INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 13, No. 2, p. 68-75, 2018
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Effect of the silver nanoparticles on the histology of albino lactating mice ovaries

Noor Mahdi*, Gazwa D. Al-Nakeeb

Department of Biology, College of Sciences for women, Baghdad University, Iraq

Article published on August 14, 2018

Key words: Silver nanoparticles, Lactating mice ovaries, Histopathological effects

Abstract

There is increasing interest and need to develop a deeper understanding of the nature, fate and behavior of nanoparticles in the environment. This is driven by the increased use of nanoparticles and the increased pressure to commercialize this growing technology. Silver nanoparticles (Ag NPs) are the most commonly nanoparticles used in various areas of research, because of their characteristic physical as: magnatic, mechanical: performance as well as antimicrobial effects as antiviral, antibacterial , so that exposure human to increase levels of nanoparticles. Therefore, this study aimed to investigate the histological effects of silver nanoparticles exposure on the ovary of the lactating albino mice. Sixty adult lactating female albino mice were divided into three main groups of (15) females exposed to 1p.p.m., (15) females were exposed by 1.5p.p.m. , (15) females were exposed to 2p.p.m. of silver nanoparticles solution for 7, 14 and 21 days as well as 3 control groups each group 5 mice contain by intra-peritoneal injection. After the end of injection periods the samples of animals were sacrificed and dissected to remove the ovaries and kept in Bouins fluids for microscopic examination treated groups exhibited different histopathological changes ,depending on the concentration of silver nanoparticles as : hyaline degeneration, hydropic degeneration, fibrous necrosis, and on the period of exposure, these changes include shrinkage of Oocyte, fatty degeneration, amyloid protein precipitation, pyknosis , necrosis ,cloudy degeneration, fusion and swelling cells, Caceous necrosis different damage effects in silver accumulations was noted in the ovary, with accumulations being significantly higher in female lactating mice, especially in the cortex, follicles, stroma.

***Corresponding Author:** Noor Mahdi ✉ Noor Mahdi ✉ no2000896@gmail .com

Introduction

Nanoparticles (NPs) are small atoms with size ranging between (1-100) nm. Silver nanoparticles (AgNPs) represent clusters of atoms with size ranged (1-100) nm.

The nano term indicates to 1 billion or 109 unites (Abou El- Nour,2010). are among the most widely used nanomaterial in consumer products, and their use in the food industry is a growing public concern in regards to safety, toxicity, and health risk (Wijnhoven *et al*., 2009). Silver metal has been used for centuries as an antibacterial agent, and the release of silver ions, in particular Ag+, at the surface of the metal is considered the source of its antibacterial activities. Silver ions are known to have toxic effects in a number of biological species, including bacteria, viruses, fungi, and some aquatic organisms (Rai *et al*., 2014). The NPs with smaller size than (50nm) are able of entering the cells and move out of the blood vessels if their size is less than 20nm (Yih and Wei, 2005).

The nanomaterial's have chemical and physical properties which differ from the product materials that have the bulk size. The silver nanoparticles represent more nanomaterial's used for its importance in medical and biological applications as anti-inflammatory, anti-viral and anti- fungal properties (Zhong, 2010).

The nanomaterial's are classified according to their origin to natural , manufactured or engineered and anthropogenic nanomaterial's resulting from exposure to dissolved silver toxic (Panyala, 2008). The nanomaterial's are used in tooth tools (Yoshida *et al,* 1999). The resin compound interferes with silver nanoparticles that contain materials with long – term inhibitory effects against *Streptococcus* mutants. The silver nanoparticles are used with cancer and neurological disease treatment (Donner, 2010) and wound dressing (Trop, 2006).

In a study the silver nanoparticles caused inflammation in rat liver, expansion of sinusoids and apoptosis, as well as it could lead to physiological changes (Sardari,2012) a decrease in the testosterone hormone levels in male albino mice serum that exposed to different concentrations after 28 days exposure.

Therefore, this study investigated the dose and time dependent effect of exposure to Ag NPs on ovarian histology.

