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EVALUATING THE IMPACT OF LITHIUM CARBONATE NANOPARTICLES ON THE ACTIVITY OF THE PITUITARY-GONAD HORMONE AND LIVER TRANSAMINASES IN THE NEONATAL PERIOD IN ADULT FEMALE RATS

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ABSTRACT

Infertility is considered as one of the most common problems in human communities. It is defined as lack of incidence of the pregnancy after one year of regular sexual intercourse and without using contraceptive methods. In addition, liver diseases are among the most common diseases in the community. As a result, many diseases, bad nutritional statuses, obesity or overweight, low mobility, consumption of alcoholic drinks, and taking some drugs are caused. The current research was conducted to examine the impact of lithium carbonate nanoparticles on pituitary-gonad activity and liver in female rats (Wistar race). In this research, 40 female rats (Wistar race) at weight range of 200-180 g were used as parent. All parent samples were randomly divided into four groups (each group containing ten rats), including control, placebo, experimental, 1 and 2 groups after the delivery. Control group received no treatment, and the placebo group received 0.5 cc of normal saline in daily basis during the breast-feeding period, and two experimental groups 1 and 2 received the values of 1.26 mg / kg of the rat weight and 1.90 mg / kg of the rat weight of lithium carbonate nanoparticles, respectively, intraperitoneally daily during breast-feeding. At the end of experiments lasted for 24 days, blood samples were taken from the heart of the female neonates (n=10 in each group). After separating the serum, concentration of the hormones estrogen, progesterone, FSH and LH, and the enzymes AST, ALT and ALP was measured and ovarian and liver tissues were separated. Then, histologic stages of slides were prepared and evaluated in terms of histology. Findings indicated that lithium carbonate nanoparticles caused significant reduction in the values of FSH, LH, estrogen and progesterone hormones and the number of primordial, pre-enteral, and antral follicles, and yellow objects and hepatocytes, and it caused significant increase in liver enzymes and atrophy follicles ($P < 0.05$). Given the characteristics of the lithium carbonate nanoparticles, it is supposed that lithium carbonate nanoparticles destruct the sexual cells, leading into reduced sexual hormones and increased liver enzymes.

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Introduction

Infertility is considered as one of the most common problems in human communities. It is defined as lack of incidence of the pregnancy after one year of regular sexual intercourse and without using contraceptive methods [1]. Various studies have evaluated the prevalence rate of the infertility in different communities in the world and reported different findings [2].

