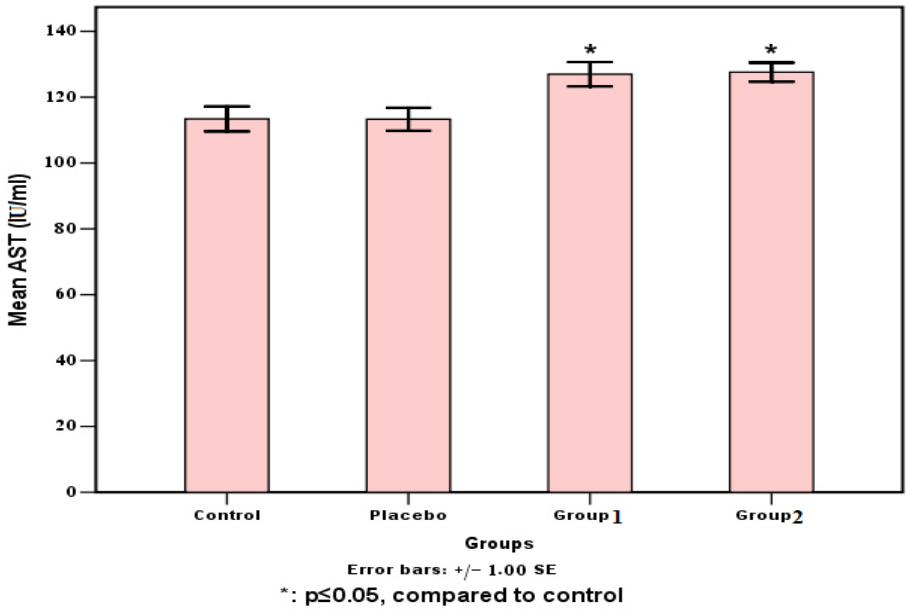
Microscopy study of liver slides showed that control and placebo groups were not different but considerable differences were observed in lithium groups.

in control and placebo groups Liver lobules were observed obviously plus romak ropes (which are aggregation of hepatocytes(H)) and central vein(CV). In some parts of slide, in lobule spaces components of triad portal (portal vein, bile duct and hepatic artery) were observed (figures 1, 2).

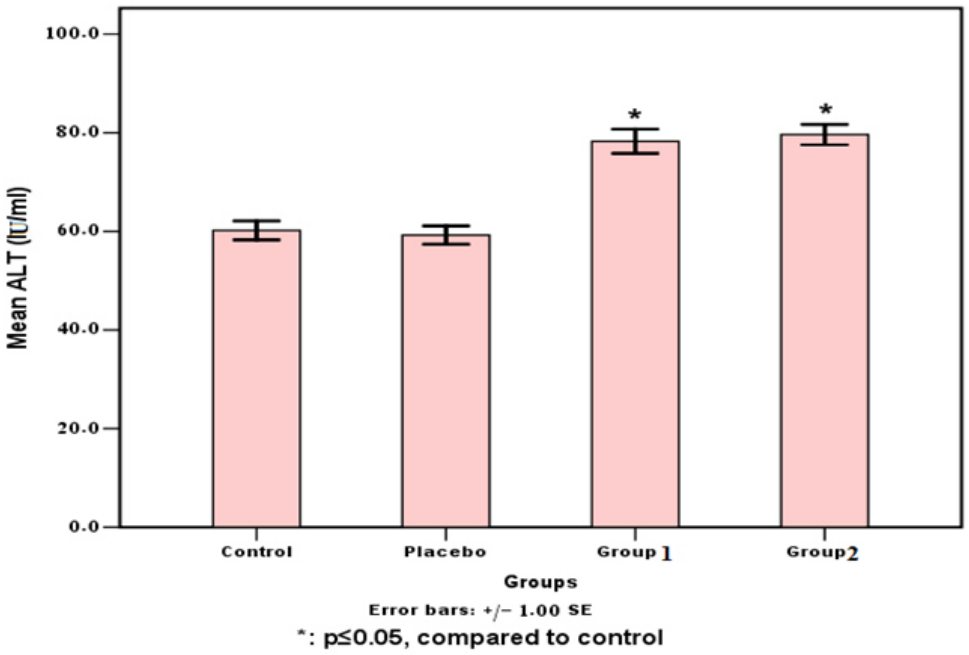
$1.26_{mg/kg}$ dose of lithium during neonatal period showed obvious acidophilus in hepatocytes(H) and somewhat high blood of central veins(CV) (Fiqure3).