Material and methods

Collection of animals

60 adult females of albino mice were collected from Pharmaceutical Supervision Department/The Ministry of Health, the infertility Institute and techniques of assisted reproduction and Iraqi center for research on cancer/The Ministry of Higher Education and Scientific Research, Which acclimated in conditions of the animals house in the infertility Institute and techniques of assisted reproduction for month before begun the study through provide favorable condition.

Control and treated groups

Sample of animals were divided into 3 treated groups, samples coloured in the tails included one group consists of 15 females exposed to 1p.p.m., 15 females were exposed by 1.5p.p.m. , 15 females were exposed to 2p.p.m. of silver nanoparticles solution for 7, 14 and 21 days as well as 3 control groups each group 5 mice contain. After the end of injection periods the samples of animals were sacrificed and dissected to remove the ovaries which there fixed by Bouins fluids for 22 hours (Bancroft , 2012).

Histological study

The histological slides were prepared by many steps including fixation, washing, dehydration, clearing, infiltration and embeddind, sectioning, staining (Bancroft, 2012) and Microscope examination and photography.

Results

In this study, the normal ovary tissue of control groups is dissected as shown in Fig. 1.

It is consist of cortex that's contain different follicles and medulla containing stoma cells, blood, nerve, lymph vessels and smooth muscles fibers.

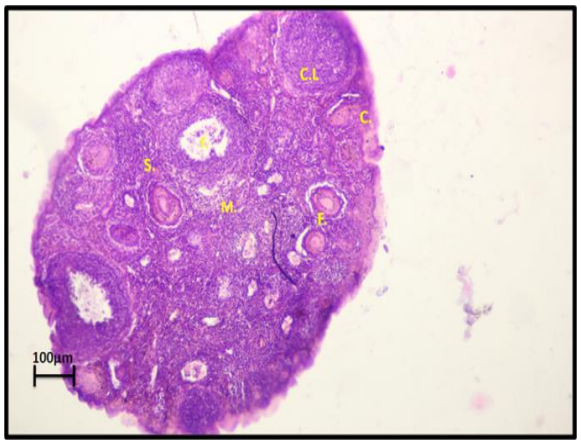

Fig. 1. Cross section of control lactation female ovary (H&E)(400X).

The result of histological sectioning of treated groups with 1p.p.m. after 7 days exposure of silver nanoparticles indicated some of histopathological changes including shrinkage in the Oocyte in growing follicle , fat degeneration, Pyknosis in all Follicle cells in granulosa layer of growing follicle Furthermore, precipitation of amyloid protein between follicle cells in growing follicle was occurred Fig. 2.

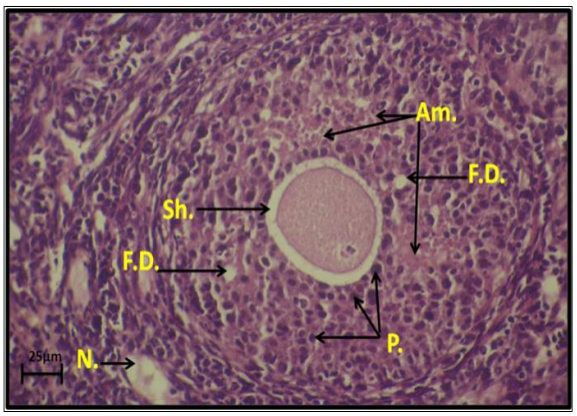

Fig. 2. Cross section of exposed lactation female ovary to 1p.p.m. after 7day Am (Amyloid), F.D. (Fatty Degeneration), P. (Pyknosis), Sh. (Shrinkage), N.(Necrosis). (H&E) (400X).

In lactating ovaries exposed to 1p.p.m. after 14 days, necrosis in Oocyte and necrosis in stroma cells , fatty degeneration in cells of granulose layer of growing follicles , Hyaline degeneration and fatty degeneration in stroma cells were occurred Fig.3.