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Multiple epidemiological studies conducted in the recent years suggest an increase in the prevalence rate of the infertility caused by high consumption of foods containing high fat, tobacco, reduced human mobility, and pollutions resulting from mechanical life in industrialized and developing countries [3]. Metals are regarded as important and toxic pollutants in various sectors of the life [4]. The mono-valent cation lithium belongs to the group of alkali metals from the group 1 in the periodic table of elements, which has close similarity with sodium and potassium. Lithium can be replaced by sodium in many sodium channels, which the most important of them include exchanger channels of sodium-hydrogen type 3 in tubules of the proximal kidney, exchanger pump of sodium-potassium type 2 and mucosal sodium channel in the central aggregating tubule of the kidney [5]. Studies have indicated that lithium might result in an increase in proliferation or stopping in the cell cycle and apoptosis in the cells of the mammals, depending on the cell type [6]. The salty form of lithium carbonate (Li2CO3) was used initially for treatment of the gout. Then, it was used as psychiatric drug to treat the mania phase bipolar disorder. In addition, other applications of the carbonate lithium include the treatment of cluster headaches, skin disorders, treatment of depression, schizophrenia, aggression, and disordered attention deficit [7]. Lithium is distributed uniformly human body. It can be found in tissues such as the brain, kidney, thyroid, bone, liver, and muscle cells. Lithium is distributed widely in the central nervous system and it is linked with some neurotransmitters. Lithium can lead into reduced release of norepinephrine and an increase in the serotonin synthesis [8]. Approximately, 95% of lithium carbonate is excreted through, 1% through stool, and 4 to 5% through sweat [9]. The action mechanism of the lithium has not been defined well. This drug inhibits several enzymes, which are involved in recycle of phosphoinositides of nerve membrane. This action might result in secondary peak source drain, namely, phosphatidylinositol bisphosphate (PIP2), reducing the inositol triphosphate (IP3) and diacyl glycerol (DOG) production. These secondary peaks are vital in neuronal transmission of the amines, including those mediated by central adrenoreceptors and muscarinic receptors [10]. Lithium carbonate nanoparticles are considered among the nanotechnology products. In many of the studies, it has been reported that using lithium during pregnancy and after it will decrease the growth of the fetus and infant [11, 12]. Using lithium is associated with multiple complications, including thyroid problems, dysfunction in regulating the urinary osmolality, allergic reactions and gastrointestinal complications. Additionally, it has been reported that great number of the patients used lithium were affected by polyurea [13]. As liver plays vital role in metabolism of the hormones, endocrine changes might be found after the liver diseases [14]. As liver damages are developed, the liver will not be able to perform metabolic actions and regulate different hormonal systems, and thus, regulation of the endocrine systems in the body will be disrupted. Therefore, deficiency in liver function will result in various endocrine axes in the body are influenced, which one of the main impacts of this deficiency in the liver function is the clinical manifestations of hypogonadism and feminization in males. These effects are more manifested as testicles atrophy (50-75%), sexual disability (79%), impairment in genital erection, reduced number of sperm, and reduced sexual desire [15]. Feminization and hypogonadism in males suffering from liver diseases are due to reduction in the level of serum testosterone and a relative increase in blood circulation estrogens. The similar impacts are created in women suffering from liver diseases, while few studies have been conducted in women, since it has no much importance clinically among the women, especially in early stages [16]. Given lithium carbonate properties and function of the nanoparticles, this research was conducted to examine the impacts of lithium carbonate nanoparticles on the activity of the pituitary-gonad axis and liver activity during the neonatal period in adult female rats.

Methodology

This experimental research was carried out in the animals' nest of Islamic Azad University of Falaverjan in April 2016. In this research, 40 female rats (Wistar race) at weights of 200 to 220 grams and the age of 90 to 100 were used as parents. Then, they were exposed to similar conditions for 14 days at temperature of 22-26 ° C and relative humidity of 40 to 60% and the natural light cycle to adapt with new environment. All of them had free access to water. In addition, 10 adult male rats (Wistar race) were used for mating. First, 100 µg of estradiol valerate was dissolved in 0.2 ml of olive oil in this research to co-cyclize the rats and it was injected to rats intramuscularly, and 50 µg progesterone was injected intramuscularly 42 hours later [17]. Vaginal smear was prepared from the rats six hours after injecting. For this purpose, vaginal sampling was conducted by using a swab wetted with physiological serum. After expanding the samples, 96% alcohol was added to the lime to stabilize them, and they were dried in free air. The slides were then stained for 15 minutes with a Giemsa solution, which has been diluted with ratio of 1 to 20 [18]. Estrus period phases were specified based on the ratios and morphology of leukocytes and epithelial cells. Accordingly, in the pro-estrus phase, nucleophilic epithelial cells were dominant, in the estrus phase, the horn cells without nucleus were dominant, and in the next phase (estrous), the same percentage of horn cells, nucleophilic epithelial cells, and leukocytes were seen in the expansion. In the di-estrous phase, leukocytes were dominant [19, 20]. Microscopic observations showed that all rats were co-cycled in the estrous phase. Then, adult female rats were divided into 4 groups. In each group, one adult male rat was kept in separate cages for 3 nights for mating. By observing the vaginal plaque, the zero day of pregnancy was specified and male rats were separated from female rats and 10 female rats were placed in one group to complete the pregnancy period. Parent samples were randomly divided into four groups after the delivery (each group containing 10 rats). These groups included control, placebo, experimental 1 and experimental 2 groups. Parent rats in the control group received no treatment and rats in the placebo group were injected 0.5 ml normal saline, as the impact of stress, in daily for 24 days, which lasted for 24 days. Experimental groups 1 and 2 received.26 mg/kg of the rat weight and 1.09