Also, studies showed that 1.9 $_{mg/kg}$ dose in neonatal period caused pathological changes such as total destruction of lobules, destruction and necrosis of hepatocyte(H). Obvious hyperemia was also sawn in central veins(CV) (figure 4).

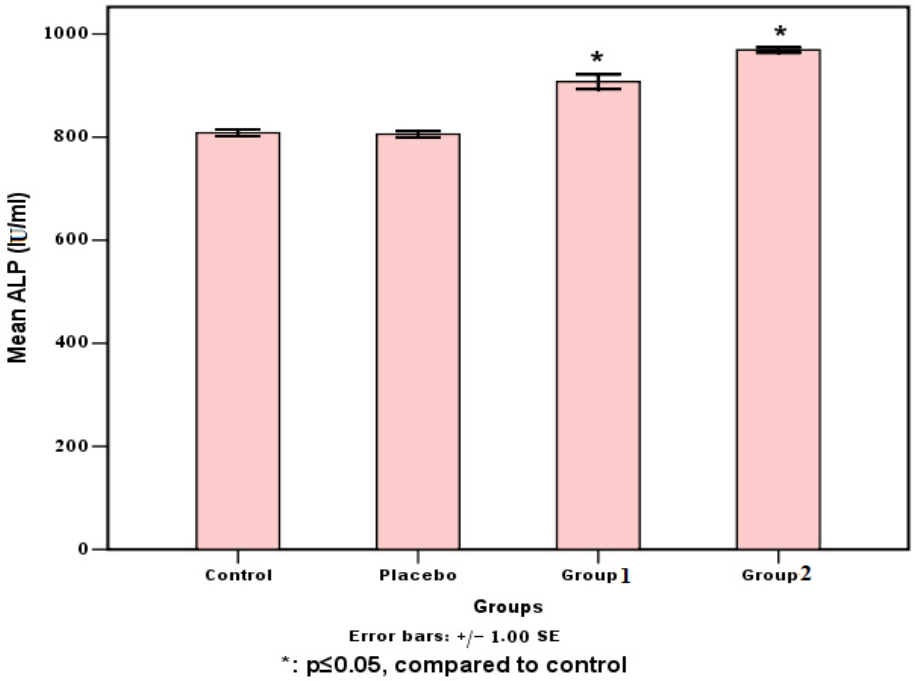
Average and standard deviation of hepatocytes are presented in graph 4. Significant reduction was observed in the number of hepatocytes of lithium groups ($p<0.05$).



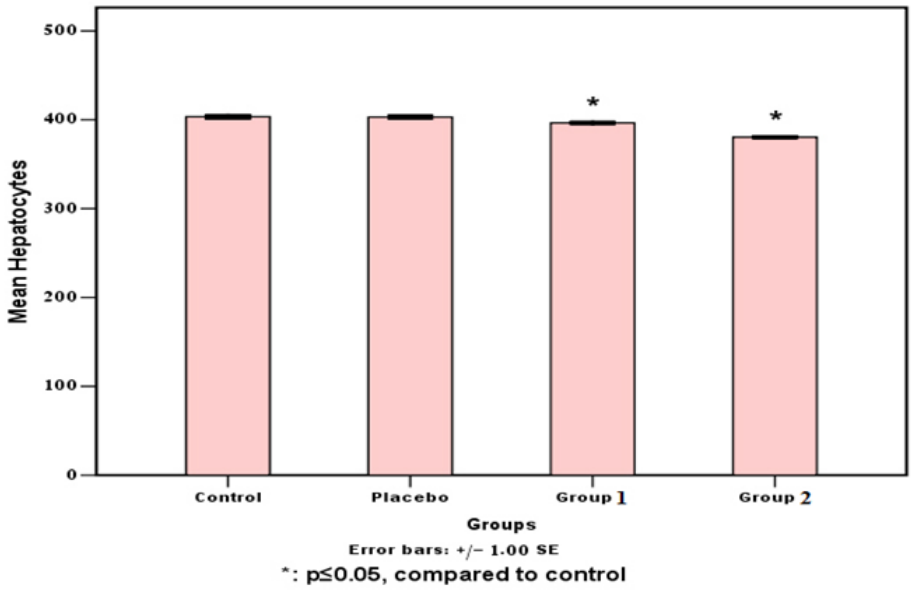
Graph1. Mean comparison of AST amount
*** significant difference between experimental groups and control (p<0.05).**