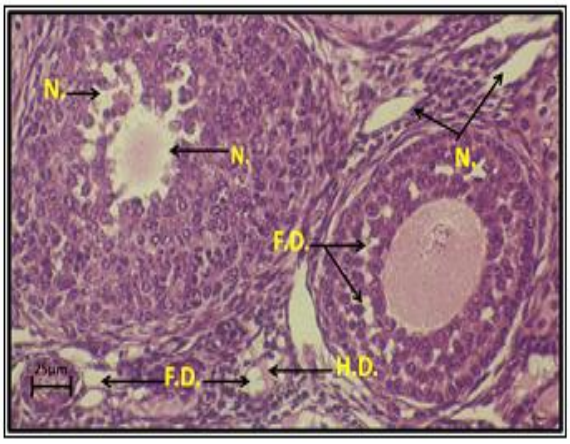

Fig. 3. Cross section of exposed lactation female ovary to 1p.p.m. after14 day F. D. (Fatty Degeneration), H.D. (Hyaline Degeneration), N.(Necrosis). (H&E) (400X).

The histopathological effects of1p.p.m. on treated groups after 21 days exposure showed a precipitation of amyloid protein in blood vessels of stroma cell and necrosis Fig. 4.

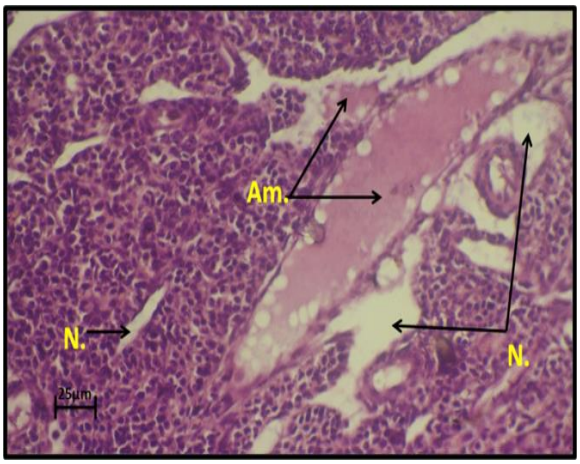

Fig. 4. Cross section of exposed lactation female ovary to 1p.p.m. after21 day N. (Necrosis), Am (Amyloid) (H&E) (400X).

The histological sections of treated groups exposed to concentration of 1.5p.p.m. After 7 days, presents swelling and fusion cells and cloudy degeneration in stroma cells Fig. 5.

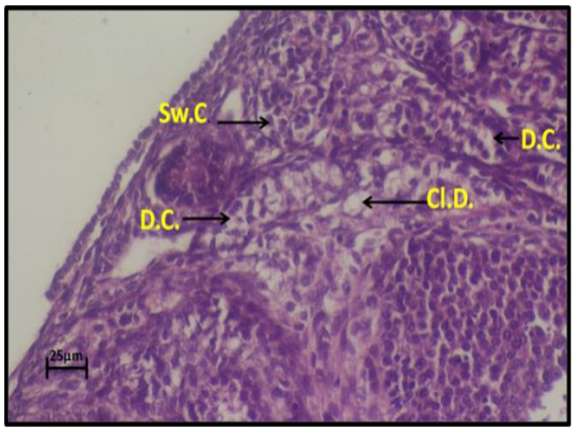

Fig. 5. Cross section of exposed lactation female ovary to 1.5p.p.m. after 7 day Sw. C. (Swelling Cell), F.C.(Fusion Cell), Cl. D.(Cloudy Degeneration) (H&E) (400X).

As well as exhibited ahydropic degeneration and fusion cell in luteal cells in treated groups with concentration of 1.5p.p.m. After 14 days Fig. 6, While, at 1.5 p.p.m. after 21 days exposure, they appeared to increase the precipitation of amyloid, hyaline degeneration , necrosis in granulosa layer of follicle and pyknosis in follicle cell Fig. 7.

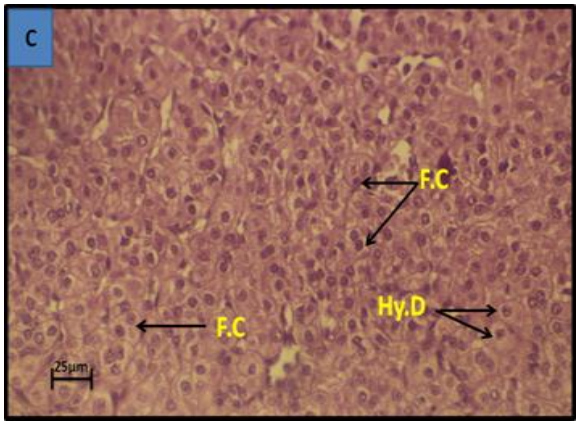

Fig. 6. Cross section of exposed lactation female ovary to 1.5p.p.m. after14 day F.C.(Fusion Cell), Hy.D.(Hydropic Degeneration) (H&E) (400X).