mg/kg of the rat weight lithium carbonate nanoparticles, respectively, in daily base during the breast-feeding period. They were prepared in the form of semi-colloid 1000 ppm with an average diameter of 10 nm from Nanozino Company of Tehran. Then, the male and female neonates were separated 24 days after their birth and they were kept until their 3-month age (puberty). Then, neonates were anesthetized blood samples were prepared from their heart by injecting 0.7 mg/kg ketamine 10% of the female neonates (n=10 in each group). The collected samples were centrifuged for 15 minutes at 3000 rpm. Then, the serum was separated from the clot. ELISA method (Stat Fax 2100) was used to evaluate the level of estrogen, and quantitative method of luminescence (SIMENS) was used to valuate FSH and LH hormones, and auto analyzer (Alpha classic) was used to evaluate ALP, AST and ALT enzymes. In addition, tissue samples of the ovary and liver were placed separately inside the formalin 10% after leaving the animal body. Then, cross-sectional tissue was taken from the samples and illuminating, paraffinization, molding, and preparation of tissue sections were conducted by micrometer with a thickness of 5 micrometers after the dehydration stages. Then, Eosin-Hematoxylin method was used for staining. After the preparing the pathology slides, they were examined fusing optical microscopy. After collecting the data, data were analyzed by SPSS software and ANOVA and Tukey post hoc test at a significant level of P <0.05.

Findings

Neonatal impacts of lithium carbonate nanoparticles on the activity of the pituitary-gonad axis in adult female rats. Based on the Table 1, significant reduction can be seen in LH, FSH, estrogen and progesterone hormones concentrations in the experimental groups 1 and 2, compared to control and placebo groups during the neonatal period (P <0.05). In addition, findings obtained by counting primitive follicles, pre-antral, antral and yellow objects indicated significant reduction (P <0.05) and counting the atrophic follicles indicated significant increase (P <0.05) in number of these cells in the experimental groups 1 and 2 compared to control and placebo groups (Table 2). Microscopy investigation of sections prepared from ovarian tissue suggests pathological variations in two experimental groups 1 (Figure 3) and 2 (Figure 4) compared to control group (Figure 1) and placebo group (Figure 2) in the form of blood congestion and atrophy of follicles.

Table 1. Comparing the mean serum level of pituitary-gonad axis hormones in adult female rats

Hormons / Groups	FSH(mIU/dl) Mean ±SD	LH(mIU/dl) Mean ±SD	Estrogen (ng/dl) Mean ±SD	Progesterone (ng/dl) Mean ±SD
Control	**0.852 ± 0.0551**	**0.844 ± 0.0490**	**58.646± 2.1860**	**8.592 ± 0.7009**
Placebo	**0.845 ± 0.0452**	**0.825 ± 0.0558**	**56.245± 3.1549**	**8.537 ± 0.7203**
experimental Group1	**0.386 ± 0.0337***	**0.447± 0.0585***	**48.427± 6.5457***	**6.378 ± 0.4658***
experimental Group2	**0.320 ±0.0402***	**0.382 ± 0.0639***	**35.182± 6.8545***	**5.384 ± 0.7675***

The sign * suggests a significant difference between experimental groups and control group (p <0.05)

Table 2. Comparing the mean number of sexual cells in adult female rats

Cells / Groups	primordial follicles	preantral follicle	Antral follicle	Corpus luteum	Atrophy follicle
Control	4.970 ± 0.3773	5.830 ± 0.4762	5.070 ± 0.4084	4.350 ± 0.4378	4.510 ± 0.6045
Placebo	4.700 ± 0.3266	5.710± 0.3213	4.970 ± 0.3020	4.620 ± 0.6233	4.570 ± 0.6617
experimental Group1	3.740 ± 0.5400*	3.780± 0.4022*	3.570 ± 0.2497*	3.470 ± 0.5034*	6.180 ± 0.5514*
experimental Group2	2.670 ± 0.4057*	2.710± 0.2846*	2.680 ± 0.3882*	2.680 ± 0.2936*	7.160 ± 0.3471*