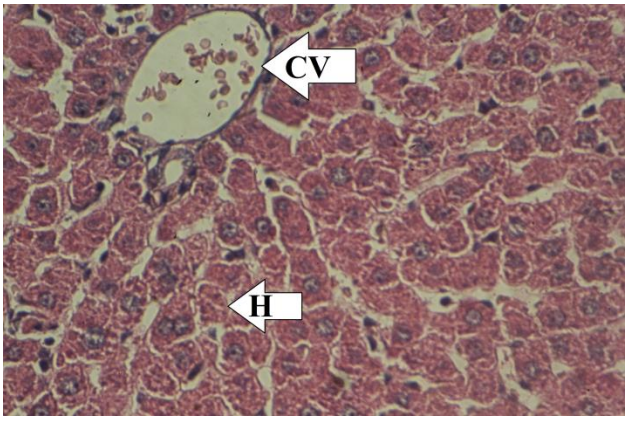
Graph2. Mean comparison of ALT amount
*** significant difference between experimental groups and control (p<0.05).**

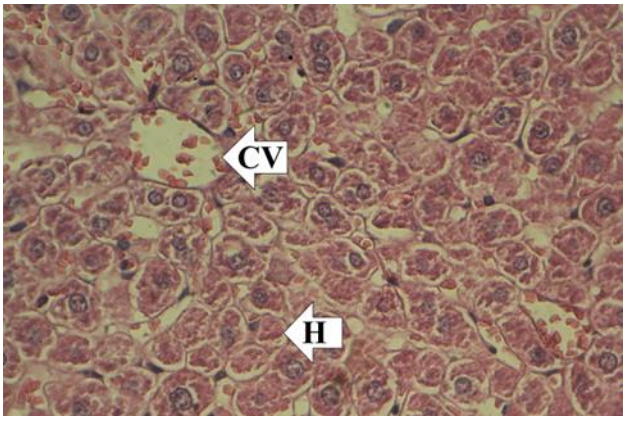


Graph3. Mean comparison of ALP amount
*** significant difference between experimental groups and control (p<0.05).**



Graph 4. Mean comparison of the number of hepatocytes
*** significant difference between experimental groups and control (p<0.05).**





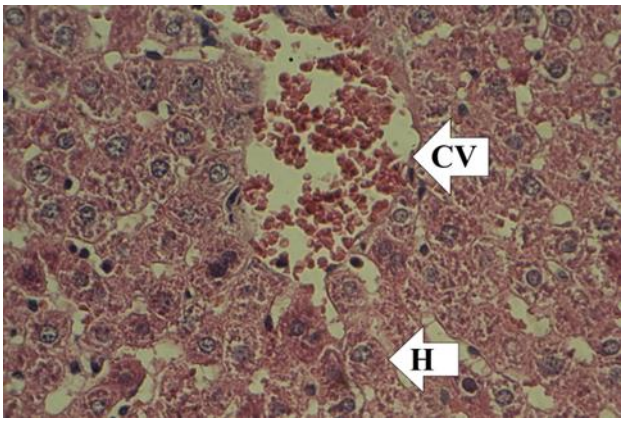


Figure1. cross section of liver tissue in control group (40x)

Figure2. cross section of liver tissue in placebo group (40x)