Whereas, the histopathological changes in lactating ovaries tissue in treated groups of silver nanoparticles at 2p.p.m. After 7 day exhibits casceous necrosis, pyknosis in luteal cells Fig. 8. Furthermore, at a concentration of 2p.p.m. after 14 days exposure, group showed fibrous necrosis, pyknosis in luteal cell of corpus luteum Fig. 9, and continuous precipitation of amyloid protein in stroma cells, shrinkage follicle cell of growing follicle and necrosis in concentration 2p.p.m. After 21 days of exposure of silver nanoparticles Fig.10.

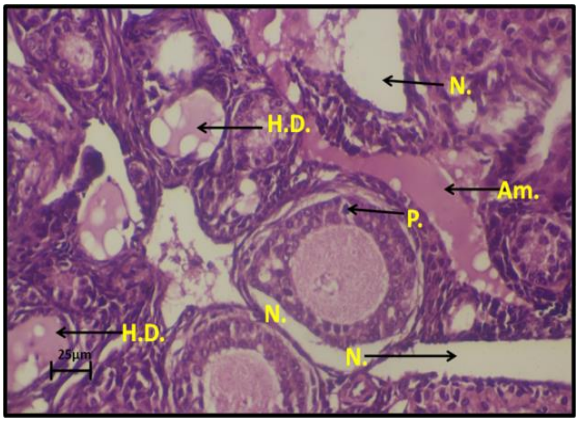

Fig. 7. Cross section of exposed lactation female ovary to 1.5p.p.m. after 21 day, H. D. (Hyaline Degeneration), N.(Necrosis), Am. (Amyloid), P. (Pyknosis), (H&E)(400X).

Discussion

The silver nanoparticles caused a toxicological effect in human and environment, the major cause of toxic effect is the ionic shape of silver in aqueous phase and release of tones of silver to the environment as an industrial wastes. When exposed to solvents, silver compounds result in toxic effects on human body organs and other organisms. Silver nanoparticles have different effects on different organs.

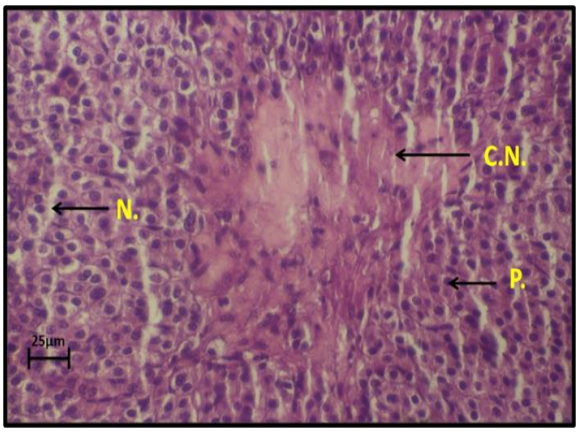

Fig. 8. Cross section of exposed lactation female ovary to 2p.p.m. After 7 day, N. (Necrosis), C. N. (Casceous Necrosis) (H&E)(400X).

The silver nanoparticles formed complex with cell proteins , when particles enter the organs and caused inflammatory reaction against nanoparticles (Attia, 2014) and exert effects were presented in respiratory tract and blood cells , this effect depends on the size of particles , diameter of silver nanoparticles as well as Brownian movement (Gavanji *et al*, 2014) that leads to release cytokines that enter the circulatory system and distributed to spleen , heart , liver , kidneys as well as to female reproductive system.