The sign * suggests a significant difference between experimental groups and control group (p <0.05)

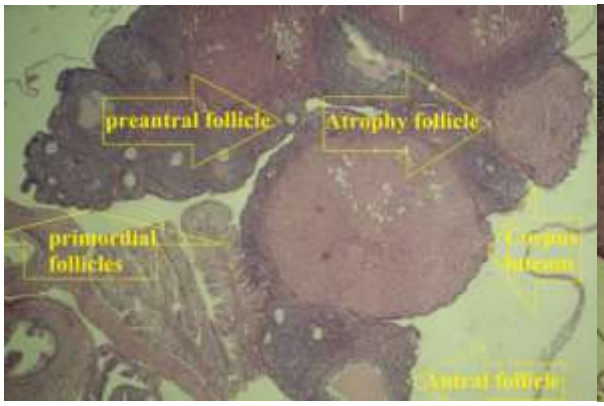

Figure 1. Cross section of ovarian tissue in control group (10X)

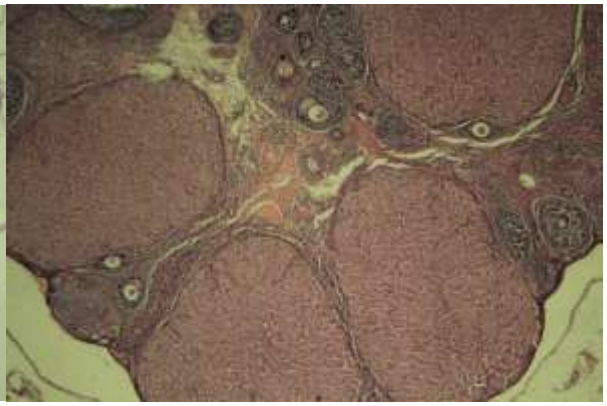

Figure 2. Cross section of ovarian tissue in Placebo group (10X)

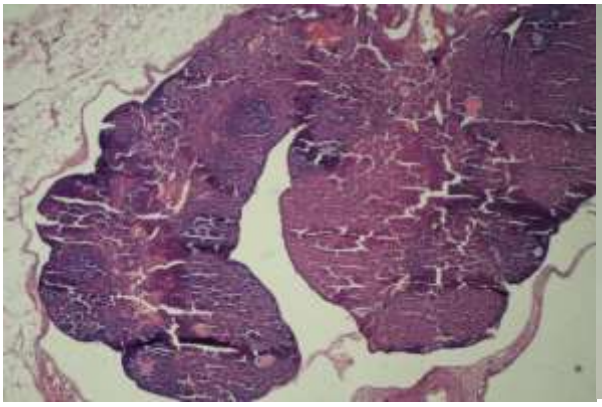

Figure 3. Cross section of ovarian tissue in experimental group 1(10X)

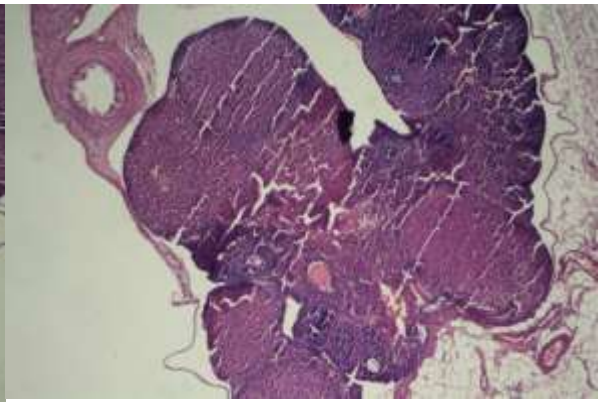

Figure 4. Cross section of ovarian tissue experimental group 2(10X)

Neonatal impacts of lithium carbonate nanoparticles on the liver function in adult female rats:

Examining the change at concentration of aspartate aminotransferase (AST) enzymes among different treatments of the experiment indicated increased serum level of this enzyme in experimental groups 1 and 2, compared to control and placebo groups during the neonatal period, which this increase was not significant in experimental group, but it was significant in the experimental group 2 ($P < 0.05$). Additionally, examining the change in mean concentration level of Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) enzymes among the different treatments showed significant increase in these enzymes in the experimental groups 1 and 2, compared to the control and placebo groups ($P < 0.05$). Findings of the tissue studies and counting the liver cell indicated no significant difference in the experimental group 1 compared to control and placebo groups. However, significant reduction was seen in the number of hepatocytes in the experimental group 2 (Table 3) ($P < 0.05$). Microscopy investigation of the sections prepared from the liver tissue indicated no difference among control group (Figure 5) and placebo group (Figure 6) and experimental 1 group (Figure 7) in the form of appearance form, but significant difference was seen in experimental group 2 (Figure 8) in this regard. In the control group, placebo group, and experimental 1 group, liver lobules along with were seen clearly and some parts of the lam, in the spaces between lobules of the triad port, that is, port vein, bile ducts and hepatic artery are seen. In the experimental 2 group, two clear acidophilus are seen in hepatocytes. In addition, pathologic variations are seen in the form of destruction of hepatocytes and their necrosis, and clear blood congestion is seen in the central veins.

Table 3. Comparing the mean serum level of liver enzymes and hepatocytes in adult female rats

Enzymes and Cells / Groups	AST (ng/ml)	ALT(ng/ml)	ALP(ng/ml)	Hepatocyte
Control	113.40 ± 11.993	42.47 ± 1.526	811.80 ± 39.673	402.00 ± 4.967
Placebo	114.80 ± 12.318	44.50 ± 1.579	827.50 ± 16.216	403.00 ± 4.784
experimental Group1	126.98 ± 11.732	54.65 ± 4.455*	944.60 ± 39.119*	395.60 ± 4.169
experimental Group2	127.60 ± 9.095*	64.45 ± 4.244*	954.80 ± 28.287*	378.00 ± 3.801*

The sign * suggests a significant difference in experimental groups compared to control group ($p < 0.05$)

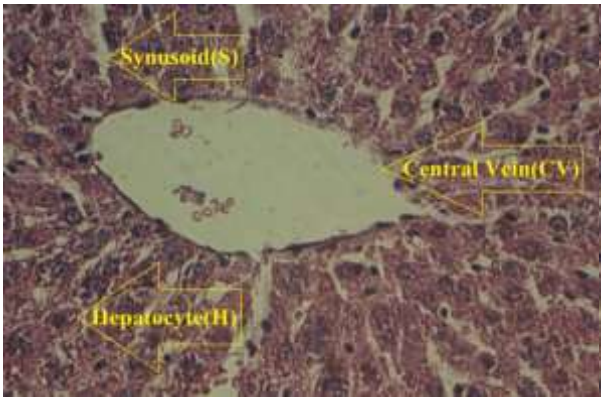

Figure 5. Cross section of liver tissue in the control group (40X)