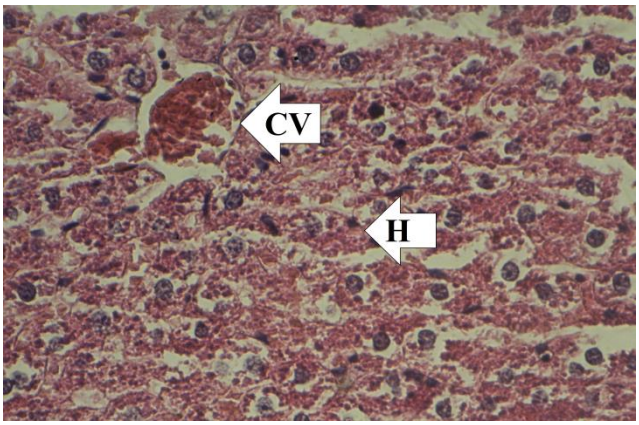


Figure3. cross section of liver tissue in first experimental group (40x)

Figure4. cross section of liver tissue in second group(40x)

DISCUSSION

Lithium is a monovalent cation from first group of periodic table which has close relationship with sodium and potassium. Lithium can replace sodium in many sodium canals which one of them is hydrogen-sodium exchange canals type III (NHE3III) in kidneys proximal tubules, sodium-potassium chloride exchange pump type II (NKEC2) in ascending part of loop of Henle and epithelial sodium channel (ENC) in renal central collector tubules. Lithium salts may increase urinary excretion of sodium acute or chronic. The most important disorders are because of interfere in aldosterone performance, which increases in ENaC gene expression in apical membrane of kidney. Lithium controls this effect and cause high excrement of sodium (Nielsen et al. 2006, Stone. 1999).

Lithium carbonate is absorbed via digestion system completely and can pass the blood - placental barrier easily so that has equal serum concentration in fetus and mother. It also can be secreted in milk which the concentration in milk is half of mother's serum concentration (Hill et al. 2008).
In a study, lithium carbonate reduced testosterone, FSH, and LH hormones in male mice (Seyedhosseinighaheh et al. 2013).

Nano particles' pollution has been proposed lately as a new, dangerous problem (Revel, 2006, Shi et al. 2006).

In this study the effects of using lithium carbonate nanoparticles on liver enzymes and liver histology were evaluated during neonatal period and results showed that the amount of AST,ALT, and ALP enzymes were increased significantly ($p<0.05$).
Rezaeizarchi (2011) studied titanium nanoparticles and reported increase in ALT amount. Bgatova et al. (2014) studied the effects of lithium carbonate nanoparticles on tumor growth and reported that injection of lithium carbonate nanoparticles in tumor margins caused tumor cells necrosis, destruction of the tumor vascular bed and attracting neutrophils and macrophages to the center of tumor.
Kim et al. (2009) in a study investigated the effect of silver nanoparticles on liver tissue of rats. Tissue damages were observed in liver lobules, copfer cells, and sinusoids. Jafarzadehsamani et al. (2015) also studied silver effects on liver and reported that dose increment increased tissue damages.
In zamani et al. study (2013), nanosilver particles enforced destructive effects on liver tissues and caused necrosis and degeneration of liver tissue plus accumulation of inflammatory cells.
Since ALT enzyme is a more specific indicator for liver injuries, and damages to liver cells releases this enzyme, probably the reason of ALT increment in this study was destructive effect of lithium carbonate nanoparticles. Also, bile duct obstruction

causes ALP increment. Increase in AST and ALT enzymes can be due to increase in their anabolism or decrease in catabolism (Christ-Crain et al. 2004).

Stability and accuracy of hepatocytes is essential for vital functions of liver (Mokhtari et al. 2007). Probably, lithium carbonate nanoparticles disrupte this stability because of their physicochemical properties and cause liver dysfunction. Many studies have been done about nanoparticles properties. For instance, silver nanoparticles had negative effects on fish cells (Wise et al. 2010). Also, they reduced viability and reproduction of liver cells because of their physicochemical properties.

Silver nanoparticles can produce reactive oxygen species (ROS) and free radicals and high ROS aggregation can start inflammatory responses and lead to mitochondrion destruction. Therefore, glutathione sulfate hydrogenase (GSH) level will be decreased because of inflammation. So, apoptosis factors such as *cytochrome-c* are released and led to cell death (Zamani et al.2013). We can conclude that probably lithium carbonate nanoparticles can act the same way.