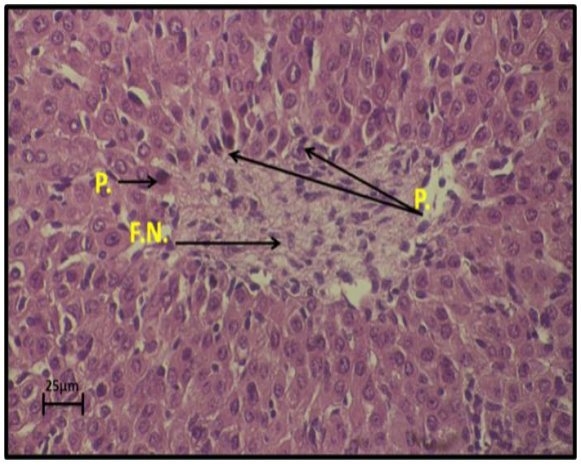

Fig. 9. Cross section of exposed lactation female ovary to 2p.p.m. after 14 day, P. (Pyknosis) , F. N. (Fibrous Necrosis) (H&E)(400X).

From the figure (5) the damage effects of silver nanoparticles in lactating mice ovaries is presented as a cloudy degeneration in the concentration 1.5p.p.m. after 7days exposure , the reason is due to change in liquid balance and damage in cell membrane that cause damage of Na+-K+ pump , and disturbance in ions balance , which caused liquid accumulation in cytoplasmic components (Stevens *et al*, 2009).

The current study showed fat degeneration state in granular layer of growing follicle after 7days exposure to1p.p.m. After 14 days, the damage appeared in stroma cell and granular layer of growing follicle from the figure (2)(3) . The major reason is due to fat degeneration that's lead to disturbance in metabolism of fatty acids and un balance of lipids in and out of the cell by damaging the cell membrane.

As a result of free silver ions, lipids were accumulated intracellular, and the cell is vaculated, the small vacuoles fuse with others to formed large vacuole found in cytoplasm of the cell , and the nucleus occupied most of the cell wall. (Kumar *et al*,2007).

The hydropic degeneration causes damage in the ovaries in lactation mice at a concentration of 1.5p.p.m. after 14 days exposure in luteal cell of corpus luteum from the figure (6). Silver nanoparticles cause swelling damage in cell, accumulation of water and Na+, Ca+ intracellular and K+ extracellular ,the reasons are due to the inhibition of glycolysis by silver ions Ag+ through the inhibition of oxidative phosphorylation, causes swelling of mitochondria and decreases of ATP production leads to inhibition of Na+, K+ ATPase of cell membrane which due to the high uptake of water and Na+ intercellular and pump out extracellular (Altunkaynak *et al*, 2016).

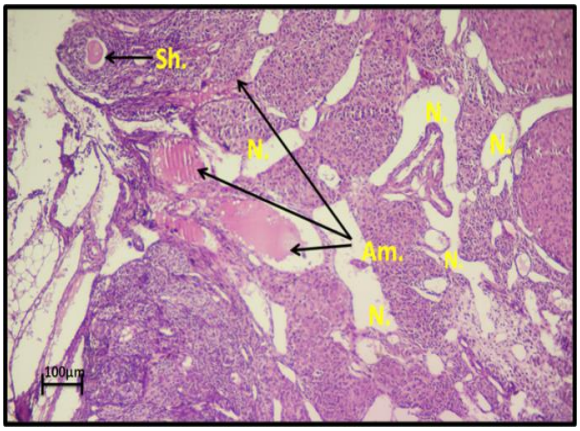

Fig. 10. Cross section of exposed lactation female ovary to 2p.p.m. after21day, N.(Necrosis), Sh. (Shrinkage), Am.(Amyloid) (H&E)(400X).

Interestingly, hyaline degeneration appeared in ovaries tissue exposed to 1p.p.m. after 14 days, and the same effect is appeared at 1.5p.p.m. after 21 days in stroma cell from the figure (3) (7) .The reason of this occurrence is due to the deposition of protein resulted from decomposition of amino acids and accumulation in the cytoplasm of cell , As a result many cells are damaged and inflammation of silver nanoparticles is occurred (Robbins and Kumar, 1987).