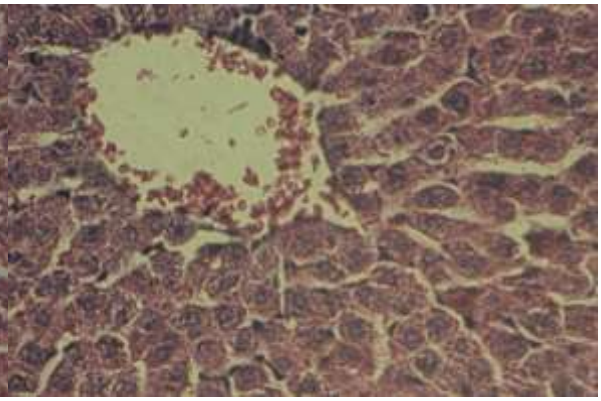

Figure 6. Cross section of liver tissue in placebo group (40X

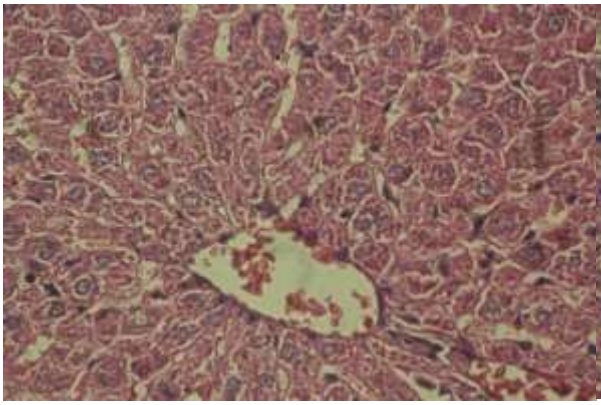

Figure 7. Cross section of liver tissue in experimental group 1 (40X)

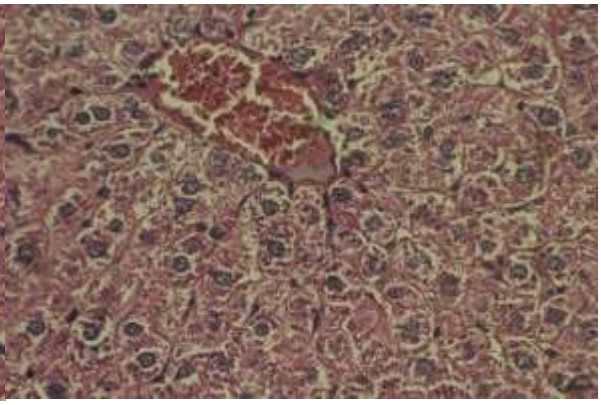

Figure 8. Cross section of liver tissue in the experimental group 2 (40X)

Discussion

Findings showed that the mean of follicle stimulating hormones (FSH), luteinizing hormones (LH), estrogen and progesterone hormones, and the number of primitive follicles, preantral, antral, and yellow objects in the neonatal period of adult female rats in experimental groups 1 and 2 (received 1.25 mg/kg and 0.91 mg/kg of the weight of rats lithium carbonate nanoparticles, respectively) reduced significantly, that in compared to the control and placebo groups, which is dependent to dose. However, number of atrophy follicles in both experimental 1 and 2 groups in the form of dose dependent showed significant increase. In line with this research results, it was reported that in that lithium led into significant reduction in the value of sexual hormones in rats and humans in experimental 1 and 2 groups. It was also reported that this drug also causes disruption in follicular development and reduction in folliculogenesis [21]. Findings of this research which was conducted on the impacts of prescribing the preantral lithium carbonate on the function of hypothalamus-pituitary-gonad axis in male infants, findings showed that lithium caused significant increase in the number of atresia follicles and reduced primary, initial, secondary follicles, graft, yellow object, sexual hormones of estrogen, progesterone, FSH and LH in children [22]. Sexual glands activity is regulated by pituitary gonadotropins FSH and LH, released under the influence of GnRH [23]. In an experimental research on the pituitary-gonad axis, lithium reduced the GnRH hormone secretion, followed by reduction in the secretion of FSH and LH [24], which this finding was in line with findings of experimental 1 and 2 groups in our study. It has been proven serotonin neurotransmitter receptors are directly involved in moderating the LH secretion in rats [25]. As serotonin has an inhibitory impact on pituitary gonadotrophin hormones through the 5HT2A receptors, an increase in serotonin will lead to reduced secretion of gonadotropin hormones from the pituitary. On the other hand, serotonin is one of the essential precursors in melatonin biosynthesis and an increase in it will result in increased secretion of melatonin from pineal cells, leading to reduced oscillating secretion of 5HT2A and as a result, reduced sexual gland activity [26]. As research has indicated that lithium increases serotonin concentration (Mahmoodi Gharai et al, 2008), lithium carbonate nanoparticles might increase the level of sexual hormones and pituitary gonadotropins and ovarian follicles through increasing the levels of serotonin. The lithium carbonate nanoparticles impact on the pituitary gonad axis and ovary activity has not been recognized so far, but it has been found that lithium act by inhibiting the e Glycogen syntheses kinase-3β enzyme (GSK-3β), which is a key molecule in the main pathway of Wnt gene expression [27]. The significance of Wnt gene pathway in the development of reproductive organs