CONCLUSION

On the whole, results of this study confirmed toxic effects of lithium carbonate on liver of progeny via lactation. More studies are proposed to predict effects and also action mechanism of this matters.

REFERENCES

1. Ahmad Shah, N. Bhat, M. Shadad, SH Saleem Itoo, M. Ahamad Shah, B.and Ahmad Khan, J. 2014. Effects of lithium carbonate on the microanatomy of thyroid gland of albino rats. International Journal of Research in Medical Sciences, 2(1):279-284.
2. Amiri, S. Nikkam, N. and Sahebinejhad, M. 2008. Statistical analysis of production technology and patents related to nanotechnology in different countries. Journal of Science and Technology Policy, 3: 1-11.(In Persian).
3. Bgatova, NP. Borodin, YI. Makarova, VV. Pozhidaeva, AA. Rachkovskaya, LN. and Konenkov, VI. 2014. Effects of Nanosized Lithium Carbonate Particles on Intact Muscle Tissue and Tumor Growth. Journal of Bulletin of Experimental Biology and Medicine, 157(1): 89-94.
4. Christ-Crain, M. Meier, Ch. Puder, J. Staub, JJ. Peter R. Huber, PR. Ulrich Keller, U. and Müller, B. 2004. Changes in liver function correlate with the improvement of lipid profile after restoration of euthyroidism in patients with subclinical hypothyroidism. EXCLI Journal, 3:1-9.
5. Csutora, P. Karsal, A. Nagy, T. Vas, B. Kovacs, G. Rideg, O. Bogner, P. and Miseta ,A. 2005. Lithium induces phosphoglucomutase activity in various tissues of rats and in bipolar patients. International Journal of Neuropsych pharmacol ,1:1-7.
6. Hill, EJ. Woehrling, EK. Prince, M. and Coleman, MD. 2008. Differentiating human NT2/D1 neurospheres as a versatile in vitro 3D mode system for developmental neurotoxicity testing. Toxicology 249(2-3): 243-250.
7. Hosseini, E . Frozanfar, M . and Payehdar, A. 2013. The effect of hydroalcoholic extract of purslane on serum concentration of esterogen, progesterone, prolactin and gonadotropins in mature female rats. Journal of Shahrekord University of Medical Sciences, 15(5): 12-21. (In Persian).
8. Hubscher, CH. Brooks, DL. and Johnson, JRA. 2005. quantitative method for assessing stages of the rat estrous cycle. Biotechnic & Histochemistry, 80(2): 79-87.
9. Iqbal, MM. Ryan, W. and Passman, TE. 2001. Effects of antimanic mood-stabilizing drugs on fetuses, neonates, and nursing infants. Southern Medical Journal, 94:304-22.
10. Jafarzadeh Samani, R. Heydarnejad, MS. and Kabiri Samani, M. 2015. A survey of acute histopathological effects of silver nanoparticles on liver, kidney with blood cells during oral administration in male mice (Mus musculus). Journal of Shahrekord University of Medical Sciences, 17(4): 97-107. (In Persian).
11. Jamil, F. Behnam Rassouli, M. Mahdavi Shahri, N. and Dehghani, H. 2013. Study of the Effects of Hyperglycemia and Insulin Therapy on Uterus Histology and Estrous cycle in Wistar Rat. Journal of Cell & Tissue, 4(2): 149-157. (In Persian).
12. Karla, C.Yogeshkumar, M. and Alexander, M. 2010. Nano silver as a new generation of nanoproduct in biomedical. Trandsin Biotecnology , 28 : 580-588.
13. Katzung, B. G. Masters, S. B. and Trevor, A. J. 2010. Basic & Clinical Pharmacology. 12th ed. California, USA, 1245 P.
14. Kim, S. Cho,i JE. Choi, J. Chung, KH. Park, K. Yi, J. et al. 2009. Oxidative stress-dependent toxicity of silver nanoparticles in human hepatoma cells. Toxicol in vitro, 23(6): 1076-1084.
15. Marcondes, FK. Bianchi, FJ. and Tanno, AP. 2002. Determination of the estrous cycle phase of the rats: some helpful considerations. Brazilian Journal of Biology, 62: 609-614.
16. Miroliyayi, A.A. Shasb, A. and Ghaeli, P. 2013. Kidney complications arising from the use of lithium. Iranian Journal of Psychiatry and Clinical Psychology, 19 (3): 241-243. (In Persian).
17. Mokhtari, M. Shariati, M. and Geshmardi, N. 2007. Oral effects of lead on thyroid hormones and liver enzymes in rats. Journal of Hormozgan University of Medical Sciences, 11(2): 115-20.
18. Monteiro-Riviere, NA. Nemanich, RJ. Inman, AO. Wang, YY. and Riviere, JE. 2005. Multi-walled carbon nanotube interactions with human epidermal keratinocytes. Toxicology Letters, 155(3): 377-84.
19. Nielsen, J. Kwon, TH. Christensen, BM. Frokiaer, J. and Nielsen, S. 2006. Lithium-induced NDI in rats is associated with loss of alpha-ENaC regulation by aldosterone in CCD. American Journal of Physiology Renal Physiology, 290(5)1222-1233.
20. Opresko, DM. Toxicitysummeryforlithium. 1995. :Availablefrom:http ://www .rislc. lsd. ornl.gov/lith.shtml.
21. Peter, HH. Irene, BH. and Oleg, VS. 2004. Nanoparticles-known and unknown health risks. Journal of Nanobiotechnology, 2(1): 12-27.
22. Revell, PA. 2006. The biological effects of nanoparticles. Nanotechnology Perceptions, 2: 283-98.
23. Rezaei Zarchi, S. 2011. Effect of Titanium Dioxide Nanoparticles on The Amount of Blood Cells and Liver Enzymes in Wistar Rats. Journal Shahid Sadoughi Univ Med Sci, 19(5): 618-26. (In Persian).
24. Sarbishegi, M. Mahmoudzadeh, Sagheb, HR. Heidari, Z. and Rafighdoost, H. 2003. Investigation of Fetotoxic Effects of Lithium Carbonate on Growth Parameters in Rat's Embryo. Tabib shargh, 5(2):115-121. (In Persian).
25. Seyedhosseini Ghaheh, H. Sohrabi, D. Mousavi, M. Ghanbari Gorgani, M. Sarshar, S. Mazoumzadeh, S.