Some of histological sections exhibit a caceous necrosis at 2p.p.m. after 7days exposure from the figure (8) , it was appeared by lipid damage and transfer fat cell into necrotic cell by accumulation of lipid in the cell that presented as a foam shape and forms a large cell contains many nucleus . This is happened by lipase releases in the cell and fatty materials damage and releases of fatty acids. The acids are fused with calcium, resulted from inflammatory process and form a foam shape, and the area appeared as white (Bhattacharya, 2016).

The study also showed a fibrinoid necrosis at 2p.p.m. after 14 days exposure in corpus luteum from the figure (9) resulted from accumulation or precipitation of proteins in ground substance of tissues through deposition of plasma proteins in blood vessels walls.

The study showed that cells are fused at 1.5p.p.m. after 7 days exposure in stroma cells and the same effect is seen after 14 days in luteal cells of corpus luteum from the figure (5)(6). This appearance is due to the presence of cells called epitheloid cells that aggregate with other to form cells contain many nucleus that have the same cytoplasm, that resulted from chronic inflammation of silver nanoparticles (Sundritter and Thomas , 1979).

The current study showed an amyloid deposition appeared in ovaries of lactation mice at concentration 1p.p.m. after 7days exposure, whereas, 1p.p.m., 1.5p.p.m. and 2p.p.m. after 21 days exposure in stroma from the figure (2)(4)(7)(10) which is represented of ahomogeneous protein cellular materials deposits in blood vessels and basal membrane . The amyloid protein deposition leads to lack of cellular functions by a avoiding of process to diffuse within extracellular tissues resulting from distribution in protein synthesis that due to the effect of silver ions released from silver nanoparticles or due to phagocytic immunoglobulin's by taken the light chains from cells after partial lysis of released protein fibers by the shape of amyloid (Steven *et al*, 2009).

The study showed that some cells are unable to adapt of changes resulted from silver nanoparticles by appearing of pyknosis, which is the first stage of necrosis, when cells are exposed to 1p.p.m. after 7 days, in granular layer of growing follicle, un like granular layer from the figure (2) , pyknosis is seen in corpus luteum of ovary when exposed to2p.p.m. after 7days from the figure (8) where as it was seen in luteal cells after 14 days exposure at 2p.p.m. from the figure(9), the granular layer of follicle cells showed necrosis of cells and death when exposed to 1.5p.p.m. of 21 days from the figure (7), thus led to activat factors of death that released from cytochrome C from mitochondria which resulted from silver ions and effect on mitochondria and then lead to lack of RNA from damaged endoplasmic reticulum caused to pyknotic nuclei and chromatin become dense and dark mass and this is one type of necrosis resulted from damaged effect of silver nanoparticles on genetic materials (Steven *et al*, 2009).

Shrinkage of nucleus is seen in growing follicle where exposed to 1p.p.m. after 7days from the figure (2) and 2p.p.m. after 21 days exposure from the figure (10) the same is happened in follicle , the nucleus appeared small in size , chromatin dense and the nucleus envelope (Al- Zahid *et al*, 2015).

Silver nanoparticles was caused a necrosis at 2p.p.m. after 7days of exposure from the figure (8) in luteal cell of corpus luteum and at 1p.p.m. after 14 days from the figure (3) cell of growing follicle and in stroma cells . At 1p.p.m., 1.5p.p.m., 2p.p.m. after 21 days from the figure (4)(7)(10), the necrosis appeared in stroma cell in ovaries tissue by pump in a large amount of water and Na+ intracellular cause swelling and damage when continued and cause mitochondrial swelling , lack of ATP release resulted from oxidative phosphorylation and caused balance distribution intracellular, cytoplasmic, nucleolus damage, release lytic enzymes caused lytic of cells. (Battacharya, 2016).

References

Abou El–Nour KMM, Eftaiha A, Al Warthan A. 2010. Synthesis and applications of Silver nanoparticles. Arabian journal of chemistry **3(3),** 135-140.

Wijnhoven SWP, Peijnenburg WJGM, Herberts CA, Hagens WI, Oomen AG, Heugens EHW, Roszek B, Bisschops J, Gosens I, Van De Meent D. 2009. Nano-silver, A review of available data and knowledge gaps in human and environmental risk assessment. Nano Toxicology **3,** 109–138.