and sexual glands has been proven [28]. As expression of various molecules in this pathway has been reported in ovarian of rat, Wnt pathway seems to play key role in follicular development of the ovary, and it is worth noting that the mechanism of the lithium effect in the ovaries is significant [29]. Considering what was said above, it could be stated that lithium carbonate nanoparticles have function similar to lithium, and accordingly, they reduce the activity of pituitary gonad axis hormones and ovarian follicles. Findings of the research show that in the two experimental 1 and 2 groups (received 1.25 mg/kg rat weight and 1.09 mg/kg rat weight of lithium carbonate nanoparticles, respectively), the concentration of aspartate aminotransferases enzymes (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) increased among different treatments. In addition, reduction in the mean number of hepatocytes was seen only in the experimental 2 group. Lithium carbonate absorbed completely through the digestive system and can easily pass through a blood - placental barrier, so that its serum concentration becomes same in mother and fetus. It can also be secreted in mother's milk, which its concentration in milk is half of the mother's serum concentration [30]. Contaminations caused by nanoparticles are today considered as new and hazardous issue [31, 32]. Durability of metal oxide nanoparticles in environment and food chain is high, leading to continued poisoning caused by them [33]. Findings of a research showed that using lithium carbonate in the long-term resulted liver degeneration and necrosis in liver tissue [34]. One research showed that using lithium in the long-term will cause hyperthyroidism, hyperplasia, and thyroiditis [9]. Research has indicated that lithium carbonate decreases superoxide dismutase (SOD) and glutathione peroxidase (GPX) and increases peroxidation of lipids in liver, resulting in disruption in antioxidant status [35]. Kim et al examined the toxicity of silver nanoparticles on liver tissue in rats. In this study, tissue damage dependent on silver nanoparticles in liver lobules, kupffer cells and sinusoids were seen in the liver tissue [36]. In a research examined the impact of silver nanoparticles on liver tissue, findings indicated that silver nanoparticles induced destructive impacts on liver tissue and caused necrosis and degeneration in liver tissue with the accumulation of inflammatory cells [37]. As ALT enzyme is a more specific marker for liver damage, damage in liver cell might increase the release of this enzyme. Thus, increased ALT enzyme might be due to destructive impact of lithium carbonate nanoparticles on liver cells. In addition, bile duct obstruction increases the serum concentration of ALP enzyme. Hence, due to liver cells damage and bile duct obstruction, ALP concentration has been increased. Increase in concentration of AST and ALT enzymes might be due to an increase in anabolism or decrease in their catabolism [38]. The stability of hepatocytes is necessary for vital functions of liver. Due to their physico-chemical properties, lithium carbonate nanoparticles will probably disrupt this stability and result in liver dysfunction. For example, research has shown that silver nanoparticles have toxic impacts on the fish cells [39]. Additionally, due to their physicochemical properties, nanoparticles reduce the bioavailability and proliferation of living cells [40, 41]. As silver nanoparticles can produce reactive oxygen species (ROS) and free radicals, over-accumulation of ROS might initiate the inflammatory responses and lead into mitochondria destruction. As a result, GSH level (Glutathione Sulfate Hydrogenase) due to inflammation decreases. Therefore, apoptotic factors, such as cytochrome C, are released, leading to cell death [37]. It could be stated that lithium carbonate nanoparticles can act similarly in two experimental 3 and 4 groups.

Conclusion

Findings of this research in general confirm transfer of toxic impacts of lithium carbonate nanoparticles from the mother body during breast-feeding on the pituitary-gonad axis activity and liver in the female gender. It is recommended that further studies to be conducted to predict the impacts and action mechanism of this substance.

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