and Hosseini, Z. 2013. Effects of Lithium on Levels of Testosterone, Pituitary Gonadotropins, and Testis Tissue in Adult Male Rats. Journal of Isfahan Medical School, 31(227): 191-199. (In Persian).

26. Sharma, SD A. and Iqbal, M.2005. Lithium induced toxicity in rats: a haematological, biochemical and histopathological study. Biological and Pharmaceutical Bulletin, 28: 834-837.
27. Shi, JW. Zhang, F. Zhao, YL. and Chai, ZF. 2006. Acute toxicity of nano- and micro-scale zinc powder in healthy adult mice. Toxicology Letters, 161(2): 115-23.
28. Shvedova, A. Castranova, V. Kisin, E. Schwegler-Berry, D. Murray, A. Gandelsman, V. et al. 2011. Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells. Journal of Toxicology and Environmental Health, 66(20): 1909-1926.
29. Stanton, B. A. Koeppen, B. M. 2017. Berne & Levy Physiology. 7th ed. Books & Journals Elsevier, 870p.
30. Stone, KA. 1999. Lithium-induced nephrogenic diabetes insipidus. Journal of the Ameeican Board of Family Medicine, 12(1):43-47.
31. Wise, JP. Sr Goodale, BC. Wise, SS. Craig, GA. Pongan, AF. Walter, RB. et al. 2010. Silver nanospheres are cytotoxic and genotoxic to fish cells. Aquatic Toxicology, 97(1): 34-41.
32. Zamani, N. Naghsh, N. and Fathpour, H. 2013. Comparing poisonous effects of thioacetamlde and silver nanoparticles on enzymic changes and liver tissue in Syrian mice. Zahedan Journal of Research in Medical Sciences, 16(2): 54-7.