Rai M, Deshmukh SD, Ingle AP, Gupta IR, Galdiero M, Galdiero S. 2016. Metal nanoparticles: the protective Nano shield against virus infection. Crit. Rev. Microbiology **42(1),** 46-56. PMID: 24754250 http://dx.doi.org/10.3109/1040841X2013.879849

Yih TC, Wei TC. 2005. Nanomedicine in cancer treatment. Nano medicine: Nanotechnology, Biology, and Medicine **1,** 191-192.

Zhong W, Xing MM, Maibach HI. 2010. Nano fibrous materials for wound care. Cutan. Ocul. Toxicology **29(3),** 143- 152.

Panyala NR, Pena-Mendez EM, Havel J. 2008 . Silver or Silver Nanoparticles: A Hazardous Threat to the Environment and Human Health. Journal of Applied Biomedicine **6**, 117-129.

Yoshida K, Tangagawa M, Matsumoto S, Yamada T, Atsuta M. 1999. Antibacterial activity of resin composites with silver containing materials. European Journal of Oral sciences **107(4),** 290 -296.

Donner A. 2010. Nanotechnology in molecular medicine trends in molecular medicine. ESof **16(12),** 551 -552.

Trop M, Novak M, Rodl SL, Lellbom B, Kroell W, Goessler W. 2006. Silver coated dressing Acticoat caused raised liver enzymes and Argyria like symptoms in burn patient. J. Trauma **60,** 648- 655.

Bancroft JD, Steven AS. 2012. Theory and practice of histological techniques. 2nd ed. Churchill living stone, Edinburgh, London: 233-250.

Sardari RRR, Zarchi SR, Talebi A, Wang S, Wang J, Liu LJ, Li J, Yuan F, Lu S, Wan Z, Chou L, Xi T. 2012. Toxicological effects of silver nanoparticles in rats. Afr. J. Microbiology Research **6(27),** 5587- 5593.

Attia A, A. 2014. Evaluation of the testicular alterations induced by silver nanoparticles in male mice : Biochemical , Histological and ultra structural studies. Research Journal of Pharmaceutical, Biological and Chemical Sciences **5(4),** 1558-1589.

Gavanji S, Sayedipour SS, Doost mhammdi M, Larki B. 2014. The effect of different concentration of silver nanoparticles on enzyme activity and liver tissue of adult male wistar rats in vivo condition. I.I.S.R.K, **4(2),** 182-188.

Stevens A, Lowe J, Scott I, Damjanov I. 2009. Core Pathology .3th ed . Elsevier. China: 442-443.

Kumar V, Abbas AK, Fausto N, Mitchell RN. 2007. Robbins Basic Pathology .8th ed .John F. Kennedy Blvd. Philadelphia , USA: 85-86**.**

Altunkaynak BZ, Akgul N, Yahyazadeh A, Altunkaynak ME, Turkmen AP, Akgul HM. Unal B. 2016. Effect of mercury vapor inhalation on rat ovary: Sterology and histopathology. J. Obstet Gynaecol , Research **42(4),** 410-416.

Robbin SSL, Kumar V. 1987. Basic Pathology .4 th Ed Saunders Company, Phildelphia , London, 787.

Bhattacharya GK. 2016.Concise pathology for exam preparation . 3rd Elsevier. RELX India, 14-15.

Sundriter W, Thomas C. 1979. Color Atlas and Textbook of Histopathology. 6th Ed. Sand Ritter, Walter. USA, 225.

Al zahid AA, Faris KJ, Kodiar OS. 2015. Histopathological study of the toxic doses of Clove Oil *Syzygium aromaticum* on ovaries of female rabbits. IOSR-JAVS **8(8),** 33-38.

Zhang S, Du C, Wang Z, Han X, Zhang K, Liu L. 2013. Reduced cytotoxicity of silver ions to mammalian cells at high concentration due to the formation of silver chloride. Toxicol. *In vitro* **27**, 739–744. [PubMed].

Hadrup N, Lam HR. 2014. Oral toxicity of silver ions, silver nanoparticles and colloidal silver—a review. Regul. Toxicol. Pharmacology **68,** 1–7. [PubMed]